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Platelets As Initiators and Mediators of Inflammation at the Vessel Wall

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Abstract

Platelets are dynamic cells with activities that extend beyond thrombosis including an important role in initiating and sustaining vascular inflammation. A role for platelets has been described in many physiologic and pathophysiologic processes such as atherosclerosis, stem cell trafficking, tumor metastasis, and arthritis. Platelet activation at sites of an intact inflamed endothelium contributes to vascular inflammation and vascular wall remodeling. Platelets secrete a wide array of preformed and synthesized inflammatory mediators upon activation that can exert significant local and systemic effects. This review will focus on the role of platelet derived mediators in vascular inflammation and vascular wall remodeling.

Introduction

Hemostasis is critical to survival and platelets have many redundant pathways to ensure that activation occurs only when necessary. Platelets can be activated by injury to the vessel wall, activation of the coagulation cascade or by activating factors released from stimulated endothelial cells and platelets (e.g. ADP, thromboxane, von Willebrand Factor). Activation of resting platelets triggers a variety of intra-platelet events including exocytosis of granules, secretion of vasoactive mediators, and conformational changes in receptors (e.g. GP IIb/IIIa). The release of vasoactive mediators also leads to the elaboration of a pro-inflammatory environment within a developing thrombus that can impact local changes in the vessel wall. Platelet functions are most commonly associated with a vessel that has lost its endothelial cell layer, but platelets also interact with an intact inflamed endothelium in either a stable or transient manner leading to the secretion of platelet derived vasoactive mediators. Platelets have been found forming non-occlusive thrombi in many models of vascular inflammation including transplant rejection and cerebral malaria (1,2) (Figure 1) and platelet derived mediators have also been found in the subendothelial vessel wall (3).

This review will focus on platelet derived inflammatory mediator interactions with the vessel wall and how these mediators can lead to changes in the vessel wall. Platelet secreted immune mediators can be broadly classified as either preformed or produced upon stimulation. Platelets have 3 types of granules released in a regulated manner upon stimulation that contain stored proteins and small molecules, many of which have major

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roles in vascular inflammation (4). Dense granules store small molecules such as ATP, ADP, serotonin, glutamate, and polyphosphates. Alpha granules contain an extensive list of proteins that have important roles in both thrombosis and inflammation. A short list includes Platelet Factor 4 (PF4/CXCL4), β -thromboglobulin (NAP-2/CXCL7 as the active breakdown product), RANTES (CCL5), IL-1 α and IL-1 β , TGF- β and TNF- α . Each has been shown to have important effects on their own in vascular inflammation, but because they are released in combination at the site of injury or endothelial inflammation, even greater effects then described experimentally are likely to occur *in vivo*.

To discuss the impact of a few platelet derived mediators on the vessel wall we will divide them into small molecules, prostaglandins, and chemokines/cytokines.

Small Molecules

Small vasoactive molecules are primarily found in dense granules. Serotonin (5-HT) has been recognized as a platelet derived product for many decades with numerous studies in the 1950s noting its role as a platelet derived vasoconstrictor (5,6). Platelets are the source of circulating serotonin, yet megakaryocytes do not have the enzyme necessary to produce serotonin (tryptophan hydroxylase, Tph). Instead platelets take up and store serotonin from its synthesis site in the duodenum (7). There are 2 isoforms of Tph, Tph-1 and Tph-2, with only Tph-1 expressed outside the brain. Tph-1^{-/-} mice are viable and specifically lack serotonin in the periphery (8). Serotonin has little effect on platelet aggregation and activation on its own, but it can amplify platelet activation when combined with other weak agonists (8). Serotonin's main peripheral effects may be in its interactions with the vessel wall. Serotonin has well described mitogenic effects on vascular smooth muscle cells (VSMC), best described in pulmonary arterial hypertension (PAH) (9). Vascular smooth muscle cells express multiple receptor subtypes for serotonin (5-HT_{1DB}, 5-HT_{2A}, 5-HT_{2B}, 5-HT₄, 5-HT₇) and serotonin mediated VSMC proliferation may be driven by G protein signaling and reactive oxygen species (ROS) signal transduction pathways (9). In addition to proliferative effects, 5-HT has also been shown to be pro-inflammatory to VSMC by driving IL-6 production (10).

The release of nucleotides such as ATP and ADP also has important roles in vascular remodeling. Prolonged exposure to ADP and ATP has been linked to vascular changes associated with accelerated graft arteriosclerosis (AGA), atherosclerosis, and hypertension (11,12). VSMCs express the ATP responsive P2X₁ receptor and several P2Y subtypes (P2Y_{2,4,6}) (13). P2Y receptor signaling mediates an increase in VSMC constriction, proliferation and inflammatory responses (14). Treatment with receptor blockers such as clopidogrel can reduce atherosclerotic lesion progression (15). This may be the result of a combined effect of clopidogrel on both platelets and VSMC.

Thromboxane and Prostaglandins

Aspirin is one of the most commonly used drugs in the world and is a first line drug in the treatment of patients at risk for cardiovascular events. Aspirin irreversibly acetylates cyclooxygenase (COX) and blocks its activity. COX is expressed in many inflammatory cells, but platelets are a major source of COX products in the vasculature. Platelets constitutively express COX-1 and during times of prolonged inflammation can express COX-2 (16). Thromboxane is the major platelet derived COX product with lesser amounts of prostaglandins such as PGE₂ and PGI₂ also produced. Platelets are the central source of TxA₂ in myocardial ischemia with little contribution from leukocytes and the vasculature (17). Platelet deposition at the vessel wall results in the local elaboration of high concentrations of thromboxane that can have effects on VSMC. VSMC express thromboxane receptors (TP) and thromboxane induces VSMC contraction, ROS production,

cell proliferation, and hypertrophy (18,19). PGE₂ has similar effects and COX-1 products accelerate atherogenesis in mice (20). Our work has demonstrated that platelet derived glutamate can induce low level COX activation through platelet kainate receptors contributing to amplified platelet activation (21). Studies such as these indicate that platelet COX-1 activation may have a potent role in vascular remodeling. However, without the use of platelet specific COX-1 knockout mice this is difficult to directly demonstrate.

Platelet Chemokines/Cytokines

The activation of platelets leads to the release of more than a dozen chemokines, most of which belong to the CC (e.g. CCL5, regulated upon activation, normal T-cell expressed, and secreted, RANTES) or CXC (e.g. CXCL4/platelet factor 4/PF4) chemokine families. In particular, platelet derived PF4, CXCL7, and IL-1 have important effects in vascular inflammation that have received recent attention.

CXCL7 and CXCL4 are found in very high concentrations in platelet releasates. There are several molecular variants of CXCL7 all of which are derived from the proteolysis from a single 128aa precursor called pre-platelet basic protein (PPBP) (22). The cleavage of the 34-residue leader sequence produces PBP (94aa) and further truncation results in connective tissue activating protein III (CTAP-III, 85aa), β -thromboglobulin (81aa), and neutrophil activating peptide 2 (NAP-2/CXCL7, 70aa) (23). CXCL7 binds to CXCR1 and CXCR2 and induces neutrophil adhesion and migration *in vitro* (24). CXCL7 may also be responsible for neutrophil accumulation around vascular tissues in combination with activated mast cells (25). However, a more broad appreciation of potentially important roles for this abundant platelet chemokine in vascular inflammation is currently lacking.

PF4 was the first described CXC class chemokine (23). The identity of PF4 receptors and its signaling is still not completely resolved or understood. The addition of free GAG chains or the removal of cell surface GAG abolishes PF4 monocyte binding and signaling (26). Proteoglycans may serve as a functional receptor for PF4, or similar to other chemokines, GAGs may allow PF4 to become localized and facilitate its binding to a chemokine receptor. An alternative splice variant of CXCR3, CXCR3B, has been reported to be a receptor for PF4 on endothelial cells and activated T lymphocytes (27). However, a more recent study demonstrated that PF4 may signal through both CXCR3A and CXCR3B (28). The described functions of PF4 can be equally confusing including both pro and anti-inflammatory and pro and anti-proliferation (29,30). *In vitro*, PF4 inhibits T-cell proliferation and cytokine release (31) and PF4 has reported anti-proliferation and angiogenesis activity on endothelial cells (32). Using an *in vivo* mouse model of cerebral malaria we have shown that PF4 is pro-inflammatory in part by driving increased T-cell CXCR3 expression and trafficking to the brain and INF- γ production, but had no effect on T-cell proliferation (1). Using this model we have also demonstrated that PF4 induced monocyte activation and trafficking in a Kruppel Like Factor 4 (KLF4) transcription factor dependent manner (33). Others have shown that PF4^{-/-} mice crossed with ApoE^{-/-} mice have reduced atherosclerotic lesions compared to control ApoE^{-/-} mice and PF4 is found within the vessel wall at lesion sites (3). PF4-RANTES complexes also appear to be very important to this process (29). These seemingly disparate effects of PF4, pro and anti inflammatory/proliferation, are likely the result of the different model systems used and *in vitro* versus *in vivo* effects, reflecting the complex nature of PF4 interactions with other molecules and cells in the vasculature. Direct effects of PF4 on VSMC has not been explored, but may also be important in atherosclerosis progression, particularly in humans where there is more VSMC proliferation and inflammation compared to mouse models.

Platelet derived IL-1 has recently received significant attention in the literature. Platelets store both IL-1 α and IL-1 β and mild stimulation leads to the translation of more platelet IL-1 β (34). Platelet derived microparticles have been identified as mediators of joint inflammation associated with arthritis in a platelet IL-1 α dependent manner (35) and platelet IL-1 α also drives cerebrovascular inflammation (36). IL-1 and its receptors are increased in atherosclerotic tissue and increased plasma IL-1 is associated with increased risk of atherosclerosis (37). Platelet interactions at the site of lesion development may be an important source of IL-1. IL-1 can drive vascular smooth muscle proliferation, endothelial adhesion molecule expression and cell trafficking all of which are important steps in the pathogenesis of atherosclerosis (38,39).

Summary

Platelets are dynamic cells with many important functions ‘beyond the clot’. Platelets may exert great effects on the vessel wall in acute and chronic inflammatory diseases. A more complete understanding of these functions is a work in progress.

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Figure 1. Non-Occlusive thrombi are present within cerebral vessels of mice with experimental cerebral malaria and within vessels of skin grafts during rejection (immunohistochemical staining for vWF). Arrowheads indicate vWF rich thrombi and arrows enmeshed leukocytes.