Platelets are small, disc-shaped cell fragments that circulate in the blood and play an important role in hemostasis. The lifespan of a platelet is ≈5 to 9 days. Emerging evidence indicates that platelets may be involved in the pathogenesis of pulmonary arterial hypertension (PAH). Platelets store secretory granules that contain growth and mitogenetic factors, cytokines, and vasoactive substances, which are released in a regulated manner on stimulation. α granules contain P-selectin, transforming growth factor-β1, platelet-derived growth factor (PDGF), B-thromboglobulin, platelet factor 4, RANTES (Regulated on Activation, Normal T Cell Expressed and SECRETED), CCL2 (chemokine (C-C motif) ligand 2), tumor necrosis factor-α, interleukin-1α, interleukin-1β, fibrinogen, and coagulation factors V and XIII. δ (dense) granules contain serotonin (5-hydroxytryptamine [5-HT]), calcium, and ADP/ATP. Activation of platelets increases the production of thromboxane A2, which, in turn, activates other platelets and promotes vasoconstriction and thrombosis.

Several platelet-derived molecules have been shown to be involved in the pathogenesis of PAH. Among them, PDGF is an extremely potent mitogen and chemoattractant for pulmonary arterial smooth muscle cells (PA SMCs). The PDGF level and its receptor expression are elevated in PA SMCs in animal models of PAH. PDGF receptor expression is enhanced in the lungs of patients with PAH. Multiple experimental studies support a role of PDGF in the pathogenesis of PAH. Inhibition of PDGF by imatinib (or Gleevec) was able to reverse pulmonary hypertension in 2 different animal models of PAH. Imatinib improved pulmonary arterial remodeling, right ventricular hypertrophy, and cardiac output and increased animal survival. Ghofrani et al reported that imatinib reversed SMC proliferation and neointima formation in a human patient with PAH. Therefore, PDGF may play a role in some forms of PAH although the imatinib in PAH, a randomized, efficacy study (IMPRES) showed that imatinib treatment was unsatisfactory. Activation of platelets also releases vasoconstrictors, such as thromboxane A2 and serotonin (Figure).

Serotonin, or 5-HT, increases vasoconstriction and impairs the endothelial–SMC cross talk. Enhanced 5-HT expression and transport in animal models of PAH have established a link between 5-HT and PAH. Some drugs (eg, anorexigenics) that alter 5-HT levels are associated with an increased risk for the development of PAH.

Platelets releases transforming growth factor-β1, which stimulates the deposition of extracellular matrix (Figure). Other growth factors released by platelets include insulin-like growth factor 1, fibroblast growth factor, and vascular endothelial growth factor. These growth factors may play a significant role in PA SMC overproliferation and PA remodeling in PAH.

On stimulation, platelets also release inflammatory cytokines, such as interleukin-1β, interleukin-1α, and tumor necrosis factor-α (Figure), which causes inflammation in endothelial cells contributing to endothelial dysfunction. Inflammation plays an important role in the pathogenesis of PAH.

Although platelets play a role in PAH, the underlying mechanism is incompletely understood. It is not known why activated platelets target pulmonary vascular cells rather than systemic vascular cells. It seems possible that molecules released from PA cells lead to aggregation of platelets to the injured PA sites, which then release vasoactive, mitogenetic, and inflammatory factors. The other possibility may be that the sensitivity of the injured PA cells to these factors is enhanced. Therefore, how vascular cells (eg, endothelial cells) activate platelets is the key to the understanding of the mechanism of PAH. Thus, attention should be paid to the communication between vascular cells and platelets in PAH. PA endothelial cells, in normal hemostasis, act to inhibit platelet activation by producing several factors, such as nitric oxide (NO), endothelial-ADPase, and prostaglandin I2. Endothelial-ADPase clears away the platelet activator, ADP.

In this issue of *Circulation Research*, Bauer et al reported the role of toll-like receptor 4 (TLR4) on platelets in the pathogenesis of experimental PAH. TLR4 detects lipopolysaccharide from Gram-negative bacteria and plays an important role in the activation of the innate immune system. TLR4 is expressed on platelets, which mediates inflammatory and immune responses. In this study, the authors demonstrated the first evidence that platelet-specific deletion of TLR4 protected the development of PAH in hypoxia-induced and Sugen/hypoxia-induced models. Interestingly, platelet-specific deletion of TLR4 and global deletion of TLR4 attenuated hypoxia-induced PAH and right ventricular hypertrophy to approximately the same degree, suggesting that it is the platelet TLR4 that is involved in the pathogenesis of PAH. Platelet-specific deletion of TLR4 abolished hypoxia-induced up-regulation of P-selectin on the surface of platelets. P-selectin functions as a cell adhesion molecule that promotes platelet...
aggregation and leukocyte infiltration to the injured site during inflammation. Hypoxia also increased serum levels of serotonin in wild-type mice, which is known to be involved in the pathogenesis of PAH. The upregulation of serum levels of serotonin was abrogated by platelet-specific deletion of TLR4, suggesting that the platelets are the primary source of the hypoxia-induced increase in circulating serotonin. Although it is known that TLR4 is involved in the pathogenesis of PAH, this study further specified a critical role of the platelet TLR4 in the development of experimental PAH. The results indicate that platelet TLR4 may be a critical link for the communication between pulmonary vascular endothelial cells and platelets. In another word, TLR4 may function as a receiver that receives signals from PA endothelial cells. However, it is not known what endogenous ligands of platelet TLR4 are released from PA endothelial cells in response to local injury (idiopathic PAH) or environmental stresses (eg, hypoxia or cold exposure). It does not seem that inhibition or blockade of platelet TLR4 is a practical therapeutic strategy for PAH because it may increase bleeding time and cause unexpected side effects. This study shows that TLR4 is essential for the maintenance of normal platelet function and hemostasis. Thus, inhibition of TLR4 on the platelets is not recommended for the treatment of PAH unless TLR4 protein expression is upregulated. A reasonable approach to the treatment of PAH is to suppress the release of the ligands specific for platelet TLR4 or to eradicate the pathogenic factors that stimulate the release of these ligands. The current known ligands for TLR4 may include hyaluronic acid, heparin sulfate, high-mobility group box 1, and some heat shock proteins. Such ligands may be released from pulmonary vascular cells (eg, endothelial cells), in response to local stresses (hypoxia or inflammation), to activate TLR4 on platelets, which further aggregates platelets to the injured vessel sites (Figure). On activation, platelets release a variety of vasoconstrictors (eg, serotonin and thromboxane A2), inflammatory cytokines (tumor necrosis factor [TNF]-α, interleukin [IL]-1α, and IL-1β), and growth factors (PDGF, transforming growth factor [TGF]-β1, vascular endothelial growth factor [VEGF]). These factors impair PA endothelial function, cause vasoconstriction, and stimulate proliferation of PA smooth muscle cells (SMCs). The production of vasoconstrictors (eg, endothelin-1) is increased in the damaged PA ECs, which facilitates aggregation of platelets to the injured sites. However, the generation of vasoconstrictors (eg, endothelin-1) is increased in the damaged PA ECs. These changes in the PA ECs not only enhance vasoconstriction but also promote SMC proliferation or PA remodeling. The result is the increased pulmonary vascular resistance and narrowing or occlusion of small Pas, which lead to PAH, right ventricular (RV) hypertrophy, and ultimately RV failure.

This study is also in agreement with the thrombotic mechanism in initiating PAH. Chronic hypoxia decreased bleeding time in wild-type mice indicating increased thrombotic tendency, which is associated with PAH. Deletion of platelet TLR4 increased bleeding time indicating decreased thrombotic tendency, which protects against the development of PAH. Platelet activation and thrombotic lesions were found in pulmonary arteries in patients with PAH even in the absence of clinical or pathological evidence of pulmonary embolism. The pressure driving flow through the pulmonary circulation is low (right ventricular systolic pressure, ≈20 mm Hg), and the pulmonary blood velocity is slow versus the systemic circulation. These unique hemodynamic features increase contact time of platelets and endothelial cells, which facilitate thrombosis in
the pulmonary circulation. Therefore, the pulmonary circulation is at a high risk for embolism, especially in small resistance pulmonary vessels where blood velocity is further reduced. Although there is no obvious thrombosis, the activated platelets aggregate on the surface of endothelial cells in small PAs and release vasoactive, mitogenic/growth, and inflammatory factors. This process is further aggravated by reduced production of NO and prostaglandin I2 when endothelial cells are damaged (Figure). In a subpopulation of patients with PAH who have lower platelet counts, the platelet membrane expression of protease activator receptor-1 and protease activator receptor–mediated surface expression of P-selectin (an adhesion molecule in inflammation and thrombosis) are increased.16 The antithrombosis treatment has positive therapeutic effects on PAH.17

PAH is a severe and life-threatening disease with poor prognosis. There is no cure for the disease primarily because of unknown pathogenesis. Although several new treatments have been developed for PAH in recent years, which have improved prognosis, the mortality remains high. Median survival rates have improved with the introduction of endothelin receptor antagonists and phosphodiesterase-5 inhibitors, but a recent estimate of survival of 3.6 years is still disappointing.18 The pathogenesis of PAH is largely unknown but likely involves multiple factors, with genetic determination and environmental stress as 2 critical components. In most cases, PAH is the result of genetic and environmental interactions. The cause and pathogenesis of PAH vary with patients although the clinical manifestations and outcomes seem similar. It should be mentioned that animal models of PAH mimic certain aspects of human PAH. None of them can fully reproduce human PAH. Therefore, the use of different animal models, each inducing the disease by a different mechanism, is recommended. The complexity of the PAH pathogenesis is increasingly recognized as new molecular pathways, and their interactions are discovered. Nevertheless, the exact molecular mechanisms of PAH are yet to be resolved. TLR4 mediates immune responses in a variety of diseases, including PAH. The present article by Bauer et al19 further deciphered the role of platelet TLR4 in hypoxia-induced PAH. Additional studies should identify specific ligands for the platelet TLR4, which may serve as therapeutic targets for PAH. The identification of the cellular source (eg, PA endothelial or SMC cells) of these ligands is equally important because the next generation of PAH therapy should involve cell-specific suppression of the synthesis and release of these ligands. Cell- and molecule-specific manipulation shall maximize the therapeutic potentials and minimize side effects associated with traditional nonspecific treatments.

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References


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