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Platelets as Central Mediators of Systemic Inflammatory Responses

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Abstract

Systemic inflammatory responses are associated with high morbidity and mortality and represent a diverse and clinically challenging group of diseases. Platelets are increasingly linked to inflammation, in addition to their well-known roles in hemostasis and thrombosis. There is agreement that traditional functions of platelets, including adherence, aggregation, and secretion of preformed mediators, contribute to systemic inflammatory responses. However, emerging evidence indicates that platelets function in non-traditional ways. In this review, we focus on new functions of platelets that may be involved in the host response to infection.

Introduction

The role of platelets in the host response to infection is increasingly appreciated. Platelet function is often compromised in diseases as diverse as human immunodeficiency virus (HIV), dengue and sepsis and disease severity and mortality correlate with the degree of thrombocytopenia that is clinically observed [1–3]. The etiology of thrombocytopenia in infectious diseases is complex but is typically due to decreased production of platelets and/or increased sequestration and clearance of activated platelets in the periphery. In regards to production, platelets are formed from megakaryocytes, which typically reside in the bone marrow but have also been observed in the circulation and lungs [4]. Several studies have shown that megakaryopoiesis and/or thrombopoiesis become disrupted during infection, contributing to reduced platelet counts [5]. There is also evidence that platelets continue to develop in the circulation [6]. In this regard, our group recently reported that circulating platelets themselves have the ability to divide and form functional progeny [7]. Whether or not the formation of progeny contributes to circulating platelet counts is unknown. However, when platelets encounter thrombin or *E. coli* they fail to produce progeny raising the possibility that this new function of platelets may contribute to the thrombocytopenia observed in infectious situations.

Although the production of platelets is unquestionably affected as the host encounters pathogens, it is more recognized that systemic infections are associated with increased platelet activation in the bloodstream. Activated platelets bind other platelets and leukocytes

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in the bloodstream setting off a cascade of events that contribute to the development, evolution, and resolution of the systemic inflammatory response (Figure 1). Abnormal sequestration of platelets in the microcirculation induces thrombocytopenia and often leads to disseminated intravascular coagulation (DIC). In critically ill patients, DIC participates in the development of multiple organ failure and often death. Understanding how platelets become activated and the downstream consequences of platelet activation will contribute to the treatment of infectious diseases and the development of new therapeutics. In this review, we briefly focus on mechanisms by which platelets become activated during infectious states, paying particular attention to the emerging role of toll-like receptors (TLRs) in this process. We then discuss downstream consequences of platelet activation, focusing on recently identified prolonged actions of platelets that are turned on during the host response to infection.

The Road to Platelet Activation in Infectious Disease

The infectious milieu provides a variety of signals that lead to platelet activation (Figure 1). It is well known that platelet-activating factor (PAF) and thrombin are generated by host cells as they encounter pathogens [8]. Both PAF and thrombin signal through G-protein coupled receptors inducing rapid changes in platelets including secretome release and translocation of P-selection to the surface of the platelets, where it serves as a tether for leukocytes. PAF and thrombin also activate integrin $\alpha_{IIb}\beta_3$, allowing it to bind fibrinogen and bridge platelets to one another. Homotypic platelet aggregates deposit in the vasculature, leading to thrombosis, DIC and consumption of platelets. Because of their established roles in platelet activation and coagulation, PAF and thrombin have been the targets of clinical trials designed to improve outcomes in patients diagnosed with sepsis [9–10].

While the effects of PAF and thrombin are well characterized, new agonists that induce platelet activation are continually being identified. Among these are TLR agonists, including lipopolysaccharide (LPS). Human and murine platelets express TLR2, -4 and -9 [11–12]. TLR5 has also been identified in human platelets [13]. Unlike classical agonists, however, TLR-dependent signaling typically does not have a direct effect on traditional platelet functions, such as aggregation or secretion [14]. However, studies in knockout mice (TLR4-/-) demonstrate that platelet TLR4 is responsible for mediating LPS-induced thrombocytopenia [15]. Furthermore, thrombocytopenic mice inadequately produce tumor necrosis alpha (TNF- α) in the presence of LPS unless they are simultaneously transfused with platelets [16]. Together, these *in vivo* data indicate that activation of platelet TLR4 is involved in the clearance of circulating platelets and the generation of proinflammatory cytokines in endotoxemia.

Recent studies from Washington and colleagues [17] demonstrate that LPS markedly increases plasma levels of sTLT-1 (soluble Triggering Receptor Expressed on Myeloid Cells-like [TREM] transcript-1) in mice. sTLT-1 is also increased in patients diagnosed with sepsis compared to healthy controls. The exact functions of TLT-1 in platelets, which is stored in α -granules and translocated to the surface in response to thrombin, collagen, and LPS, are still emerging. To date, it is known that activated platelets release sTLT-1, which binds fibrinogen and augments platelet aggregation. Mice that lack TLT-1 have defects in platelet aggregation and more readily succumb to challenges with LPS. TLT-1 deficient mice also display higher plasma levels of TNF- α and D-dimers when compared to their wild-type counterparts, indicating that platelet-derived TLT-1 functions to dampen the inflammatory response to infection.

Recently Identified Consequences of Platelet Activation in Infectious Disease

Activated Platelets Synthesize and Secrete Pro-inflammatory Cytokines

In professional phagocytes, such as neutrophils and macrophages, LPS signaling is linked to *de novo* gene expression. A decade ago, it was heretic to think that platelets were capable of regulated gene expression. However, our thinking has evolved. It is now well accepted that platelets have central roles in inflammation. In addition to rapid release of inflammatory mediators that are stored in granules and immediately released upon activation, platelets also synthesize proteins in response to extracellular cues [18]. Our first clue into the role of protein synthesis by platelets was in 1998 when we demonstrated that thrombin-activated platelets synthesize B-cell lymphoma 3 (Bcl-3) [19]. Synthesis of Bcl-3 begins within 15 minutes of activation and lasts for at least 8 hours, demonstrating that stimulated platelets continue to function over time. As Bcl-3 is synthesized by platelets, it binds to Fyn and regulates cytoskeletal-mediated processes [19]. In follow-up investigations, we demonstrated that targeted deletion of Bcl-3 prevents platelets from retracting fibrin-rich clots [20]. This suggests that protein synthetic events in platelets may be important for recanalization of thrombosed vessels.

In addition to Bcl-3, platelets synthesize other proteins that play critical roles in the host response to infection. One of these is interleukin-1 β (IL-1 β). In 2005, we discovered that circulating platelets contain precursor mRNA (pre-mRNAs) for IL-1 β [21]. In response to activating signals, platelets splice IL-1 β pre-mRNA into mature message. Spliced IL-1 β mRNA is subsequently used as a translation template to produce new protein. In our original studies, we used thrombin to induce pre-mRNA splicing in platelets. More recently, Shashkin and colleagues demonstrated that LPS is a potent inducer of pre-mRNA splicing in platelets [22]. Using IL-1 β and cyclooxygenase-2 (COX-2) as index messages, these investigators demonstrated that the magnitude of pre-mRNA splicing and the ensuing translational response is far greater in LPS-stimulated platelets compared to thrombin-stimulated platelets. After it is synthesized, IL-1 β is primarily packaged into microparticles that promote the adherence of polymorphonuclear leukocytes (PMNs) to endothelium [23]. Our group and others [24–26] have also demonstrated that human platelets express pre-mRNA for tissue factor (TF), a gene that drives coagulation responses in sepsis [26]. Several agonists active in the septic milieu induce TF pre-mRNA splicing and translation of the mature message into bioactive protein, including PAF, thrombin, gram negative and positive bacteria, α -toxin, and LPS ([26] and manuscript submitted).

Activated Platelets Mediate Responses in Target Leukocytes

As described above, agonists found in the infectious environment induce traditional and non-traditional activation responses within platelets that contribute to the pathogenesis of sepsis and related disorders. Activated platelets also drive responses in target leukocytes that modulate the host response to infection. As platelets become activated, they express P-selectin on their surface [27]. Membrane-expressed P-selectin engages its receptor, P-selectin glycoprotein-1 (PSGL-1), on PMNs and monocytes [28]. Zarbock and coworkers demonstrated that selectin-mediated platelet-PMN interactions are a critical step in activation and recruitment of leukocytes to the lung in acute lung injury (ALI) [29]. Their work demonstrated that platelet P-selectin mediates PMN sequestration and lung injury, linking platelets directly to inflammatory lung disease [29].

Interactions between platelets and PMNs are critical for cell trafficking, but they also serve as a means for the delivery of molecular signals. A prime example is the formation of neutrophil extracellular traps (NETs) in sepsis. Clark and colleagues recently demonstrated

that platelets signal PMNs to extrude DNA-rich NETs, although the signaling factor in platelets that triggers this response is unknown [30]. NETs are extracellular chromatin lattices that are studded with elastase and other antimicrobial factors [31]. The primary function of NETs is to ensnare and kill microbes. Plasma from sepsis patients facilitates platelet-induced NET formation and studies in sepsis mouse models indicate that TLR4-dependent platelet activation is involved in this process [30,32]. In these studies, the authors also observed interactions between platelets and NETs in the pulmonary circulation. These NETs, however, also damaged endothelial cells raising the possibility that platelet-induced NET generation may ignite microvascular injury in sepsis [32]. Recent work also suggests that NETs recruit red blood cells and promote fibrin deposition, providing another link from platelets to thrombus formation [33].

In addition to interacting with PMNs, platelets use P-selectin to bind monocytes. In *in vitro* models, platelets adhere to monocytes for hours [34–35]. Consistent with this observation, platelet-leukocyte aggregates are markedly increased in the bloodstream of patients diagnosed with sepsis [36]. One consequence of prolonged interactions between platelets and monocytes is the expression of genes that encode for inflammatory proteins and enzymes involved in infectious processes. These include the synthesis of the proinflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1), IL-1 β , and IL-8 [30,34]. Platelet-monocyte aggregates also produce COX-2, matrix metalloproteinase-9 (MMP-9), and TF [34–35,37–38].

Conclusions

Systemic inflammatory responses are complex clinical phenomena that present a major challenge to medicine. Specifically, sepsis presents us with a paradox where innate inflammatory responses evolved to fight infection do significant damage to the host, and DIC leads to platelet consumption and prolonged clotting times in the presence of thrombosis. These complexities provide us with a unique opportunity to study the dynamic role played by the host in the pathogenesis of this disease. We are reminded that platelets are not simple cytoplasts with the sole role of maintaining hemostasis over the course of minutes. Instead, platelets contribute to diverse processes that occur over hours to days and, during this time, change their own phenotype as well as the phenotype of nearby cells. The mechanisms by which diverse inputs differentially regulate platelet outputs are growing. Upcoming discoveries will undoubtedly cement the role of platelets as central mediators of systemic inflammatory responses, shine new insight into their functions and provide opportunities for clinical intervention in the future.

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Figure 1. Platelets integrate a variety of inputs to mediate pathology in systemic inflammatory responses

Thrombin, PAF, and microorganisms are commonly observed in infectious situations. Each of these factors modulates platelet function, inducing both rapid and prolonged responses that include the formation of homotypic and heterotypic aggregates. Platelet-platelet aggregates and platelet-leukocyte aggregates contribute to DIC, the formation of NETs, and the synthesis and secretion of proinflammatory cytokines that are commonly observed as the host responds to infection. These responses may help fight off infection, but they can also contribute to the pathology of the disease. Abbreviations: DIC, disseminated intravascular coagulation; NETs, neutrophil extracellular traps; PAF, platelet activating factor; PAFR, PAF receptor; PAR, protease activated receptor; TLR, Toll-like receptor.