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Platelets: versatile modifiers of innate and adaptive immune responses to transplants

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Abstract

Purpose of review—Over the last decade, there has been mounting experimental data demonstrating that platelets contribute to acute vascular inflammation and atherosclerosis. This review focuses on recent findings that link platelets to inflammatory responses of relevance to transplants.

Recent findings—Although it has been known that platelets modify vascular inflammation by secretion of soluble mediators and release of microparticles, new aspects of these mechanisms are being defined. For example, platelet-derived RANTES (CCL5) not only functions in homomers, but also forms more potent heteromers with Platelet Factor 4 (CXCL4). This heteromer formation can be inhibited with small molecules. New findings also demonstrate heterologous interactions of platelet microparticles with leukocytes that may increase their range of impact. By attaching to neutrophils, platelet microparticles appear to migrate out of blood vessels and into other compartments where they stimulate secretion of cytokines. Contact of platelets with extracellular matrix also can result in cleavage of hyaluronan into fragments that serve as an endogenous danger signal.

Summary—Recent findings have expanded the range of interactions by which platelets can modify innate and adaptive immune responses to transplants.

Keywords

Platelets; cytokines; microparticles; ischemia-reperfusion; rejection

Introduction

Platelets were noted together with fibrin as a component of hyperacute and severe acute rejections in early clinical transplants [1]. These findings were consistent with the known thrombotic functions of platelets in injured vessels. As these severe forms of rejection became less frequent, attention to platelets as a factor in rejection diminished except in xenotransplantation. The inconspicuous and transient presence of platelets in biopsies from grafts with acute and chronic rejection also deflected attention from the possible inflammatory interactions of platelets with endothelial cells and leukocytes (Fig 1). More recently, there has been mounting experimental data demonstrating platelets have multiple inflammatory functions in experimental models of acute vascular injury and atherosclerosis. These pathological processes have similarities to antibody-mediated rejection and chronic

allograft vasculopathy that have been increasingly recognized as important impediments to transplant survival. At least one mechanistic link between alloantibodies to transplants and platelet activation has been established by Lowenstein and colleagues [2], who have demonstrated that antibodies to HLA cause endothelial cells to release von Willebrand factor and P-selectin from Weibel-Palade bodies. Release of these adhesion molecules has been correlated with intravascular platelet aggregates in animal models of antibody-mediated rejection [3–7]. Clinically, intracapillary platelet aggregates have been noted in both antibody- and cell-mediated rejection of renal transplants [8]. In addition to possible contributions to rejection, platelets can participate in other processes that are pertinent to transplants from deceased donors, including brain injury, stroke and ischemia-reperfusion. The increasing data connecting platelets to inflammation have prompted a reassessment of platelet functions in vascular pathology and transplantation.

In the past few years, we have reviewed some of these aspects of platelet biology as they pertain to transplants [9, 10], and others have reviewed the contribution of platelets to acute vascular inflammation and atherosclerosis [11–13]*.

This review will focus on more recent findings that link platelets to innate immune responses elicited in transplants.

Overview of platelet functions relevant to transplantation

Platelets are strategically equipped to modify interactions between leukocytes and endothelial cells. In the laminar flow of arteries, the small discoid platelets are enriched close to the endothelial cells lining blood vessels. Platelets carry a plethora of immunologically potent mediators, some of which are packaged in cytoplasmic granules at the time the platelets bud off megakaryocytes in the bone marrow whereas others are imbibed from plasma as the platelets circulate [14]. Activated human platelets also synthesize IL-1 β by splicing pre-mRNA transcripts [15] and produce inflammatory mediators through the cyclooxygenase pathway. Platelets contain 3 morphologically distinct types of secretory granules with different contents: alpha granules, dense granules and lysosomes [9]. Two groups have identified subsets of alpha granules based on differences in contents [16, 17]. In addition, platelets express many receptors and ligands on their plasma membranes. As shown in Figure 2, platelets can interact with endothelial cells and leukocytes through 3 major mechanisms: exocytosis of soluble mediators from storage granules [18], extrusion of microparticles containing membrane and cytosolic proteins [19–22], and contact through membrane bound ligands and receptors [6, 11].

Platelets are a major conveyor of soluble mediators

The number of mediators that platelets secrete (the ‘secretome’) has been extended to over 300 by proteomic arrays [23, 24]. Activated platelets can release several chemokines that attract neutrophils, monocytes and lymphocytes. These include IL-8 (CXCL8), MIP-1 α (CCL3), RANTES (CCL5) and MCP-3 (CCL7), as well as Platelet Factor 4 (PF4; CXCL4) and beta-thromboglobulin (CXCL7) that are characteristic of platelets. Because PF4 is a prototypical product of platelet activation, Gleissner et al [25]* cultured human peripheral blood monocytes with recombinant PF4 and compared the gene expression array to monocytes stimulated with macrophage colony-stimulation factor (M-CSF). The transcriptome induced by PF4 was distinct from either inflammatory or alternatively activated macrophages. PF4 did induce some genes involved in inflammation and antigen presentation, notably TNF, IL-6, CCL22, MHC class II and the costimulatory molecule, CD86. Interestingly, CCL22 can feed back on platelets to induce expression of P-selectin.

Individual chemokines can have multiple overlapping synergistic or antagonistic functions reflecting the multiple receptors that many chemokines bind. For example, platelet secretion of RANTES (CCL5) can activate monocyte and T cell subsets by binding to CCR1 or CCR5. An added layer of complexity has been introduced recently by the finding that CCL5 can form heteromers with PF4 (CXCL4). These heteromers form in platelet alpha-granules, and have enhanced capacity to recruit monocytes. More recently, Koenen et al [26] have also shown that small molecules can be designed to bind to CCL5 and prevent heteromer formation with PF4. Human and mouse peptides have been demonstrated to inhibit CCL5 adhesion of monocytes to endothelial cells in vitro. The mouse peptide also decreased atherosclerosis in ApoE knockout mice fed a high-fat diet. Unlike a complete deficiency in CCL5, small molecule inhibition of CCL5 heteromer formation with CXCL4 did not decrease antigen specific or mitogen-induced proliferation, or clearance of herpes simplex virus 2 infection in vivo.

In addition to cytokines, platelets contain enzymes that are potentially relevant to transplants. A recent study by de la Motte et al [27]** demonstrated that platelets contain hyaluronidase 2 but not hyaluronidase 1. As a result, platelets can cleave hyaluronan, a component of extracellular matrix, into intermediate-sized fragments but not complete the degradation. These incompletely degraded fragments of hyaluronan are significant because they act as endogenous danger signals for cells that express Toll-Like Receptors (TLRs) 2 and 4. Such hyaluronan fragments have been found to stimulate chemokine production by macrophages and to initiate inflammatory responses in acute lung injury [28]. In contrast, undegraded hyaluronan signals through TLR2 and 4 on epithelial cells to prevent apoptosis. Therefore, hyaluronidase 2 favors inflammation over quiescence [29]. Platelets may respond to hyaluronan fragments because they also express TLR 2 and 4 [30–32].

Platelets express integrins, ligands and receptors that promote intercellular adhesion and activation

Activated platelets express glycoprotein IIb/IIIa (GPIIb/IIIa; CD41/CD61), P-selectin, CD40 and CD154 on their surface. These receptors and ligands form a highly interconnected web that promotes adhesion and signaling interactions among platelets, neutrophils, monocytes, lymphocytes and endothelial cells. They also can modulate the maturation and function of macrophages and dendritic cells. CD154 was originally named CD40 ligand because it interacts with the CD40 receptor on macrophages and endothelial cells, however, it also binds to GPIIb/IIIa to activate platelets and Mac-1 (CD11b/CD18) to activate neutrophils and macrophages. These interactions have been the subject of numerable experimental and clinical studies in acute vascular inflammation and particularly atherosclerosis [11]. This is a very complex area for study or intervention because of the multiple cells that express each of the ligands and receptors on their membranes. However, in a mouse model of vascularized heterotopic heart transplantation, Kirk and colleagues demonstrated that human platelets expressing CD154 or recombinant CD154 trimers restored allograft rejection in CD154 knockout mice [33]. Furthermore, treatment with monoclonal antibody to human CD154 prevented rejection in this model.

Platelet expand their sphere of influence through the release of microparticles

Platelets microparticles are 0.1–1.0 μm packets of cytosol surrounded by membrane. These microparticles strategically increase in number and surface area the solid phase expression of P-selectin. As a result, microparticles can facilitate interactions among leukocytes in the blood or on the vascular endothelium [19]. Microparticles can also circulate to the spleen to

alter interactions in secondary lymphoid tissues. The interaction of P-selectin on platelet microparticles with PSGL-1 on monocytes causes their activation and expression of CD11a/CD18 and CD11b/CD18 as well as tissue factor. Mause, et al [20] found that platelet microparticles contain CCL5 that can be transferred to vessels as platelets roll on or make firm adhesions with endothelial cells. Blocking antibodies to P-selectin or glycoprotein Ib (GPIb; CD 42b) decreased rolling and firm adhesion of the microparticles, as well as CCL5 deposition and subsequent monocyte attachment on carotid arteries.

In addition to soluble mediators, platelet microparticles can transfer receptors. Salanova, et al [21] demonstrated that platelet microparticles can transfer GPIIb/IIIa receptors to neutrophils that co-localize with β 2-integrins and cooperated in NF- κ B activation. This transfer of membrane proteins is bidirectional. Microparticles from monocytes and macrophages can transfer tissue factor to platelets [22]

In most studies, microparticles have been isolated from plasma. Recently, this concept was expanded by Boilard et al [34] ** in studies of inflammatory arthritis. They found CD41-positive platelet microparticles in synovial fluids from patients with rheumatoid arthritis and juvenile idiopathic arthritis, but not in fluid from patients with osteoarthritis. These experiments describe an inflammatory loop that is initiated by microparticle formation. Fibroblast-like synoviocytes and the extracellular matrix that line the joint cavity stimulated formation of microparticles, and the microparticles stimulated the synoviocytes to secrete the inflammatory cytokines IL-6 and IL-8. In this study, an animal model was used to demonstrate further that microparticle formation was dependent on GPVI, a collagen receptor specific for the platelet lineage. This interaction required IL-1 expression by the microparticles and IL-1 receptor on synoviocytes. In an accompanying commentary, Zimmerman and Weyrich elaborate on possible mechanisms for the movement of microparticles into extravascular sites [35]*. They envision that the known propensity for microparticles to adhere to activated neutrophils or monocytes provides a means of mobility. This concept has significant implications for transplantation because it provides a mechanism for platelet microparticles to enhance interactions among T cells, macrophages and dendritic cells in the interstitium of transplanted organs.

Platelet interactions with innate immunity relevant to ischemia-reperfusion

The contribution of platelets to ischemia-reperfusion injury has been studied more extensively in lung and liver than other transplants. More than a decade ago, experiments linked P-selectin to the sequestration of platelets and neutrophils in ischemic organs [36, 37]. Weiss et al [38] reported that CD41 positive deposits of platelets were more frequent one hour after transplantation in human livers from brain dead donors compared to living donors. Two recent prospective clinical studies measured soluble P-selectin levels before, during and after lung transplantation. In one [39] soluble P-selectin and soluble CD154 levels were found to increase significantly after lung transplantation but not after thoracotomy. This study also reported a greater increase in platelet-monocyte conjugates after lung transplantation than after thoracotomy alone. The second study [40] of 376 lung transplant recipients reported an association of higher postoperative soluble P-selectin levels with an increased risk of primary graft dysfunction at 72 h after transplantation.

Platelet interactions with innate and adaptive immunity relevant to rejection

Intracapillary platelet aggregates have been demonstrated by immunohistology to be a frequent finding in human renal transplants undergoing acute antibody and cell-mediated rejection [8]. In experimental models, antibody-mediated rejection is associated with release of von Willebrand factor and attachment of platelets to vascular endothelium in cardiac allografts [6]. A causative relationship between antibody binding to vascular endothelial

cells and platelet rolling was established in skin allografts in mice [7]. In these experiments, MHC incompatible skin grafts were transplanted to athymic recipients and early inflammatory responses to skin grafts were allowed to subside before antibodies to the incompatible MHC antigens were administered. A single transfer of alloantibodies caused release of von Willebrand factor and decreased platelet velocity in the capillaries of the skin graft but not in the adjacent recipient skin. This effect was transient and diminished within 2 days. However, repeated administrations of antibody prolonged the effect. Alloantibodies induced platelet activation that was reflected in circulating platelets by increased expression of P-selectin. Moreover, the activation of platelets was accompanied by increased intravascular accumulations of leukocytes in the skin allografts. The localization of leukocytes to the skin grafts was inhibited by depletion of platelets with antibodies to CD41. T cells can also initiate platelet activation in allografts. To demonstrate this interaction, skin allografts were transplanted to athymic mice that were reconstituted with naïve T cells after the grafts were revascularized [41]**. In this model, the T cell response to skin grafts activated platelets as evidenced by CXCL4 secretion. In turn, the platelets caused increased T cell infiltrates into the skin allografts and accelerated rejection. In addition, a novel method of inhibiting platelet contributions to transplant rejection through inhibition of glutamate receptor signaling was examined.

Clinically, P-selectin and other markers associated with platelet activation have been associated with acute rejection of cardiac and renal transplants [42, 43]. However, other studies report that markers of platelet activation are elevated in transplant recipients without evidence of rejection [44].

Autoantibodies that react with platelets may also contribute to graft rejection. In recent studies, Rose and colleagues [45] have found that IgM autoantibodies to vimentin from cardiac transplant recipients can increase the formation of platelet-leukocyte conjugates. These conjugates resulted from exposure of vimentin, a nonpolymorphic intermediate filament protein, on the plasma membrane of activated platelets and macrophages, as well as on apoptotic neutrophils and lymphocytes. Exteriorization of vimentin permits IgM autoantibodies to bind activated leukocytes and propagate activation and interaction of leukocytes and platelets in vitro. This mechanism may underly the correlation that they established previously between the development of cardiac allograft vasculopathy and autoantibodies to vimentin [46]. In a mouse model of chronic rejection, they demonstrated that immunizing cardiac allograft recipients to vimentin resulted in localization of platelet-leukocyte conjugates in the grafts and accelerated vasculopathy [47]**.

Two recent experimental models using segmental artery allografts indicate that the ADP receptor on platelets, P2Y₁₂, may be an effective target to limit inflammation. In one study, MHC compatible carotid arteries were transplanted to P2Y₁₂ receptor knockout recipients [48]. In the 2 month observation period, fewer leukocytes and less neointima were found in the arterial grafts to the knockout recipients compared with the normal controls. In addition, fewer platelets expressed CD154 and fewer platelet-leukocyte aggregates were detected in the circulation at 2 weeks after transplantation in the knockout recipients. In the second paper, mice with MHC incompatible aortic allografts were treated for 30 days with clopidogrel [49]. Clopidogrel treatment resulted in decreased neointima formation with fewer infiltrating macrophages. Because no other immunosuppression was used, the implications of this model may be more related to injury repair mechanisms than to chronic rejection [50].

Conclusion

Platelets can interact with endothelial cells and leukocytes through 3 major mechanisms: exocytosis of soluble mediators from storage granules, extrusion of microparticles containing membrane and cytosolic proteins, and contact through membrane bound ligands and receptors. New aspects of these mechanisms have been found that are relevant to transplantation. These include the discovery that cytokines can form heteromers in platelet granules that have modified functions. Of potential therapeutic relevance is the finding that cytokine heteromer formation can be inhibited with small peptides.

Recent studies of platelet microparticles suggest that they can migrate out of blood vessels by attaching to neutrophils thereby increasing their range of impact. Considering the fact that platelet microparticles express p-selectin and CD154, extravascular distribution of microparticles could influence the responses of graft infiltrating cells.

The potential roles of platelets in transplantation need to be tested in more transplant models.

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Abbreviations

PF4	Platelet Factor 4
GPIb	glycoprotein Ib
GPIIb/IIIa	glycoprotein IIb/IIIa

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Figure 1.

Platelet staining by routine hematoxylin and eosin compared with immunohistology. Serial sections from a kidney transplant removed from a recipient with circulating donor specific alloantibodies. Because platelets lack a nucleus and have little cytoplasm, they are inconspicuous in capillaries and glomerulus of kidney transplant stained by routine hematoxylin and eosin (upper left panel). Immunohistological stain for P-selectin (CD62P) of a serial section demonstrates darkly stained intracapillary aggregates of platelets at low (upper right panel) and high power (lower two panels). Some of the platelets appear to be attached to leukocytes (arrows).

Hsiao-hsuan Kuo (co-author) original

Figure 2.

Platelets can interact with endothelial cells and leukocytes by 3 mechanisms. Platelets can interact with endothelial cells and leukocytes by exocytosis of soluble mediators from storage granules, extrusion of microparticles containing membrane and cytosolic proteins, and contact through membrane bound ligands and receptors. Platelets can also be stimulated by all 3 of these mechanisms.

William Baldwin (co-author) original