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Clinical Relevance of Microparticles from Platelets and Megakaryocytes

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Abstract

Purpose of review—Platelet microparticles were identified more than forty years ago and are the most abundant circulating microparticle subtype. Yet fundamental questions about their formation and role in human disease are just beginning to be understood at the cellular and molecular level. This review will address mechanisms of platelet microparticle generation and evaluate our current understanding of their clinical relevance.

Recent findings—New evidence indicates that the majority of CD41⁺ microparticles circulating in healthy individuals derive directly from megakaryocytes. CD41⁺ microparticles also form from activated platelets upon loss of cytoskeleton-membrane adhesion, which occurs in a multitude of disease states characterized by elevated platelet microparticle levels. More recent studies have demonstrated that platelet microparticles function as a transport and delivery system for bioactive molecules, participating in hemostasis and thrombosis, inflammation, malignancy infection transfer, angiogenesis, and immunity. The mechanism of platelet microparticle participation in specific disease entities such as rheumatoid arthritis has been elucidated.

Summary—Continued research into how platelet microparticles are generated and function as a transcellular delivery system will advance our basic understanding of microparticle physiology and may enable new strategies for treatment of select disease entities.

Keywords

microparticle; platelet; megakaryocyte

Introduction

Many cells, including platelets, endothelial cells, leukocytes, and erythrocytes, shed small (0.1-1 micron) fragments of their plasma membranes into the circulation. There is increasing evidence that these submicron fragments, termed microparticles, have important physiological roles. Platelet microparticles are the most abundant microparticles in the bloodstream constituting approximately 70% to 90% of circulating microparticles [1-3]. Evidence that platelet microparticles participate in thrombus formation is derived from

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several sources. Elevated platelet microparticle levels are associated with many disease states including heparin-induced thrombocytopenia [4], arterial thrombosis [5,6], idiopathic thrombocytopenic purpura, thrombotic thrombocytopenia [7], sickle cell disease [8], uremia [9], malignancy [10], and rheumatoid arthritis [11]. Platelet microparticles have also been implicated in the pathogenesis of atherosclerosis as well as the regulation of angiogenesis [12,13]. Despite their apparent participation in important physiological and pathological processes, fundamental aspects of platelet microparticle physiology remain unexplored.

The aim of this review is to highlight the concept of platelet microparticles as a cell-to-cell communication system. Special emphasis will be placed on the role of microparticles in the pathogenesis of disease.

The origin of “platelet” microparticles

Plasma-derived microparticles were first identified as an activity supporting thrombin generation in platelet poor plasma and were referred to as ‘platelet dust’ [14]. Subsequent marker studies demonstrated that plasma-derived microparticles can originate from leukocytes, erythrocytes, or endothelial cells as well as platelets. These microparticles are defined primarily on the basis of their surface antigens. Those labeled with anti-CD41 (or anti-CD42b) antibodies have been termed platelet microparticles [15,16]. CD41⁺ microparticles are less than a micron in diameter and typically express anionic phospholipids on their extracellular surface. They are distinguished from platelet exosomes, which are derived from endocytosis and are released from multivesicular endosomes [17]. Platelet microparticles derived from activated platelets have been separated into size classes that are heterogeneous when characterized by proteomic and functional studies [18]. Interestingly, platelet microparticle size classes differ significantly in their contents of growth factors, chemokines, and plasma membrane receptors.

The origin of CD41⁺ microparticles was first assumed to be activated platelets. Microparticles are readily formed following activation of platelets with strong physiological platelet agonists such as thrombin and collagen or non-physiological agonists such as Ca²⁺ ionophore. Microparticles have also been demonstrated to form in stored platelets [19], under shear [20,21], following exposure to complement proteins C5b-9 [22], or with induction of apoptosis [23,24]. Although generated under different conditions *in vitro*, specific mechanisms are thought to underlie microparticle formation from platelets. Included in these processes are loss of plasma membrane asymmetry and loss of cytoskeleton-plasma membrane adhesion. These mechanisms are active in the formation of platelet microparticles in disease states.

Microparticles that stain with platelet-specific markers, however, are abundant in healthy individuals whose platelets do not express activation markers. Furthermore, the few studies evaluating the half-life of microparticles indicate that they are cleared rapidly following introduction into the circulation [25,26]. These observations suggest that microparticles would need to be constantly shed from platelets in healthy individuals in the absence of platelet activation in order to produce the concentration of CD41⁺ microparticles circulating in the plasma of healthy individuals. An alternative possibility is that CD41⁺ microparticles in healthy individuals are continuously derived from megakaryocytes, the precursor cells of platelets. According to this hypothesis, CD41⁺ microparticles consist of two populations, those derived from platelets (platelet microparticles, PMP) following activation and those continuously derived from megakaryocytes (megakaryocyte-derived microparticles, MKMPs). Recent results support this possibility.

Circulating megakaryocyte-derived microparticles

It has been known for over a decade that cultured megakaryocytes shed microparticles. In evaluating mature human megakaryocytes derived from CD34⁺CD38⁺ bone marrow cells, Cramer et al. noted numerous particles 0.1-0.3 μm in diameter. These microparticles expressed $\alpha_{\text{IIb}}\beta_3$ and could be identified by flow cytometry [27]. More recently, high-resolution videomicroscopy revealed that a distinct population of cultured, mouse megakaryocytes formed microparticles. These microparticles formed as submicron beads along the length of thin micropodia. Microparticle-forming megakaryocytes developed dynamic blebs that decorated the surface of the cells. Blister-like protrusions appeared and rapidly disappeared from the surface of cells. Visualization of microparticle-forming megakaryocytes at higher resolution with thin-section electron microscopy revealed 0.2-0.5 micron particles extending from the mouse megakaryocyte surface (Fig. 1). Organelles appeared to be excluded from blebs. Similar structures were also observed in cultured, human megakaryocytes generated from cord blood [28]. Inhibition of actin polymerization and stimulation of actin depolymerization augmented microparticle production and blebbing from megakaryocytes [28]. These observations support the concept that weakening the link between the membrane and the cytoskeleton causes microparticle shedding. This supposition is consistent with a body of data suggesting that dynamic plasma membrane blebbing critically depends on filamentous actin integrity [29-31]. In line with this model, both talin deficiency and PIP5 kinase $\text{I}\gamma$ deficiency in megakaryocytes, which cause disruption of the membrane-cytoskeleton interaction, also cause bleb formation [30]. An intriguing possibility is that megakaryocyte blebbing and microparticle shedding is driven by the Rho-Rock myosin pathway, which is active in megakaryocytes [32].

To evaluate the possibility that circulating CD41⁺ microparticles may derive directly from megakaryocytes, markers that distinguished PMPs from MKMPs have been identified. CD62P and LAMP-1 were expressed on microparticles derived from activated murine platelets, but not from cultured murine megakaryocytes [28]. Like MKMPs, microparticles isolated from murine plasma demonstrated negligible CD62P and LAMP-2. Full length filamin A, which links the GPIIb α membrane glycoprotein to the actin cytoskeleton, is cleaved following platelet activation and was not found in microparticles derived from activated platelets [28]. However, both MKMPs and microparticles isolated from circulating plasma demonstrated full length filamin A. Similarly, microparticles isolated from human plasma demonstrated full length filamin A in the absence of CD62P expression. These marker studies suggest that the majority of circulating CD41⁺ microparticles more closely resemble MKMPs than PMPs.

Additional evidence that circulating microparticles can be derived directly from megakaryocytes comes from clinical research. Studies evaluating total CD41⁺ microparticles, CD62P⁺ microparticles, and CD63⁺ microparticles suggested that only the CD62P⁺ and CD63⁺ microparticle populations correlated with history of myocardial infarction or peripheral vascular disease. The fact that total CD41⁺ microparticle counts did not increase lead the authors to speculate that circulating CD41⁺ microparticles lacking activation markers may derive directly from megakaryocytes [33]. Consistent with these results, recent studies showed that the majority of CD62⁺ microparticles in plasma from healthy individuals was CD62⁻ and contained full-length filamin A, indicating a megakaryocytic origin [28].

Role of microparticles in cell-to-cell communication

An increasing number of studies indicate that CD41⁺ microparticles contribute to intercellular communication [34]. While the physiological significance of this “platelet dust”

may have been overlooked for many years, recent work suggests that these tiny blebs from cells may play an important role in the transport and delivery of bioactive molecules and signals throughout the body. Microparticles may affect target cells either by stimulating them directly via surface-expressed ligands [35,36] or by transferring surface receptors from one cell to the other [37,38]. Janowska-Wieczorek et al. reported that hematopoietic stem-progenitor cells covered with PMPs expressed several new platelet membrane receptors such as CXCR4, CD41, CD62, and PAR-1. These authors also demonstrated that PMPs facilitated the engraftment of progenitor cells [39]. Microparticles from activated platelets transfer receptors to the membranes of normal as well as malignant cells and this transfer affects recipient cell function. For example, the transfer of CD41 from platelet derived microparticles to hematopoietic cells increased the adhesion of hematopoietic cells to fibrinogen [35].

Since microparticles engulf cytoplasm during their formation, they acquire proteins and RNA that originate from the cytosol of the parent cell. An increasing body of evidence suggests that after attachment or fusion with target cells, microparticles deliver cytoplasmic proteins and RNA to recipient cells [34]. This process can be mediated either through receptor-ligand interactions or through internalization by recipient cells via endocytosis [40-42]. This phenomenon may contribute to the reprogramming of target cells. Ratajczak et al demonstrated that microparticles derived from embryonic stem cells are able to reprogram hematopoietic progenitors by horizontal transfer of mRNA and protein delivery [43]. Subsequent studies showed that microparticles from endothelial progenitor cells are able to trigger angiogenesis both in vitro and in vivo by a horizontal transfer of mRNA to human microvascular and macrovascular endothelial cells [44]. Whether MKMPs could act as functional messengers of genetic information remains to be determined, but the recent identification of differentially expressed microRNAs in plasma microparticles of healthy individuals lends credence to this hypothesis [45]. Significant differences were found in microRNA expression between platelets, peripheral blood mononuclear cells and plasma microparticles, the majority of which are CD41⁺ [45]. The possibility that MKMPs within this CD41⁺ population can deliver microRNAs to recipient cells remains to be explored.

Platelet microparticles and immunity

A body of experimental data suggests that platelets play an integral role in modulating innate and adaptive immunity [46]. While there is support for the concept that platelet activation plays a role in the regulation of immunity, the underlying mechanisms by which they provide early signals to immune cells are not fully understood. Previous work has focused on the role of CD154 (CD40L), which is critical to the start and proliferation of the adaptive immune response. Sprague et al has shown that platelet microparticles activate adaptive immune cells in specific tissue compartments in response to cues that trigger antibody synthesis and alter lymphocyte activities [47]. Using an adoptive transfer model, the authors show that platelet-derived membrane vesicles can communicate activation signals to B-cells. Platelet-derived microparticles transport and deliver CD154 to turn on antigen-specific IgG production and germinal center formation. The release of platelet microparticles from sites of thrombosis in disease states may, therefore, stimulate an immune response.

Platelet Microparticles in Disease States

Hemostasis and thrombosis

PMPs appear to function in hemostasis. Castaman's defect, an isolated deficiency in the ability to generate platelet microparticles, is associated with a bleeding tendency [48,49]. Platelets from patients with Scott's syndrome also demonstrate an impaired ability to

generate platelet microparticles and display a bleeding diathesis. A role for PMP in activation of vascular cells has previously been demonstrated. Exposure of platelet microparticles to phospholipase A₂ results in the release of arachidonic acid, which is subsequently metabolized by the platelet to thromboxane A₂ [50]. This process results in the transactivation of platelets and endothelial cells and promotes monocyte-endothelial cell interactions [51]. Berckmans et al. demonstrated that most cell-derived microparticles circulating in healthy individuals are CD41⁺ and that these microparticles promote the generation of small amounts of thrombin, even in the presence of inhibitory antibodies to tissue factor and FVII. The authors hypothesize that this low grade thrombin generation in healthy individuals may lead to the activation of protein C and thus have an anticoagulant effect [3]. Whether CD41⁺ microparticles have coagulant or anticoagulant properties is yet unknown.

Since platelet microparticles are highly procoagulant, it has been proposed that they may contribute to the pathogenesis of arterial thrombotic disease. Several studies have suggested that circulating microparticles provide a potential prognostic marker for atherosclerotic vascular disease [52]. P-selectin and CD63-exposing platelet microparticles reflect platelet activation in peripheral arterial disease and myocardial infarction [33]. Michelsen and colleagues have demonstrated increased levels of platelet microparticles in survivors of myocardial infarction [53]. They demonstrate a significant independent association between large platelet microparticles and plasma thrombinantithromin complexes and soluble CD40 ligand (sCD40L) in patients with myocardial infarction, but not in healthy controls. In a recent study, Chironi et al demonstrated that internal and external carotid artery diameter correlated negatively with microparticles derived from platelets, endothelial cells, and leukocytes [54]. It remains unclear whether the microparticles are contributing to the remodeling of the artery or are simply representing the activation status of the cells from which they are derived.

Cancer and Angiogenesis

Numerous studies have shown that platelet microparticles have the capacity to induce angiogenesis and are involved in the metastasis of cancer [13]. Platelet microparticles promoted the proliferation, survival, migration, and tube formation of human umbilical vein endothelial cells, suggesting that platelet microparticles may promote the formation of new blood vessels during tumor growth. This effect was mediated by the concerted action of FGF-2, VEGF, and a lipid factor. Platelet microparticles also induced sprouting of blood vessels both in vitro and in vivo [55]. Furthermore, intra-myocardial injection of platelet microparticles markedly elevated the amount of new capillaries formed in the heart muscle in the background of ischemia.

While the role of platelet microparticles in cancer development is unknown, it is well-established that some tumor cells have the capacity to activate platelets and induce platelet aggregation. Thus, it may be expected that the concentration of platelet microparticles at the sites of tumors may be abnormally increased. In gastric cancer, platelet microparticle levels are better predictors of metastasis than plasma levels of IL-6, RANTES, and VEGF [56]. Helley and colleagues have recently demonstrated that platelet microparticle levels are highly correlated with aggressive tumors and a poor clinical outcome [57]. Dashevsky and colleagues showed that platelet microparticles can stimulate the secretion of MMP-2 via prostate cancer cells in vitro, promoting their passage through collagen, a major component of the extracellular matrix [58]. The secretion of MMP-2 was not mediated by the platelet angiogenesis regulatory proteins PF-4, VEGF, or bFGF. These results suggest that platelet microparticles may promote tumor invasiveness by stimulation of MMP-2 production.

The concept of intracellular microparticle-based receptor transfer contributing to disease has recently been demonstrated in models of cancer [59]. Al-Nedawi et al. recently found that microparticles generated from a human glioma cell line expressing an oncogenic form of the EGF receptor can transfer the receptor to tumor cells that express the wild-type receptor. The multivesicular transfer of the oncogenic EGF receptor lead to the transfer of oncogenic activity and the activation of transforming signaling pathways. Tumor cells expressing the oncogenic form of the EGF receptor also showed increased microparticle formation. These studies clearly demonstrate that microvesicles derived from tumor cells can contribute to a horizontal propagation of oncogenes.

Infectious Disease

Microparticles from activated platelets may serve as vectors that transfer receptors to recipient cells, rendering them susceptible to infection. Rozmyslowicz et al demonstrated that microparticles derived from megakaryocytes and platelets were able to transfer the chemokine coreceptor CXCR4, which is essential for the entry of X4 HIV strains, to the surface of CXCR4 negative cells. The recipient cells became susceptible to infection by the X4 HIV strain [38]. These studies raise the possibility that MKMP may influence cell function by binding to and transferring receptors to recipient cells. These studies also suggest that both platelet and megakaryocyte-derived microparticles may play an important role in spreading the infection of HIV-1. Recently, Corrales-Medina demonstrated that platelet microparticle levels were significantly higher among HIV-infected patients than controls. Thus, increased levels of microparticles in patients may be a mechanism that leads to the spreading of infection [60].

Rheumatoid Arthritis

While many different studies have highlighted the relationship between inflammation and thrombosis [61], a recent study links platelet microparticles to rheumatoid arthritis. Rheumatoid arthritis is a chronic, systemic inflammatory disorder that afflicts 1% of the world's population and principally attacks synovial joints. In rheumatoid arthritis, the joint synovium becomes inflamed with immune cells and blood vessels that are dilated. The progression of the disease involves both the adaptive and innate arms of the host immune system. Previous work has suggested that platelets accumulate in the joints of patients with rheumatoid arthritis and that increased numbers of microparticles are found in the synovial fluid of patients [62,63]. However, while earlier studies have suggested platelets may play a role in inflammatory joint disease, a mechanistic link between platelets and rheumatoid arthritis has not been established. Boilard et al demonstrate that platelets are crucial for the development of inflammatory arthritis [11]. They show that microparticles released from platelets amplify the rheumatoid arthritis. Using both pharmacologic and genetic approaches, they identified the collagen receptor glycoprotein VI and its associated gamma chain of the Fc receptor as key triggers for platelet microparticle generation in arthritis pathophysiology. Fibroblast-like cells lining the joint cavity triggered microparticle shedding. In support of their findings in the lab, clinical studies showed that synovial fluids from patients with rheumatoid arthritis contained platelet microparticles, whereas fluid from patients with osteoarthritis lacked microparticles. Boilard et al. also established that microparticles from the joint fluid of rheumatoid arthritis patients can reciprocally activate the fibroblast-like synoviocytes, stimulating synoviocytes to secrete inflammatory cytokines. IL-1 packaged into shed microparticles appears to play a major role in amplifying inflammation.

Conclusion

Considering their diverse functions, it is remarkable that platelet microparticles escaped the attention of many hematologists until recently. Now, our understanding of platelet microparticles is accelerating rapidly, and we are beginning to define their specific roles in select disease processes. Understanding how platelet microparticles impact different diseases will enable identification of new cellular pathways that are amenable to therapeutic manipulation. In addition, platelet microparticles show promise as diagnostic biomarkers for diseases and potentially as a delivery system for therapeutics. While it is difficult to predict which of these potentials will be actualized and when, the substantial growth in this area towards patient-based studies underscores its clinical relevance.

References

1. Horstman LL, Ahn YS. Platelet microparticles: a wide-angle perspective. *Crit Rev Oncol Hematol*. 1999; 30:111–142. [PubMed: 10439058]
2. Joop K, Berckmans RJ, Nieuwland R, Berkhout J, Romijn FP, Hack CE, Sturk A. Microparticles from patients with multiple organ dysfunction syndrome and sepsis support coagulation through multiple mechanisms. *Thromb Haemost*. 2001; 85:810–820. [PubMed: 11372673]
3. Berckmans RJ, Nieuwland R, Boing AN, Romijn FP, Hack CE, Sturk A. Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. *Thromb Haemost*. 2001; 85:639–646. [PubMed: 11341498]
4. Hughes M, Hayward CP, Warkentin TE, Horsewood P, Chorneyko KA, Kelton JG. Morphological analysis of microparticle generation in heparin-induced thrombocytopenia. *Blood*. 2000; 96:188–194. [PubMed: 10891450]
5. Mallat Z, Benamer H, Hugel B, Benessiano J, Steg PG, Freyssinet JM, Tedgui A. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation*. 2000; 101:841–843. [PubMed: 10694520]
6. Lee YJ, Jy W, Horstman LL, Janania J, Reyes Y, Kelley RE, Ahn YS. Elevated platelet microparticles in transient ischemic attacks, lacunar infarcts, and multiinfarct dementias. *Thromb Res*. 1993; 72:295–304. [PubMed: 8303669]
7. Galli M, Grassi A, Barbui T. Platelet-derived microvesicles in thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. *Thromb Haemost*. 1996; 75:427–431. [PubMed: 8701402]
8. Tomer A, Harker LA, Kasey S, Eckman JR. Thrombogenesis in sickle cell disease. *J Lab Clin Med*. 2001; 137:398–407. [PubMed: 11385360]
9. Ando M, Iwata A, Ozeki Y, Tsuchiya K, Akiba T, Nihei H. Circulating platelet-derived microparticles with procoagulant activity may be a potential cause of thrombosis in uremic patients. *Kidney Int*. 2002; 62:1757–1763. [PubMed: 12371977]
- 10**. Varon D, Shai E. Role of platelet-derived microparticles in angiogenesis and tumor progression. *Discov Med*. 2009; 8:237–241. [PubMed: 20040277] [This important review highlights the key studies demonstrating a role for platelet-driven microparticles in regulating new blood vessel growth]
- 11**. Boilard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, Massarotti EM, Remold-O'Donnell E, Farndale RW, Ware J, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science*. 2010; 327:580–583. [PubMed: 20110505] [This elegant study describes a critical role for platelet-derived microparticles in the development of the autoimmune disease rheumatoid arthritis. The authors identify platelet microparticles in the joint fluid of patients with rheumatoid arthritis. The platelet microparticles were pro-inflammatory and the collagen receptor GPVI is identified as a key trigger for the microparticle generation. It is unclear how the microparticles get into the joint fluid]
12. Tan KT, Lip GY. The potential role of platelet microparticles in atherosclerosis. *Thromb Haemost*. 2005; 94:488–492. [PubMed: 16268460]
13. Kim HK, Song KS, Chung JH, Lee KR, Lee SN. Platelet microparticles induce angiogenesis in vitro. *Br J Haematol*. 2004; 124:376–384. [PubMed: 14717787]

14. Wolf P. The nature and significance of platelet products in human plasma. *Br J Haematol.* 1967; 13:269–288. [PubMed: 6025241]
15. George JN, Thoi LL, McManus LM, Reimann TA. Isolation of human platelet membrane microparticles from plasma and serum. *Blood.* 1982; 60:834–840. [PubMed: 7115953]
16. George JN, Pickett EB, Saucerman S, McEver RP, Kunicki TJ, Kieffer N, Newman PJ. Platelet surface glycoproteins. Studies on resting and activated platelets and platelet membrane microparticles in normal subjects, and observations in patients during adult respiratory distress syndrome and cardiac surgery. *J Clin Invest.* 1986; 78:340–348. [PubMed: 2942561]
17. Heijnen HF, Schiel AE, Fijnheer R, Geuze HJ, Sixma JJ. Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. *Blood.* 1999; 94:3791–3799. [PubMed: 10572093]
- 18*. Dean WL, Lee MJ, Cummins TD, Schultz DJ, Powell DW. Proteomic and functional characterisation of platelet microparticle size classes. *Thromb Haemost.* 2009; 102:711–718. [PubMed: 19806257] [This study contains very interesting data showing that platelet microparticle size classes differ significantly in their contents of membrane receptors, chemokines and growth factors, as well as their effects in functional assays]
19. Bode AP, Orton SM, Frye MJ, Udis BJ. Vesiculation of platelets during in vitro aging. *Blood.* 1991; 77:887–895. [PubMed: 1704268]
20. Miyazaki Y, Nomura S, Miyake T, Kagawa H, Kitada C, Taniguchi H, Komiyama Y, Fujimura Y, Ikeda Y, Fukuhara S. High shear stress can initiate both platelet aggregation and shedding of procoagulant containing microparticles. *Blood.* 1996; 88:3456–3464. [PubMed: 8896411]
21. Chow TW, Hellums JD, Thiagarajan P. Thrombin receptor activating peptide (SFLLRN) potentiates shear-induced platelet microvesiculation. *J Lab Clin Med.* 2000; 135:66–72. [PubMed: 10638696]
22. Wiedmer T, Shattil SJ, Cunningham M, Sims PJ. Role of calcium and calpain in complement-induced vesiculation of the platelet plasma membrane and in the exposure of the platelet factor Va receptor. *Biochemistry.* 1990; 29:623–632. [PubMed: 2159784]
23. Shcherbina A, Remold-O'Donnell E. Role of caspase in a subset of human platelet activation responses. *Blood.* 1999; 93:4222–4231. [PubMed: 10361119]
24. Dale GL, Friese P. Bax activators potentiate coated-platelet formation. *J Thromb Haemost.* 2006; 4:2664–2669. [PubMed: 16970802]
25. Flaumenhaft R. Formation and fate of platelet microparticles. *Blood Cells Mol Dis.* 2006; 36:182–187. [PubMed: 16466949]
26. Rand ML, Wang H, Bang KW, Packham MA, Freedman J. Rapid clearance of procoagulant platelet-derived microparticles from the circulation of rabbits. *J Thromb Haemost.* 2006; 4:1621–1623. [PubMed: 16839364]
27. Cramer EM, Norol F, Guichard J, Breton-Gorius J, Vainchenker W, Masse JM, Debili N. Ultrastructure of platelet formation by human megakaryocytes cultured with the Mpl ligand. *Blood.* 1997; 89:2336–2346. [PubMed: 9116277]
- 28*. Flaumenhaft R, Dilks JR, Richardson J, Alden E, Patel-Hett SR, Battinelli E, Klement GL, Sola-Visner M, Italiano JE Jr. Megakaryocyte-derived microparticles: direct visualization and distinction from platelet-derived microparticles. *Blood.* 2009; 113:1112–1121. [PubMed: 18802008] [This study demonstrates that microparticles can be generated directly from megakaryocytes. The authors directly visualize the formation of microparticle from living megakaryocytes. A role for the actin cytoskeleton in microparticle formation is established]
29. Cunningham CC. Actin polymerization and intracellular solvent flow in cell surface blebbing. *J Cell Biol.* 1995; 129:1589–1599. [PubMed: 7790356]
30. Wang Y, Litvinov RI, Chen X, Bach TL, Lian L, Petrich BG, Monkley SJ, Critchley DR, Sasaki T, Birnbaum MJ, et al. Loss of PIP5K1gamma, unlike other PIP5KI isoforms, impairs the integrity of the membrane cytoskeleton in murine megakaryocytes. *J Clin Invest.* 2008; 118:812–819. [PubMed: 18188447]
31. Charras GT, Hu CK, Coughlin M, Mitchison TJ. Reassembly of contractile actin cortex in cell blebs. *J Cell Biol.* 2006; 175:477–490. [PubMed: 17088428]

32. Fackler OT, Grosse R. Cell motility through plasma membrane blebbing. *J Cell Biol.* 2008; 181:879–884. [PubMed: 18541702]
33. van der Zee PM, Biro E, Ko Y, de Winter RJ, Hack CE, Sturk A, Nieuwland R. PSelectin- and CD63-Exposing Platelet Microparticles Reflect Platelet Activation in Peripheral Arterial Disease and Myocardial Infarction. *Clin Chem.* 2006
34. Ratajczak J, Wysoczynski M, Hayek F, Janowska-Wieczorek A, Ratajczak MZ. Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. *Leukemia.* 2006; 20:1487–1495. [PubMed: 16791265]
35. Baj-Krzyworzeka M, Majka M, Pratico D, Ratajczak J, Vilaire G, Kijowski J, Reca R, Janowska-Wieczorek A, Ratajczak MZ. Platelet-derived microparticles stimulate proliferation, survival, adhesion, and chemotaxis of hematopoietic cells. *Exp Hematol.* 2002; 30:450–459. [PubMed: 12031651]
36. Morel O, Toti F, Hugel B, Freyssinet JM. Cellular microparticles: a disseminated storage pool of bioactive vascular effectors. *Curr Opin Hematol.* 2004; 11:156–164. [PubMed: 15257014]
37. Mack M, Kleinschmidt A, Bruhl H, Klier C, Nelson PJ, Cihak J, Plachy J, Stangassinger M, Erfle V, Schlondorff D. Transfer of the chemokine receptor CCR5 between cells by membrane-derived microparticles: a mechanism for cellular human immunodeficiency virus 1 infection. *Nat Med.* 2000; 6:769–775. [PubMed: 10888925]
38. Rozmyslowicz T, Majka M, Kijowski J, Murphy SL, Conover DO, Poncz M, Ratajczak J, Gaulton GN, Ratajczak MZ. Platelet- and megakaryocyte-derived microparticles transfer CXCR4 receptor to CXCR4-null cells and make them susceptible to infection by X4-HIV. *Aids.* 2003; 17:33–42. [PubMed: 12478067]
39. Janowska-Wieczorek A, Majka M, Kijowski J, Baj-Krzyworzeka M, Reca R, Turner AR, Ratajczak J, Emerson SG, Kowalska MA, Ratajczak MZ. Platelet-derived microparticles bind to hematopoietic stem/progenitor cells and enhance their engraftment. *Blood.* 2001; 98:3143–3149. [PubMed: 11698303]
40. Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, Geuze HJ. B lymphocytes secrete antigen-presenting vesicles. *J Exp Med.* 1996; 183:1161–1172. [PubMed: 8642258]
41. Denzer K, van Eijk M, Kleijmeer MJ, Jakobson E, de Groot C, Geuze HJ. Follicular dendritic cells carry MHC class II-expressing microvesicles at their surface. *J Immunol.* 2000; 165:1259–1265. [PubMed: 10903724]
42. Clayton A, Turkes A, Dewitt S, Steadman R, Mason MD, Hallett MB. Adhesion and signaling by B cell-derived exosomes: the role of integrins. *Faseb J.* 2004; 18:977–979. [PubMed: 15059973]
43. Ratajczak J, Miekus K, Kucia M, Zhang J, Reca R, Dvorak P, Ratajczak MZ. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. *Leukemia.* 2006; 20:847–856. [PubMed: 16453000]
44. Deregibus MC, Cantaluppi V, Calogero R, Lo Iacono M, Tetta C, Biancone L, Bruno S, Bussolati B, Camussi G. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood.* 2007; 110:2440–2448. [PubMed: 17536014]
- 45*. Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, Xiao T, Schafer J, Lee ML, Schmittgen TD, et al. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS One.* 2008; 3:e3694. [PubMed: 19002258] [This article describes the first evaluation of miRNA in plasma microparticles and compares the miRNA content of platelets and peripheral blood mononuclear cells to that of plasma microparticles]
- 46*. Semple JW, Freedman J. Platelets and innate immunity. *Cell Mol Life Sci.* 2010; 67:499–511. [PubMed: 20016997] [This comprehensive review highlights the emerging roles of platelets in innate immunity]
- 47*. Sprague DL, Elzey BD, Crist SA, Waldschmidt TJ, Jensen RJ, Ratliff TL. Platelet-mediated modulation of adaptive immunity: unique delivery of CD154 signal by platelet-derived membrane vesicles. *Blood.* 2008; 111:5028–5036. [PubMed: 18198347] [The elegant studies described in this article demonstrate that platelet microparticles are capable of stimulating antigen-specific IgG production and modulating germinal-center formation in cooperation with

- CD4+ cells. These studies show that CD4+ cells are not solely responsible for delivering CD154 to B cells and demonstrate a role for platelet microparticles in orchestrating immune responses]
48. Castaman G, Yu-Feng L, Rodeghiero F. A bleeding disorder characterised by isolated deficiency of platelet microvesicle generation. *Lancet*. 1996; 347:700–701. [PubMed: 8596424]
 49. Castaman G, Yu-Feng L, Battistin E, Rodeghiero F. Characterization of a novel bleeding disorder with isolated prolonged bleeding time and deficiency of platelet microvesicle generation. *Br J Haematol*. 1997; 96:458–463. [PubMed: 9054648]
 50. Barry OP, Pratico D, Lawson JA, FitzGerald GA. Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. *J Clin Invest*. 1997; 99:2118–2127. [PubMed: 9151784]
 51. Barry OP, Pratico D, Savani RC, FitzGerald GA. Modulation of monocyte-endothelial cell interactions by platelet microparticles. *J Clin Invest*. 1998; 102:136–144. [PubMed: 9649567]
 52. Boulanger CM, Amabile N, Tedgui A. Circulating microparticles: a potential prognostic marker for atherosclerotic vascular disease. *Hypertension*. 2006; 48:180–186. [PubMed: 16801490]
 53. Michelsen AE, Brodin E, Brosstad F, Hansen JB. Increased level of platelet microparticles in survivors of myocardial infarction. *Scand J Clin Lab Invest*. 2008; 68:386–392. [PubMed: 18752144]
 - 54*. Chironi GN, Simon A, Boulanger CM, Dignat-George F, Hugel B, Megnien JL, Lefort M, Freyssinet JM, Tedgui A. Circulating microparticles may influence early carotid artery remodeling. *J Hypertens*. 2009; 28:789. [PubMed: 20032788] [Microparticles play a role in early vessel remodeling before the onset of major atherosclerosis]
 55. Brill A, Dashevsky O, Rivo J, Gozal Y, Varon D. Platelet-derived microparticles induce angiogenesis and stimulate post-ischemic revascularization. *Cardiovasc Res*. 2005; 67:30–38. [PubMed: 15878159]
 56. Kim HK, Song KS, Park YS, Kang YH, Lee YJ, Lee KR, Ryu KW, Bae JM, Kim S. Elevated levels of circulating platelet microparticles, VEGF, IL-6 and RANTES in patients with gastric cancer: possible role of a metastasis predictor. *Eur J Cancer*. 2003; 39:184–191. [PubMed: 12509950]
 - 57*. Helley D, Banu E, Bouziane A, Banu A, Scotte F, Fischer AM, Oudard S. Platelet microparticles: a potential predictive factor of survival in hormone-refractory prostate cancer patients treated with docetaxel-based chemotherapy. *Eur Urol*. 2009; 56:479–484. [PubMed: 18585841] [This paper extends the concept that platelet microparticles contribute to cancer. Platelet microparticle levels are correlated with aggressive tumors, poor clinical outcome, and increased platelet counts]
 - 58**. Dashevsky O, Varon D, Brill A. Platelet-derived microparticles promote invasiveness of prostate cancer cells via upregulation of MMP-2 production. *Int J Cancer*. 2009; 124:1773–1777. [PubMed: 19101987] [This interesting study extends the concept that platelet microparticles contribute to the invasiveness of cancer. The authors show that platelet microparticles can induce the secretion of matrix metalloproteinase-2 by prostate cancer cells in an in vitro system]
 - 59**. Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, Rak J. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. *Nat Cell Biol*. 2008; 10:619–624. [PubMed: 18425114] [This elegant study shows that tumor cells can use microparticle formation as a mechanism to transfer the oncogenic epidermal growth factor receptor to tumor cells that lack this receptor. The microparticle-based sharing of this oncogenic receptor leads to transfer of oncogenic activity. This work clearly shows that tumor-derived microvesicles can contribute to the horizontal propagation of oncogenes]
 - 60*. Corrales-Medina VF, Simkins J, Chirinos JA, Serpa JA, Horstman LL, Jy W, Ahn YS. Increased levels of platelet microparticles in HIV-infected patients with good response to highly active antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2010; 54:217–218. [PubMed: 20505474] [This study demonstrates that platelet microparticle levels are significantly higher in HIV-infected subjects than controls. This may provide a mechanism to increase the spread of virus]
 61. Wagner DD, Burger PC. Platelets in inflammation and thrombosis. *Arterioscler Thromb Vasc Biol*. 2003; 23:2131–2137. [PubMed: 14500287]
 62. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med*. 2001; 344:907–916. [PubMed: 11259725]

63. Knijff-Dutmer EA, Koerts J, Nieuwland R, Kalsbeek-Batenburg EM, van de Laar MA. Elevated levels of platelet microparticles are associated with disease activity in rheumatoid arthritis. *Arthritis Rheum.* 2002; 46:1498–1503. [PubMed: 12115179]

Figure 1. Microparticle generation from mouse megakaryocytes

Thin-section electron micrograph showing the surface of a mouse megakaryocyte. Multiple, small projections were observed emanating from the surface. Blebs at various stages are visualized along the plasma membrane. Scale bar, 500 microns.