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Chapter 7

Platelets, Inflammation and Respiratory Disease

Brian P. O'Sullivan and Steven W. Kerrigan

Additional information is available at the end of the chapter

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1. Introduction

The lungs have several characteristics that separate them from other organs; they receive the total cardiac output, they have two sources of perfusion, pulmonary and bronchial arteries, and they are exposed to the external environment, making them vulnerable to damage caused by inhaled microorganisms and airborne particulates. Chronic obstructive pulmonary disease (COPD), consisting of both chronic bronchitis and emphysema, is caused by inhalation of tobacco combustion products that cause inflammatory changes that overwhelm the body's natural anti-inflammatory defense mechanisms. Inhaled bacteria and viruses lead to infection and infiltration of polymorphonucleocytes (PMN) whose exo-products including superoxides, hyopchlorous acid, and elastases damage lung tissue even as they repel the infecting organisms. Additionally, events such as sepsis and shock can set off a systemic inflammatory cascade involving the lungs.

Virtually all lung diseases (with the exception of congenital malformations) involve some degree of inflammation of the airways, the alveoli, or the parenchyma. Given the increasing recognition of the role of platelets in inflammation, it is not surprising that platelet biology has become an active area of interest for respirologists around the world. Evidence of this is the publication in the last several years of comprehensive reviews of platelet and lung biology, platelets and innate immunity, and the contribution of platelets to specific disorders such as asthma, acute lung injury, and cystic fibrosis.[1-6] In this chapter we will review the basic biology of the platelet-lung interaction and work that demonstrates the important part these cell-like structures play both in maintenance of lung health and in multiple pulmonary diseases.

2. Platelet genesis in the lung

Megakaryocytes emerge from the bone marrow and travel to the lung where they are trapped in the microvasculature and form elongated processes known as proplatelets. Shear forces or other factors then cause proplatelets to release individual platelets.[7] Platelet release occurs in bone marrow, but there is mounting evidence that much platelet release from proplatelets occurs in the pulmonary circulation, making the lungs the birthplace of the platelet.[1] Evidence supporting this includes the observation of megakaryocytes in pulmonary vascular beds and an increased number of platelets in pulmonary venous blood as compared to pulmonary arterial blood. The presence of a large pool of newly formed platelets in the pulmonary circulation means that there is ample opportunity for interaction with other blood cells. PMN, which like megakaryocytes have trouble navigating the pulmonary microvasculature due to their resistance to deformation, accumulate in the pulmonary circuit creating a pool of marginated white blood cells. This leads to intimate contact between these cells and the potential for cell-cell interactions.

3. Platelets in Inflammation

Platelets are anucleate cytoplasts that for many years were felt to play a role exclusively in hemostasis. More recent studies suggest that platelets also play an important role in inflammation, including promoting the inflammatory response to influenza virus.[8, 9] Platelets are derived from the same myeloid stem cell as traditional inflammatory cells and therefore have retained the ability to serve as inflammatory cells. For example they can undergo chemotaxis, contain and release adhesive proteins, activate other inflammatory cells, release vasoactive substances, and have the capacity to express or release pro-inflammatory mediators such as thromboxane (TX) A_2,, platelet activating factor (PAF), brain derived neurotrophic factor (BDNF), and platelet factor 4 (PF4, also known as, CXCL4), as well as a host of other chemokines and chemokine receptors (Table 1).[10-13] Platelets contain the largest amount of transforming growth factor-β (TGFβ) in the body and express pattern recognition toll-like receptors.[14] Furthermore, direct cell-cell contact by specific adhesion molecules facilitates transcellular metabolism of arachidonic acid (AA) by both PMN and platelets.[15, 16] Platelets provide free AA to PMN, which enhances production of PMN-derived leukotriene (LT) B_4 and the cysteinyl leukotrienes, LTC_4, LTD_4, and LTE_4. At the same time, platelet-derived enzymes such as 12-lipoxygenase act on PMN eicosanoid products to produce other lipid mediators, including the anti-inflammatory lipoxins (LXs).[17] Although platelets do not have nuclei, they do contain substantial amounts of mRNA and are recognized as being capable of *de novo* synthesis of a growing number of proteins including adhesion molecules, CD40L, and interleukin-1β.[14, 18, 19]

Platelets are a linking element between hemostasis, inflammation, and tissue repair.[12, 14] Not only are they activated via traditional pathways (thrombin, adenosine diphosphate (ADP), TXA_2), they can be stimulated by antigens, antigen-antibody complexes, microorganisms, and

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bacterial endotoxins, including lipopolysaccharide from gram negative bacteria such as, *Pseudomonas aeruginosa*.[20] Platelet granules store a large number of biologically active substances that can be released upon activation. It has been suspected that platelets can undergo differential granule release of pro- or anti-inflammatory mediators depending on specific circumstances.[14] Release of mediators stored in platelet granules and *de novo* platelet production of other mediators can enhance the inflammatory response. Histamine and serotonin increase vascular permeability; ADP increases the agonist-induced oxidative burst in PMN; platelet-derived growth factor (PDGF) stimulates chemotaxis for monocytes and primes eosinophils to produce superoxide anion; and, PF4 induces PMN to adhere to unstimulated vascular endothelium, induces the release of histamine from basophils, and stimulates the adherence of eosinophils to vascular walls.[11] Platelets can also release RANTES (regulated upon activation, normal T-cell expressed and secreted), which plays an important role in recruitment and adhesion of monocytes to activated endothelium.[21] Once leukocytes are recruited to tissue, platelets potentiate the inflammatory process by inhibiting apoptosis of PMN, monocytes, and eosinophils.[22, 23]

Inflammatory mediator	Platelet location	Mediator type	Inflammatory role
TXA_2	Synthesized	Eicosanoid	Platelet amplification, Monocyte activation & T-cell differentiation
IL-1β	Synthesized	Cytokine	Endothelial cell activation
PDGF	α-granules	Growth factor	Neutrophil, monocyte and macrophage recruitment
PF4	α-granules	Chemokine	Eosinophil, neutrophil and T- cell recruitment,
TGF-β	α-granules	Growth factor	Fibroblast and monocyte recruitment
PDHRF	α-granules	Chemokine	Eosinophil recruitment
P-selectin	α-granules	Selectin receptor	Adhesion of platelets to monocytes, neutrophils, lymphocytes; complement activation
CD63	α-granules	Tetraspanin receptor	Neutrophil, monocyte recruitment
MIP-1α	α-granules	Cytokine	Eosinphil and Neutrophil activation, B-cell immunoglobulin production
VEGF	α-granules	Growth factor	Lymphocyte & endothelial cell activation
RANTES	α-granules	Chemokine	T-celll & monocyte migration and recruitment
5-HT	δ-granules	Transmitter	Dendritic cell & lymphocyte activation
ADP	δ-granules	Nucleotide	Leukocyte, platelet & endothelial cell activation
Histamine	δ-granules	Transmitter	Activation of the endothelial cells; increase in permeability
Glutamate	δ-granules		Lymphocyte trafficking

Platelets metabolize arachidonic acid (AA) via the cyclooxygenase and lipoxygenase pathways to produce inflammatory mediators, the most abundant of which are $TXA_{2,}$ via the cyclooxygenase pathway, and 12-S-HETE, via the 12-lipoxygenase pathway.[24] Platelet 12-lipoxygenase interacts with the products of AA metabolism by other cells (notably PMN) to produce lipoxins.[17] In addition, PMN stimulated by endotoxin release PAF, which activates platelets,[25] that in turn recruit more PMN to the inflamed area. Platelets also provide positive feedback mechanisms for their own activation. ADP secreted from dense granules and TXA_2 formed from AA bind to P2Y and TP receptors on the platelet surface, complete initial platelet activation, and recruit additional platelets into the activated fraction. Thus, once the inflammatory cascade has been initiated, leukocytes and platelets combine to propagate and amplify it.

In addition to the active role platelets play in producing inflammatory mediators – and perhaps even more important – is the role they play in the process of migration of WBC from the vascular compartment to the site of tissue injury or inflammation. In order for leukocytes to invade inflamed or infected tissue, the white blood cell must first be tethered to the vessel wall and roll along the endothelial surface, then attach firmly to the endothelium, and finally migrate through the endothelium into the tissue. The final two steps, firm attachment and diapedesis, are the result of up regulation of integrin molecules, particularly integrin $\alpha_M\beta_2$ (Mac-1, CD11b/CD18). However, tethering and rolling are dependent upon the function of selectins, especially P-selectin.[26] P-selectin is stored in α-granules of platelets and Weibel-Palade bodies of endothelial cells, from which it is translocated to the cell surface membrane upon activation of the cell. Once expressed, P-selectin binds to leukocytes via P-selectin glycoprotein ligand-1 (PSGL-1).[27] P-selectin glycoprotein ligand-1 is constitutively expressed on leukocytes and allows for formation of neutrophil-platelet, monocyte-platelet, and eosinophil-platelet aggregates if P-selectin is trafficked to platelet cell membranes. Although endothelial expression of P-selectin alone can lead to leukocyte rolling, this process (which is a necessary precursor to firm attachment and diapedesis) is much more efficient in the presence of platelet P-selectin in part due to platelet-leukocyte aggregates which amplify the ability of WBC to be recruited to the endothelial surface by cross-linking (figure 1).[28] Expression of platelet membrane P-selectin and release of their chemoattractants enhances leukocyte recruitment into the lung tissue. The number of platelets adherent to pulmonary vessels (the marginated pool) need not be large in order to affect vascular permeability and PMN recruitment. A small number of activated platelets can signal PMN-platelet and platelet-platelet interactions that lead to an increased number of platelet-PMN aggregates, which can become tethered to the pulmonary vascular endothelium.

Thus, platelets fit the definition of traditional inflammatory cells in many ways -- they are capable of phagocytosis and elaboration of pro-inflammatory cytokines, chemokines, and lipid mediators, and are vital for the process of leukocyte tethering and rolling, which are necessary fist steps in recruitment of leukocytes to areas of inflammation. Animals made deficient in platelets or in whom platelet P-selectin is blocked or deficient are less capable of mounting an inflammatory response and have fewer white blood cells in target organs and humans with inflammatory processes have increased numbers of platelet-leukocyte aggregates and

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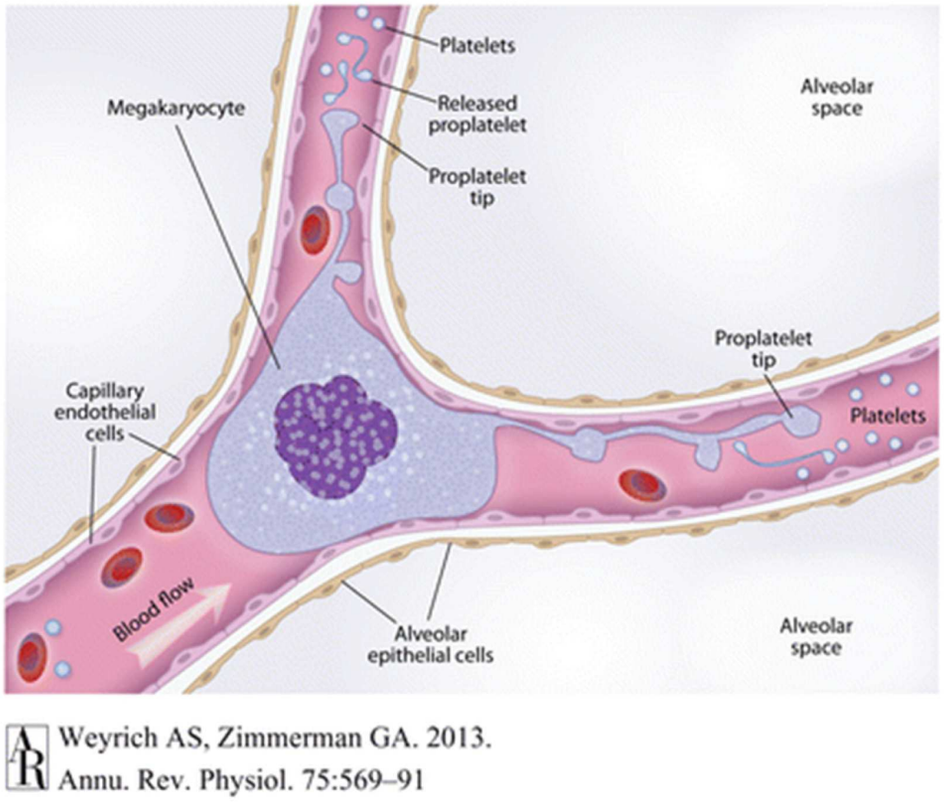

Figure 1. Megakaryocytes are found in human lung microvessels and may spawn platelets and platelet precursors in this location. Reproduced from Weyrich and Zimmerman 2013. Platelets in Lung Biology. Annu. Rev. Physiol. 75:569-91 with permission from Annual Review of Physiology, Volume 75 © 2013 by Annual Reviews.

increased expression of platelet P-selectin, both markers of platelet activation.[29, 32] In summary, platelets are a key component of the inflammatory response in pulmonary tissue.

4. Platelets and lung health

In addition to the pro-inflammatory properties of platelets outlined above, these tiny blood constituents are essential effector cells in lung hemorrhage and in vascular barrier function. As reviewed by Weyrich and Zimmerman, platelets play at least 5 roles in maintaining endothelial barrier function in the pulmonary circulation: release of soluble molecules that enhance barrier function, physical obstruction of gaps, maintenance of structural features of endothelial cells, stimulation of endothelial growth, and neutralization of agents that might enhance endothelial permeability.[1] Thrombocytopenia, therefore, can have a detrimental effect on barrier function beyond simple hemorrhage. Among the secreted products that enhance endothelial cell-cell interactions and promote vascular integrity are shingosine-1-phosphate (S1P), serotonin, and angiopoietin-1. S1P appears to be particularly important in stabilizing pulmonary endothelium by enhancing adherens junctions and tight junctions.[8] There is evidence that a critical number of platelets are necessary for basal barrier integrity. Platelets are also important for vascular repair and remodeling. The vasoactive substances that

platelets release may play protective or damaging roles depending on the circumstances surrounding platelet activation. Platelets are active effector cells in wound healing and can also play roles in angiogenesis and vascular repair and remodeling.[33] Some of these actions, when dysregulated, may lead to pulmonary hypertension, smooth muscle proliferation, and pathological remodeling of lung tissue.

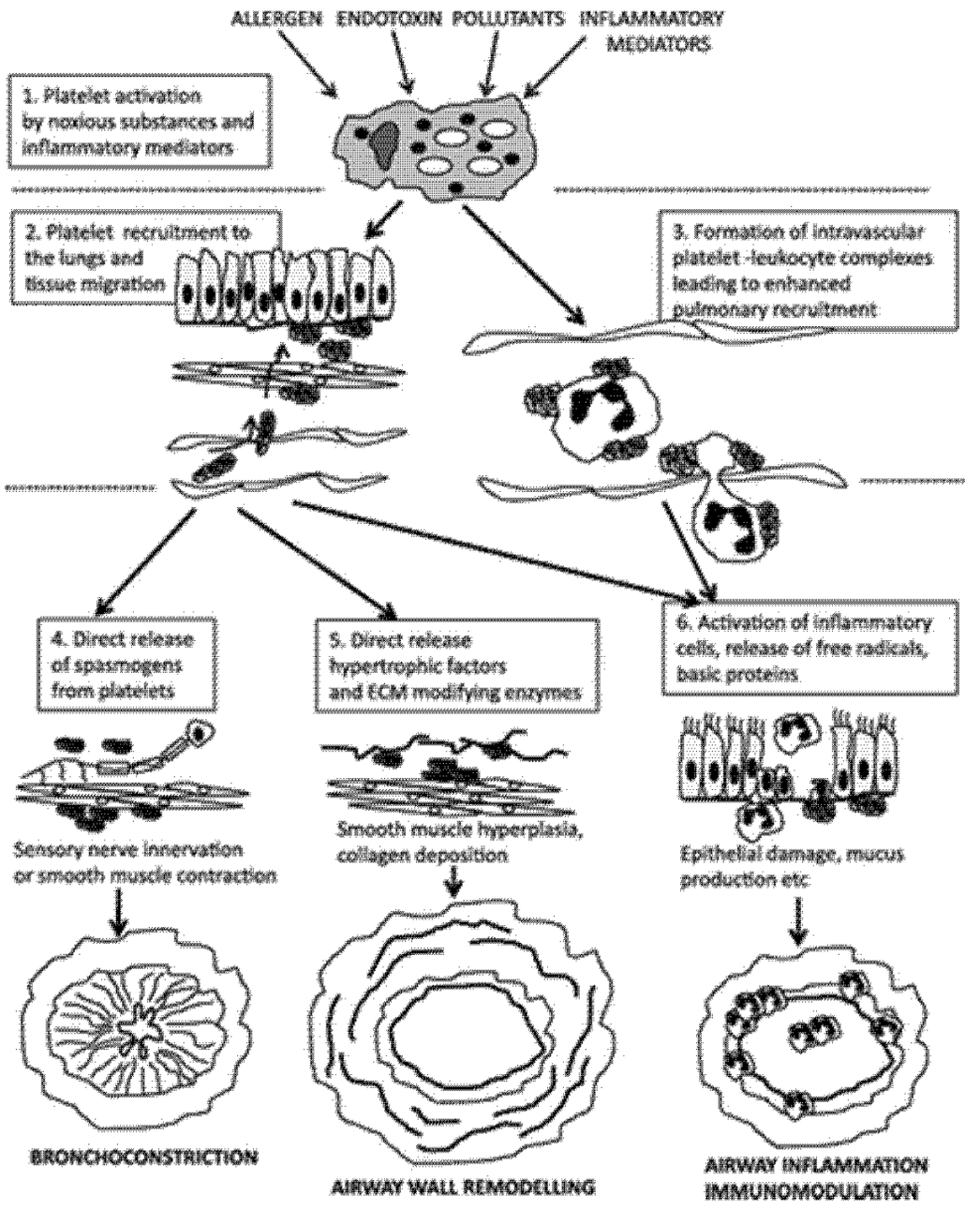

Figure 2. Platelet activation leading to airway inflammation. 'Reproduced from Page & Pitchford, Platelets Coming of Age: Implications for Our Understanding of Allergic Inflammation, Am J Respir Crit Care Med 2013;187:459-460, with permission of the American Thoracic Society. Copyright © 2015 American Thoracic Society.

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5. Platelets and asthma

Over a century ago Sir William Osler recognized that asthma was an inflammatory disease. Much work has been done since then looking for causes and treatments of airway inflammation in patients with asthma.[34] However, despite the fact that as long ago as 1967 it was recognized that platelets could release the vasoactive substance serotonin in response to a hypersensitivity challenge[35] it is only recently that the importance of platelets in this disease process has been recognized and explored in detail.

In a series of elegant animal and human experiments spanning more than a decade, investigators have shown that platelets play a fundamental role in the recruitment of leucocytes to the lungs following exposure to allergens and play a key role in the onset and perpetuation of airway inflammation in asthma.[2] Studies have shown that animals deficient in platelets, platelet receptors, or factors activating platelets have abrogation of responses to allergic challenges.[29, 32, 36, 37] De Sanctis et al[38] looked at ovalbumin sensitized mice that were P-selectin deficient and found reduced numbers of eosinophils and lymphocytes in lung lavage fluid compared to wild type mice, indicating the importance of this platelet-expressed adhesion molecule in mediating allergen induced pulmonary inflammation. Following this work, Ulfman and colleagues[39] showed that P-selectin bearing platelets were integral in tethering of eosinophils to activated endothelium in an ex-vivo perfusion model. Further studies have shown the importance of platelet P-selectin and soluble P-selectin in eosinophil attachment to VCAM-1.[40] These authors concluded that their findings were, "...compatible with a scenario whereby P-selectin, on eosinophil-associated activated platelets or acquired from plasma or from prior interactions with endothelial cells or platelets, activates eosinophil $\alpha 4\beta 1$ integrin and stimulates eosinophils to adhere to VCAM-1 and move to the airway in asthma."[40] Antibodies that block P-selectin have been shown to decrease leukocyte recruitment in animal models of asthma and inflammation.[2] P-selectin appears to be a necessary component of the allergic response in sensitized animals. Platelets also appear to play a role in causing disease in the lung parenchyma itself, with evidence of platelet diapedesis into lungs in animals sensitized to allergens.[41] Platelets that have trans-migrated into tissue can then release their abundant pro-inflammatory mediators and exacerbate allergic inflammation, a possibility that could explain non-eosinophilc airway inflammation that has been reported in some human asthmatic patients.

Human studies substantiate the finding of the importance of platelets seen in allergic animal models. There is a decreased half-life of platelets in atopic patients, implying increased platelet activation and turnover. Increased circulating neutrophil-platelet aggregates and monocyte-platelet aggregates have been seen in atopic humans and up to 25% of eosinophils may be attached to platelets in such subjects.[40] As noted in animal studies, these attachments increase expression of surface markers (integrins), which enhance leukocyte adhesion to blood vessels. Moritani and colleagues[42] were able to demonstrate an association between platelet activation as assessed by P-selectin expression and RANTES release with an asthmatic diathesis in humans. Similarly, Durk and colleagues[43] have shown increased serotonin in segmental lavage fluid from asthmatic subjects when challenged locally, implying a role for

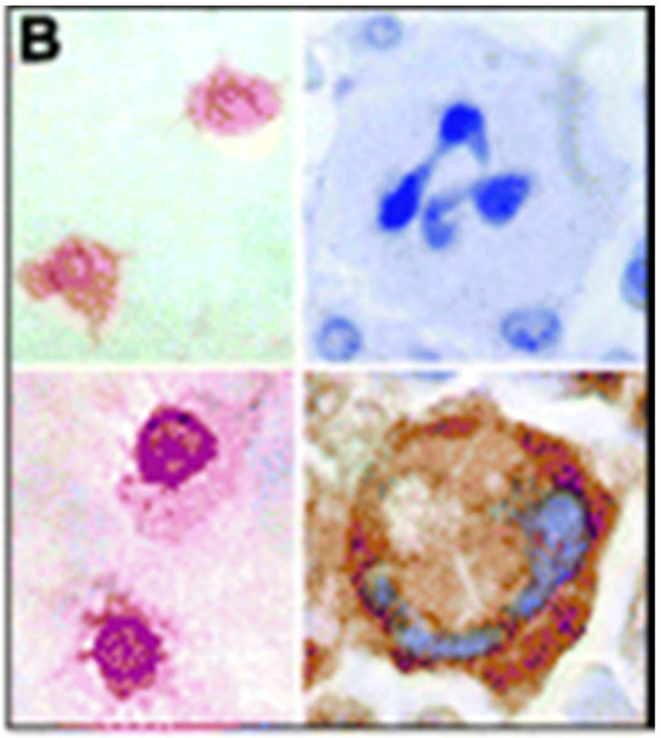

Figure 3. Immunocytochemical detection of CFTR in human PLTs (top left: irrelevant antibody; bottom left: anti-CFTR antibody) and bone marrow megakaryoblasts (top right: irrelevant antibody; bottom right: anti-CFTR antibody). Reproduced from Mattosccio et al., Cystic fibrosis transmembrane conductance regulator (CFTR) expression in human platelets: impact on mediators and mechanisms of the inflammatory response. FASEB J 2010;24:3970-3980. with permission of the Federation of American Societies for Experimental Biology. © 2010 FASEB

activated platelets, the major source of serotonin in the human lung, in allergic inflammation. These authors suggest that peripheral serotonin production could be a viable target for future asthma therapy. Others have shown a temporal relationship between circulating platelet-derived factors (included soluble P-selectin) and early and late asthmatic response in allergic subjects. Lommatzsch, et al[10] have looked at another, less well-studied platelet secretion, brain-derived neurotrophic factor (BDNF). This group found that patients with asthma had higher levels of BDNF in serum, platelets, and plasma compared to non-asthmatic matched controls. BNDF levels correlated with parameters of airway obstruction and hyper-reactivity, making this mediator of neuronal plasticity one more platelet product capable of affecting the asthmatic airways.

Associations between platelets and metabolites of arachidonic acid have been identified in asthma patients. Lipoxins play a role in the resolution phase of inflammation and are the product of interaction between PMN-associated arachidonic acid and platelet-derived 12-lipoxygenase. Oxidative stress in asthma is correlated with decreased LXA_4 in patients with severe asthma and reflects a loss of anti-inflammatory balance in these patients. In this group of patients ex-vivo incubation of cells with an inhibitor of LX degradation significantly inhibited the PAF-induced platelet-leukocyte aggregates in peripheral blood that contribute to persistent airway inflammation.[44] It is conceivable that drugs that increase LXs or other

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pro-resolution mediators could offer an alternative or adjunct to corticosteroid therapy in asthma.

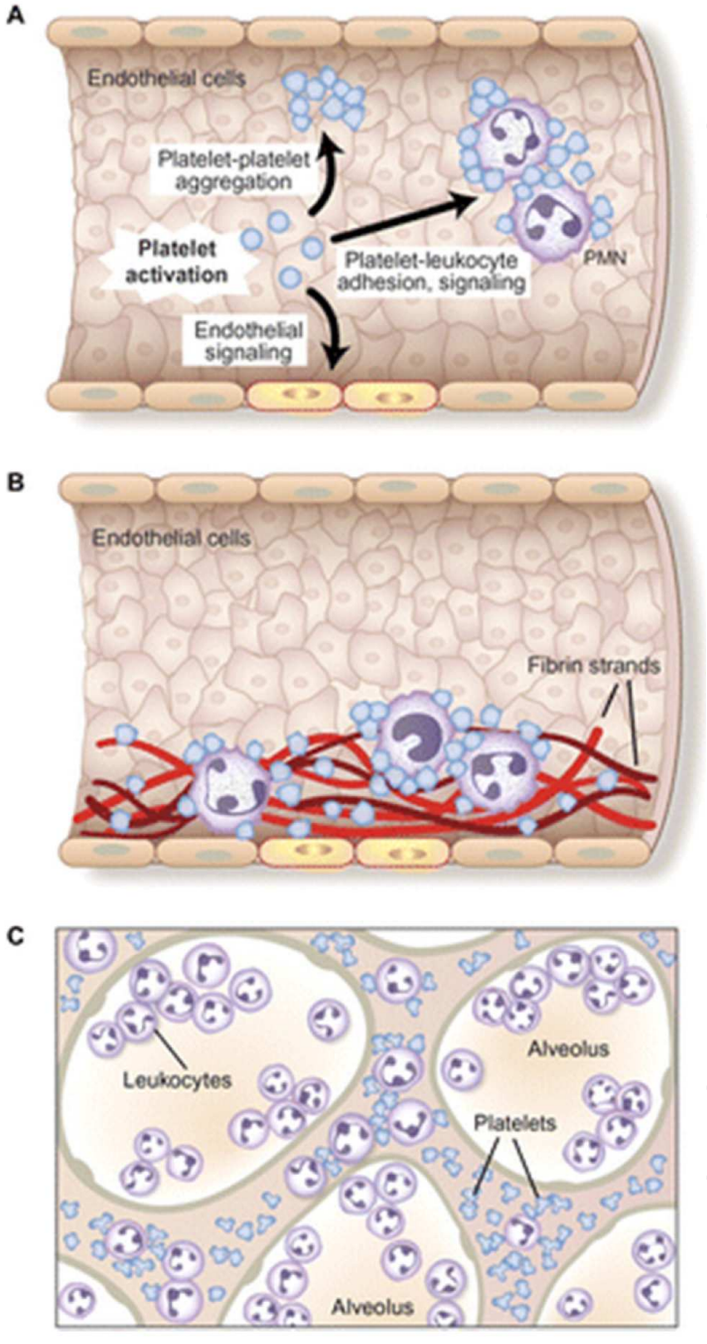

Figure 4. Platelet-leukocyte interactions in respiratory endothelium in acute lung Injury. Platelet activation upregulates adhesion molecules. (B) Platelets and PMN accumulation in microvasculature is enhanced by fibrin deposition in response to enodthelial injury. (C) Platelets facilitate accumulation of PMN in alveoli and interstitial spaces and contribute to alveolar leak syndromes. 'Reproduced from Bozza et al., Amicus or adversary: platelets in lung biology, acute injury, and inflammation. Am J Respir Cell Mol Biol 2009;40:123-134, with permission of the American Thoracic Society. Copyright © 2015 American Thoracic Society.'

In a series of human studies specifically looking at aspirin exacerbated respiratory disease, examination of nasal polyps from aspirin sensitive subjects demonstrated extravascular platelets adjacent to leukocytes. Platelets appeared to contribute an inordinate amount of LTC_4 synthase activity in these subjects compared to non-aspirin sensitive subjects.[45] LTC_4 synthase converts AA-derived LTA_4 to the bioactive bronchospastic LTC_4. Further mouse studies by this group have shown a potential role for anti-platelet drugs in aspirin induced asthma. Reduced production of melatonin by platelets also has been seen in aspirin-sensitive asthmatics.[46] This deficit could, through several mechanisms, lead to increased platelet activation and be a factor in these patients' intolerance to aspirin.

It is possible that the coagulation cascade may also play a role in the airway remodeling seen in chronic asthmatic patients.[47] Although some early studies using selectin inhibitors have been reported, human studies of the role of anti-platelet agents in the control of asthma are lacking. Growing evidence in human and non-human models clearly makes this a promising area to pursue.

6. Platelets and cystic fibrosis

Cystic fibrosis (CF) is an autosomal-recessive illness that is the most common lethal inherited disease in the Caucasian population. CF is caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on the long arm of chromosome 7.[48] The CFTR protein acts as a chloride and bicarbonate channel, among other functions. Loss of chloride channel function leads to dehydration of the airway surface liquid, which leads to thickened airway secretions, chronic respiratory infection, neutrophilic lung infection, pulmonary destruction, and premature death.[48] The majority of CF patients also have pancreatic insufficiency, predisposing them to malnutrition and fat-soluble vitamin deficiency. The CFTR protein acts predominantly as a chloride channel, but plays multiple other roles in cellular metabolism, including influencing long chain fatty acid synthesis.[49] A hyper-inflammatory state – whether innately due to CFTR dysfunction or a result of chronic infection – contributes to lung destruction over time in CF patients. This inflammatory process is characterized by PMN infiltration of airways and pulmonary parenchyma with elaboration of free neutrophil elastase and pro-inflammatory cytokines.[50] Platelets play a crucial role in facilitating the recruitment of PMN into the CF lung, and as active members of the innate immunity family release their own pro- and anti-inflammatory agents. The process of PMN recruitment in CF is similar to that described above for asthma.

By the end of the 1980s a number of studies had been performed assessing the function of platelets from CF patients. These studies were done prior to the discovery of the CFTR gene and with an incomplete understanding of the underlying molecular defect in these patients. A better picture of the role of platelets in CF has emerged with improved techniques for evaluating platelet function and a more complete knowledge of CFTR function.[5, 51] Cross-sectional studies showing an inverse relationship between platelet activation and pulmonary function provide evidence for a role for platelets in CF lung disease. These studies have shown

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that: 1) increased platelet numbers correlate with decreased arterial blood oxygen tension (P_aO_2),[52] 2) an increase in urinary concentration of thromboxane metabolites (a marker of platelet activation *in vivo*) correlates with decreased forced expiratory volume (FEV_1),[53, 54] and 3) increased plasma concentrations of soluble CD40 ligand (sCD40L).[55]

Several groups have demonstrated increased reactivity of CF platelets. Ciabattoni and colleagues[54] showed enhanced lipid peroxidation, which contributes to platelet activation in CF patients. They documented increased urinary excretion of TxB_2 metabolites and isoprostanes, consistent with oxidative stress and increased arachidonic acid turnover in CF patients and hypothesized that these factors led to platelet aggregation and activation and contributes to lung disease in CF patients. O'Sullivan and co-workers[56] showed that washed platelets from CF subjects express increased agonist-induced surface P-selectin expression (a marker of platelet activation) compared to those from control subjects. Also, platelets from CF patients incubated in plasma from non-CF subjects were more reactive to agonists than control platelets in the same plasma. Even though CF plasma upregulated function of both CF and non-CF derived platelets, CF patients' platelets were more reactive than their matched controls.[56] In addition, McGivern and colleagues demonstrated that CF patients' platelets were defective in release of their dense granules. This observation was critical as platelet dense granules contain many mediators responsible for the recruitment of inflammatory cells. Thus CF patients may have a muted inflammatory cell mobilization.[57] Together these studies support platelet activation in CF. Given the known interaction between platelets and leukocytes in the pulmonary circulation and the abundance of PMN in CF bronchoalveolar lavage and biopsy/autopy specimens, it is apparent that platelet activation mediates the inflammatory cell infiltration to at least some degree.

CFTR protein expression was first described in epithelial cells from nasal epithelium and sweat glands in humans. Since that time, CFTR expression has been identified in circulating blood cells including PMN, leukocytes, and monocytes. It is feasible that platelets also express CFTR and that its absence might explain some of the abnormalities in platelet function seen in CF patients. There have been reports of indirect evidence of loss of CFTR function in CF platelets. Agam et al.[58] demonstrated abnormal platelet volume changes in response to PGE_1, reflecting down regulation of a cAMP-regulated channel, of which CFTR is one. In another indirect evaluation of CFTR function in CF platelets, Ulane and collaborators [59] demonstrated an intrinsic increase in turnover of platelet membrane phospholipids (specifically, phosphatidylcholine), which they ascribed to CFTR dysfunction. Although CFTR protein and mRNA have not been detected in control or CF platelets by some investigators,[56] Mattoscio et al[51] did find evidence of CFTR in platelets and megakaryocytes using flow-cytometry, immunohistochemical stains, Western blot, and RT-PCR. Elegant studies by this group on CF platelet proteomics has shown distinct characteristics of CF platelets that predispose them to promoting pulmonary inflammation. In particular, they showed constitutive overexpression of beta-3 integrin, which contributes to platelet activation and which can activate the NF-KB pathway in PMN.[60]

Platelets have been found to contain an excess of the long-chain polyunsaturated fatty acid known as Mead acid.[56] When Mead acid is metabolized by platelet 12-lipoxygenase an end-

product is generated with PGE_2-like activity, which enhances platelet aggregation.[61, 62] Platelet 12-lipoxygenase also acts on arachidonic acid released from PMN cell membranes to form members of the anti-inflammatory lipoxin family. CF patients have a defect in lipoxin synthesis that appears to be directly related to a lack of normal function of CFTR in platelets.[51] In addition to their anti-inflammatory properties LXs also contribute to maintenance of normal airway surface liquid depth.[63] Loss of this normal airway lubrication is one of the fundamental pathogenic features of CF and appears to be exacerbated by the lack of normally functioning CFTR protein in platelets. Furthermore, cells from respiratory epithelium of CF patients have a decreased amount of another long chain polyunsaturated fatty acid, docosahexaenoic acid (DHA).[49] DHA is a precursor to a group of anti-inflammatory compounds known as resolvins, which are important in leading to the resolution of the normal host response to infection or injury.[64] Thus, intrinsic defects in CF platelets enhance the inflammatory process, inhibit its resolution, and contribute to the underlying defect in airway surface liquid. It appears that when normal CFTR function is lost in those with CF results in patients with CF it results in upregulation of inflammation and a corresponding loss of the counterbalancing resolution phase of inflammation.

In addition to intrinsic platelet abnormalities, there is abundant evidence of the presence of pro-inflammatory mediators in CF patients' plasma. Increased levels of sCD40L, LTB_4, immune complexes, interleukins, ATP, and tumor necrosis factor-α have all been documented.[55, 65, 67] It is possible that platelet activation in CF is merely an "innocent bystander" effect due to the fact that the platelets are immersed in a pool of platelet-activating substances. In fact, there is evidence that platelets from non-CF subjects are activated when incubated in CF plasma.[56] Many of these mediators both come from and activate platelets; therefore, it is hard to know if platelet activation causes inflammation or if inflammation causes platelet activation. In summary, loss of normal CFTR function can indirectly increase platelet activation through its affect on plasma factors such as specific cytokines, fatty acids, ATP, and vitamin E, and loss of CFTR function specifically in platelets can directly increase platelets activation. The final result, irrespective of cause of activation, is increased recruitment and activation of leukocytes into the CF lung, elaboration of mediators that cause tissue damage, and enhanced loss of lung function

7. Platelets and acute lung injury

The acute respiratory distress syndrome (ARDS) is defined as acute onset of hypoxemia in response to insults including sepsis, trauma, aspiration, and toxins. In 1994 the American-European Consensus Conference defined ARDS as acute onset of hypoxemia with bilateral infiltrates on chest roentgenogram and no evidence of left atrial hypertension.[68] The American-European group defined another entity, acute lung injury (ALI), as similar lung disease but with a lesser degree of hypoxemia. Although a 2012 task force[68] did not include ALI as a distinct entity, instead subsuming it in a sub-category of ARDS, many still use the combined term ALI/ARDS to describe those suffering from acute, severe respiratory distress.

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Despite multiple new therapeutic interventions based on well done clinical trials, mortality in the most severe category of ARDS still hovers around 50%.[69]

A hallmark of ARDS is increased capillary leak and influx of neutrophils and fluid into the lung interstitium. Recent reviews have emphasized the role of platelets in instigating this pulmonary vascular leak.[3, 8] Sepsis and trauma in particular lead to systemic inflammation and vascular permeability. Interestingly, several of the strategies employed in critical care units that have led to decreased mortality from ALI/ARDS include interventions that decrease platelet activation; these include low volume ventilation, nitric oxide administration, and anti-inflammatory/anti-platelet medications.[3] This insight has led to a trial of the anti-platelet agent aspirin in ARDS, the Lung Injury Prevention Study with Aspirin.[70]

The interplay between coagulation and inflammation has become an increasingly active area of investigation in acute lung injury. Activation of the coagulation pathway, including platelet activation, and decreased fibrinolysis contributes to ALI/ARDS. The maintenance of a normal endothelial cell barrier is a critical component of the fluid and protein diffusion balance in the pulmonary circulation. In health, platelets play an important role in systemic and pulmonary vascular integrity (see above). A number of studies show that platelet depletion can lead to vascular leakage and that reconstitution with infused platelets can correct the barrier defect (reviewed in reference 1). Platelets may take up radical oxygen species and release preformed mediators such as serotonin, which contribute to endothelial barrier stability. Among these mediators is sphingosine 1-phosphate (S1P), a lipid growth factor that promotes endothelial integrity and which may be a therapeutic agent in capillary leak syndromes.[71, 72] Thus, platelets – by direct thrombotic action and through release of specific mediators – can provide protection of respiratory function by decreasing the likelihood of capillary leak in response to injurious effectors.

Platelets may provide a degree of protection for the injured lung through their hemostatic mechanisms; however, observations suggest a negative role for platelets in experimental alveolar injury. A hallmark of ALI/ARDS pathology is the presence of microthrombi in the pulmonary circulation. Studies have shown increased numbers of platelets and leukocytes in pulmonary capillaries and platelet-fibrin thrombi have been detected in autopsy specimens from patients dying of ALI/ARDS.[3] Thrombocytopenia is a poor prognostic factor in sepsis and may represent sequestering of activated platelets in the microcirculation.[3] Post-mortem and biopsy samples have shown thrombi within pulmonary arteries, arterioles, and capillaries from patients suffering from ALI/ARDS and can explain in part the ventilation-perfusion mismatching that contributes to the severe hypoxemia seen clinically.[73] These thrombi are the result of activation of platelet hemostatic mechanisms, which have the capability to be both beneficial and harmful to an ill patient. Activated human platelets synthesize tissue factor and provide signals that induce tissue factor production by monocytes.[8] Tissue factor then leads to formation of microthrombi and clots that then occlude pulmonary vessels. The ability to inhibit thrombus formation may explain some of the clinical effect of inhaled nitric oxide (NO) in critically ill patients. NO has been shown to decrease platelet aggregation and improve oxygenation in a small cohort of ALI/ARDS patients, which the investigators attributed to its anti-thrombotic effect[74] and in vitro studies by another group showed an NO dose-depend-

ent decrease in ADP and collagen induced platelet aggregation, P-selectin expression, and fibrinogen binding.[75] NO administration to ALI/ARDS patients demonstrated similar reductions in platelet aggregation and P-selecting expression. It is possible that the transient in oxygenation seen with NO administration lies in part in its anti-platelet action and not just as a vasodilator.

Systemic inflammation caused by sepsis activates circulating cells and leads to formation of heterotypic aggregates.[76] These platelet-leukocyte interactions lead to production and release of factors that can disrupt vascular integrity. The major effector cell in ALI/ARDS is the PMN, which causes endothelial damage, plasma leakage, and hypoxemia.[77] The importance of neutrophils in syndromes of acute alveolar damage is underscored by animal studies where lung injury is abolished by induction of neutropenia. Since platelets play such a key role in PMN tethering and rolling it is not surprising that they play an active role in the inflammatory process accompanying ALI/ARDS. Since platelets are integral to PMN tethering and rolling it is not surprising that they play an active role in sepsis. Using a mouse model of sepsis-induced ALI caused by inhaled lipopolysaccharide (LPS) Grommes and colleagues[78] demonstrated that animals deficient in either platelets or neutrophils had marked reduction in neutrophilic infiltration and protein/fluid leak into alveoli. In separate experiments these investigators depleted either platelets or neutrophils. Further work showed that treatment with monoclonal antibodies to the platelet-derived chemokines CCL5 and CXCL4 led to decreased neutrophil recruitment, plasma exudation, and protease release. The mechanism for this pulmonary protection appeared to be disruption of the platelet-leukocyte heteromers formed by these chemokines. Surprisingly, these authors did not find that blocking adhesion molecules commonly associated with platelet-neutrophil aggregation, specifically P-selectin and GPIIb/IIIa, led to decreased diffuse alveolar damage.

Although the work by Grommes et al implicated CCL5 and CXCL4 and not P-selectin as the critical surface markers in heterotypic aggregate formation, others found that selective disruption of platelet P-selectin led to decreased platelet-PMN aggregates, decrease TXA_2 release, and decreased lung injury in an acid-induced model of ALI.[79] Further evidence of the fundamental role of platelet-leukocyte aggregates in ALI/ARDS comes from work by Ortiz-Munoz and colleagues[80] who showed that the aspirin-induced LX, 15-epi-lipoxin A_4, has a protective effect in LPS induced lung injury through its ability to disrupt heterotypic aggregate formation. Using intravital microscopy they were able to show formation of platelet-neutrophil aggregates in response to LPS administration and a sharp decline in aggregate formation, platelet activation, and lung injury following aspirin administration.[80] Thus a large number of studies indicate the importance of activated platelets and platelet-leukocyte aggregates in the development of ALI/ARDS in animal models. Evidence of P-selectin upregulation on the surface of circulating platelets in humans with ALI/ARDS supports the theory that these cytoplasts play and active role in human disease, too.[3, 30] Patients with sepsis have been shown to have increased surface P-selectin expression on platelets and increased plasma levels of products of α-granule release.[76] It is well recognized that expression of P-selectin is a marker of platelet activation and, the animal study of Grommes et al[78] not withstanding, leads to formation of platelet-leukocytes heterotypic aggregates. Although this likely repre-

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sents an adaptive mechanism of enhancing leukocyte recruitment to areas of infection or injury, a maladaptive consequence of platelet activation is the tissue injury and alveolar leakage that results from release of their cytoplasmic contents and recruitment of activated PMN.

LPS from gram-negative bacteria is a potent activator of platelets and platelet-leukocyte aggregates. In addition to producing mediators that can trigger platelet activation, both gram-negative and gram-positive bacteria can interact directly with platelets. In their thrombotic role, platelets adhere to exposed sub-endothelial matrix at the site of vascular injury as the first line of defense against hemorrhage. This places them in perfect position to act as sentinels against invading microorganisms. In fact, platelets are now recognized as part of the innate immune system patrolling the endothelium and capable of rapid release of an array of immunomodulatory cytokines.[6] Furthermore, platelets express toll-like receptors and interactions with bacteria through these pattern recognition receptors can lead to aggregation and/or activation of platelets directly, or indirectly through the action of plasma proteins.[4, 81] Infected thrombi formed by bacterial-platelet adhesion can seed the microcirculation of the lung or stick to exposed surfaces such as damaged heart valves.[82] Human platelets can engulf, but do not kill bacteria due to a lack of myeloperoxidase and their inability to form "killing chambers" isolated from the cytoplasm and the cell's exterior.[83] However, upon activation at the site of endothelial injury, platelet granules release thrombin-inducible platelet microbiocidal proteins, which act against a broad range of microbes. The released anti-microbial proteins include platelet activating factor-4 (CXCL4), RANTES, and β-defensins.[6, 82] Platelets then have a Janus-like character acting either for the betterment or detriment of the host; which it is depends on the timing and location of the stimulus. Platelet activation and release of anti-microbial proteins may halt invasion by bacteria or may cause tissue damage by upregulating inflammation by recruiting and activating more platelets, leukocytes, and heterotypic aggregates. Recent work implicates a specific single nucleotide polymorphism linked to platelets, which is associated with ARDS risk at least partially mediated via effects on platelet count. [84]

There is growing evidence implicating the role of neutrophil extracellular traps (NETs) in the genesis of lung damage in sepsis.[81, 85] NETs are extracellular lattices, which are capable of capturing and killing bacteria. Clark et al[81] were able to show that NETs are released from PMN in response to binding of platelet TLR4 to neutrophil TLR4 ligand but that stimulation of PMN with bacterial LPS in the absence of platelets did not induce NET release. Presumably an aspect of the innate immune system that protect organisms from bacterial infection, NETs are primarily released in small capillaries and are capable of causing damage to endothelial tissue. Whereas platelets contribute to the integrity of the endothelial barrier and promote bacterial killing, they also promote endothelial damage directly by release of their own pre-formed and rapidly transcripted mRNA products and from activation of leukocytes, whose inflammatory products and NETs have adverse effects on the pulmonary parenchyma.

Transfusion related acute lung injury (TRALI) is the leading cause of transfusion-related mortality and is defined as acute lung injury that occurs during or within 6 hours of transfusion of one or more units of blood or blood components.[86] Known risk factors for developing TRALI include several recipient factors indicative of severity of underlying illness (including

high pressure mechanical ventilation, a trigger for platelet activation) and donor factors such as female sex, HLA class II antibodies, and volume of anti-human neutrophil antigens.[86] Patients with TRALI have evidence of systemic inflammation with increases in circulating neutrophils and a decrease in platelets compared with controls, similar to those with the systemic inflammatory response syndrome seen in sepsis.[87] It is likely that TRALI is caused by infusion of anti-neutrophil antibodies and/or biologically active lipids (potentially platelet derived) that interact with recipient neutrophils that are already primed by the underlying illness.[8, 88] Activation of platelets and neutrophils then leads to the cascade of endothelial injury and permeability seen in other forms of acute lung injury. A study showed that infusion of stored platelet concentrates, rich in lipid mediators, could cause ALI when infused into rats pretreated with LPS.[88] Treatment with aspirin, which induces lipid mediator such as LXs with antagonistic actions on platelet activation and platelet-neutrophil aggregation, abrogated the TRALI response in a murine model of TRALI.[80]

8. Platelets and chronic obstructive pulmonary disease

The vast majority of studies on chronic bronchitis and emphysema have focused on the role of tobacco smoke exposure as the causative agent. Clinical trials tend to look at the effect of various bronchodilators or antibiotics in the treatment of COPD exacerbations. However, dampening the response to the inciting constituents of tobacco smoke has the potential to alleviate many of the downstream consequences of cigarette smoking. A handful of studies have examined platelet counts and platelet volumes in COPD, with sometimes contradictory results.[89, 90] PMN have been shown to play a fundamental role in the development and progression of COPD and their transmigration across pulmonary endothelium is critical to their function in COPD.[91, 92] The process of activation of platelets and adherence of inflammatory cells to pulmonary endothelium is enhanced by cigarette smoke.[93] Given the role of PMN in the destructive nature of COPD and the known role of platelets in facilitating transmigration of these cells in the pulmonary circulation, it is intuitive to believe that platelets must play a role in COPD just as they do in asthma and CF.

9. Summary

Platelets clearly play a major role in the development of multiple acute lung injury syndromes including transfusion-related pulmonary problems. Activation and aggregation of platelets plays a crucial role in the recruitment and activation of leukocytes to the site of lung injury/infection. Additionally, platelets are part of the innate immune system and are capable of triggering immune responses via release of pro-inflammatory cytokines and chemokines. It is not surprising, therefore, that they are an integral part of the inflammatory cascade in a host of pulmonary diseases. In addition to the specific processes discussed in detail in this chapter, platelets are undoubtedly active participants in a myriad of other lung processes that involve cellular infiltration and tissue destruction. The platelet is ubiquitous in pulmonary disease

genesis and sustainability. Further work with platelet inhibitors, specific and non-specific, may lead to novel approaches to therapy of chronic and acute lung disease.

Author details

Brian P. O'Sullivan[1*] and Steven W. Kerrigan[2]

*Address all correspondence to: osullivb@hitchcock.org

1 Geisel School of Medicine at Dartmouth, Hanover, NH, USA

2 Royal College of Surgeons in Ireland, Dublin, Ireland

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