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Review Article

Function and Clinical Significance of Platelet-Derived Microparticles

Shosaku Nomura

First Department of Internal Medicine, Kansai Medical University, Osaka, Japan

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Abstract

Microparticles released from platelets (PMPs) may play a role in the normal hemostatic response to vascular injury because they demonstrate prothrombinase activity. PMPs were first observed as released vesicles from platelets following adhesion to vessel walls, and flow cytometry is now the most widely used method for studying PMPs. PMPs are thought to play a role in clinical disease because they express phospholipids that function as procoagulants. High shear stress can initiate both platelet aggregation and shedding of procoagulant-containing PMP, suggesting that PMP generation by high shear stress occurs in small diseased arteries and arterioles under various clinical conditions. In addition, the possibility that PMPs evoke cellular responses in their immediate microenvironments has recently been suggested. Despite many interesting findings, the significance of PMPs in various clinical conditions remains controversial. For example, it is not known whether PMPs found in peripheral blood vessels cause thrombosis, or if they are the results of thrombosis. There has been some question about whether the PMPs found in thromboses are consumed locally, meaning that PMPs circulating in the peripheral blood are not functionally important. Currently, the number of clinical disorders associated with elevated PMPs is increasing. *Int J Hematol.* 2001;74:397-404.

Key words: Microparticle; Procoagulant activity; High shear stress; Flow cytometry; Clinical disorder

1. Introduction

One response of activated platelets to certain stimuli is shedding of microparticles. Microparticles released from platelets (PMPs) may play a role in the normal hemostatic responses to vascular injury because they demonstrate prothrombinase activity [1]. It is also possible that local generation of microparticles in small atherosclerotic arteries or arterioles promotes acute arterial occlusion by providing and propagating catalytic surfaces for the coagulation cascade.

PMPs carry several antigens characteristic of intact platelets, chiefly glycoproteins (GP) IIb/IIIa ($\alpha_{IIb}\beta_3$) and GPIb/IX. PMPs cannot be detected by standard platelet counting methods but they can be detected by other means. PMPs were first described by Wolf [2], and Warren et al [3] demonstrated the release of vesicles from platelets following the adhesion of platelets to vessel walls. Platelet-derived procoagulant activity was previously called platelet factor 3 (PF3) [4]. The discovery of PMPs answered some questions concerning PF3. Extensive studies of PMPs were made possible by flow cytometry, which is now the most widely used method for studying PMPs because of its simplicity and the wealth of information it provides about the population under study [5-7]. Despite many interesting findings, the significance of PMPs in various clinical conditions remains controversial.

Pathologic levels of fluid shear stress may occur in small arteries or arterioles partially obstructed by atherosclerosis or vasospasm and may induce activation and aggregation of circulating platelets [8-11]. This type of platelet aggregation may play a crucial role in thrombogenesis in various pathological states [9,10,12]. High shear stress can initiate both platelet aggregation and shedding of procoagulant-containing microparticles [13,14], suggesting the possibility that microparticles are generated by high shear stress in small diseased arteries and arterioles.

We present here a literature review regarding PMPs, including the growing list of clinical disorders associated with elevated PMP levels.

Correspondence and reprint requests: Shosaku Nomura, MD, First Department of Internal Medicine, Kansai Medical University, 10-15 Fumizonocho, Moriguchi, Osaka 570-8507, Japan; 81-6-6992-1001; fax: 81-72-833-3990 (e-mail: shosaku-n@mbp.sphere.ne.jp).

2. PMP Composition

PMPs can range in size from 0.02 μm to 0.1 μm, and they have no clear definition. It is unclear whether PMPs arise from complete conversion of a few platelets or from partial conversion of many or most platelets, but it is likely that either scenario occurs. There is a growing body of evidence that platelets comprise a heterogeneous population that is not attributable solely to senescence [15-17]. George et al [18] quantified many different GPs on PMPs and many are present routinely, notably $\alpha_{IIb}\beta_3$ and GPIb/IX. Sims et al [19] characterized PMPs and reported the presence of $\alpha_{IIb}\beta_3$, GPIb/IX, and α-granule membrane protein-140 (P-selectin or CD62P). In addition, an activation-dependent epitope of $\alpha_{IIb}\beta_3$ was found on complement-activated platelets but not on PMPs [20]. Other researchers have also reported differences in the composition of membrane proteins between activated platelets and PMPs [20-22].

Gemmel et al [23] reported that at least 1 of the $\alpha_{IIb}\beta_3$ adhesion ligands is involved in the mechanism of PMP generation. However, this finding is controversial [24,25]. PMPs contain molecules in addition to GPs, such as platelet-activating factor (PAF)[26], β-amyloid precursor protein [27], Ca^{2+}-dependent protease calpain [28,29], and many phospholipids [30-32], which are particularly important because they are related to the function of PMPs.

3. Mechanism of PMP Production

3.1. Production of PMP by Complement Components

Release of PMPs follows activation of platelets by strong agonists such as thrombin and collagen or by an increase in the intracellular calcium concentration induced by complement components C5b-9, calcium ionophores, or high shear stress [1,13,14,32]. Thus, PMP generation is stimulated by various agonists. Variations in the type of PMP produced by these agents, even at concentrations considered saturating with respect to aggregation, indicate that platelet activation and PMP generation are not simply all-or-none phenomena [33,34].

Sims et al [35] have extensively investigated platelet activation and associated PMP production caused by exposure of platelets to purified complement components. They speculated that recovery of membrane potential may follow shedding of PMPs carrying the C5b-9 pore (membrane attack complex [MAC]), on the basis of the finding that PMP from platelets exposed to MAC carried the majority of available MACs [35]. This process was dependent on external Ca^{2+}, which presumably moved through MAC pores. Wiedmer et al [36,37] investigated the roles of Ca^{2+} and calpain in MAC-induced vesiculation and subsequently clarified the role of protein kinases is this process. Cytoplasmic Ca^{2+} levels were increased by MAC regardless of the presence of protein kinase or phosphatase. However, PMP formation was reduced by kinase or calmodulin inhibitors, suggesting the role of platelet myosin light chain kinase or another Ca^{2+}/calmodulin-regulated membrane component in PMP generation.

3.2. Roles of the Cytoskeleton and Calpain in PMP Production

The cytoskeleton in platelets is spectrin-rich and is closely associated with $\alpha_{IIb}\beta_3$. Platelets have a network of filaments throughout their cytoplasm that exhibit distinct changes in composition and structure following platelet activation or aggregation [38,39]. Fox et al [40] presented evidence that the membrane skeleton in resting platelets stabilizes platelets against vesiculation and that shedding of microparticles is correlated with the extent of membrane skeleton disruption. These findings were extended by Basse et al [41] who used calpeptin to inhibit calpain-mediated cytoskeleton proteolysis. They found morphological differences in the filopods of activated platelets in the presence or absence of calpeptin following activation with A23187, a calcium ionophore, and they concluded that PMPs resulted from fragmentation of such filopods (pseudopods). Yano et al [42] concluded that PMPs are formed by fracture of budding pseudopods upon activation and noted that cytochalasin D nearly eliminated the PMP fraction. They also investigated the effects of protein phosphatase inhibitors (calyculin A and okadaic acid) on PMP formation. In the presence of phosphatase inhibitors, the number of PMPs arising from A23187 activation doubled, further supporting a key role for cytoskeleton dynamics in PMP formation. Pasquet et al [43] recently reported that PMP formation after A23187 administration is associated with activation of μ-calpain, increased protein tyrosine phosphatase activity, and decreased tyrosine phosphorylation.

3.3. PMP Generated by Shear Stress

Miyazaki et al [13] examined the mechanisms involved in PMP production induced by high shear stress and showed that binding of von Willebrand factor to GPIb, influx of extracellular calcium, and activation of platelet calpain were required to generate PMPs under conditions of high shear stress. Activation of protein kinase C (PKC) promoted shear-dependent PMP formation. Iwamoto et al [26] investigated the mechanism of release of platelet activating factor (PAF) from platelets and found that it is released from activated platelets in conjunction with the formation of PMPs. In addition, they found that PAF is concentrated in PMPs released from platelets activated by high shear stress [44]. Chow et al [45] suggested that thrombin formed in the vicinity of primary hemostatic plugs in areas of elevated shear stress plays a major role in the propagation of thrombi by potentiating shear-induced platelet microvesiculation.

4. Functions of PMPs

4.1. Procoagulant Effects of PMPs

PMPs were initially thought to be related to disease because they express phospholipids, which are procoagulants. The outer membrane leaflet of platelets is rich in choline phospholipids (sphingomyelin and phosphatidylcholine), which do not support coagulation, whereas the inner leaflet is rich in amino phospholipids (phosphatidyl-

ethanolamine and phosphatidylserine), which are more negative (anionic) at physiologic pH. Zwaal et al [46,47] showed that a key event in platelet activation is translocation of anionic phospholipids from the inner leaflet, especially phosphatidylserine, to the outer leaflet, where their exposure promotes cell-to-cell interactions and supports coagulation in the presence of calcium. The PMPs shed during activation are also enriched in phosphatidylserine, which accounts for the PF3 activity. It has been shown that collagen induces external exposure of anionic phospholipids in the resulting PMPs [31].

PMPs contain surface receptors for both factor VIII, a cofactor in the tenase enzyme complex [48], and factor Va, which combines with factor Xa to form the prothrombinase complex [49]. Whereas transient factor VIII binding to platelets has been reported, stable expression of factor VIII and factor Va has been reported for PMPs [48]. High- and low-affinity binding sites for activated factor IX are also present on PMPs [50]. These findings suggest that PMPs can exert procoagulant effects distant from the site of platelet activation and for a period longer than that for activated platelets.

4.2. Anticoagulant Roles of PMP

It was argued by Tans et al [51] that some PMP species may inhibit coagulation by accelerating inactivation of factor Va by activated protein C. Because PMPs are subject to the same platelet stimulation reactions, PMPs possess both pro- and anticoagulant properties. The relative distributions of pro- and anticoagulant activities in platelets and PMPs are similar. Furthermore, a recent study reported that protein C inhibitor, a member of the serpin family selected from activated platelets, binds preferentially to the phosphatidylethanolamine in platelet membranes and PMPs and efficiently inhibits phospholipid-bound activated protein C [52]. PMPs do not always display procoagulant phospholipids, possibly due to an incomplete flip-flop of phospholipids between the membrane leaflets or to scramblase activity. These observations underscore the heterogeneity of PMPs. The density of aminophospholipids has also been shown to be greater on PMPs than on remnant platelets [53,54].

4.3. PMPs Influence Endothelial and Monocyte Functions

It is now understood that platelets and neutrophils are intimately involved in coagulation, inflammation, and hemostasis, either directly through cell-to-cell contact (adhesion) or indirectly via cytokines or tissue factors [55-58]. Recently, the possibility that PMPs evoke cellular responses in the microenvironments where they are formed has been suggested [59,60]. Bary et al [60] reported that PMP may activate platelets, monocytes, and endothelial cells and that PMPs, which are known to increase in number during inflammation, may facilitate adhesive interactions between monocytes and endothelial cells [59,60]. PMPs, generated via activation of human platelets by thrombin or A23187 in the presence of the cyclooxygenase (COX) inhibitor indomethacin, dose-dependently increase platelet aggregation, intracellular movement of Ca^{2+} in platelets and monocytes, and inositol phosphate (IP) formation [59]. Thus, PMPs evoke biological responses irrespective of the COX activity of their precursors. PMPs also demonstrate transcellular delivery of unmetabolized arachidonic acid, and PMP activation of human vascular endothelial cells (HUVECs) and U-937 cells induces de novo expression of COX-2 but not COX-1 [59,61].

4.4. PMP in Cell-to-Cell Interactions

Studies with pharmacological inhibitors suggest that PMP-induced HUVEC activation is not mediated via activation of thromboxane, platelet-activating factor, or α-adrenergic receptors [59]. The concentrated delivery of PMP bioactive lipids may modulate multicellular interactions that occur in the early stages of atherogenesis. The significance of platelet activation in atherogenesis is unclear. Because circulating PMPs have been reported in human platelet activation and inflammation syndromes [28,62], the role of PMPs in modulating interactions between monocytes and endothelial cells have been investigated. Recently, MPs of monocytic and lymphocytic origin have been shown to be present in atherosclerotic plaques but not in the underlying arterial walls [63]. In addition, Combes et al [64] reported that MPs formed after induction of tumor necrosis factor may participate in the dissemination of proadhesive and procoagulant activities in thrombotic disorders. A role of PMPs in atherosclerosis has also been suggested. PMPs can alter adhesive interactions among endothelial cells, monocytes, and monocytoid cells. Barry et al [60] reported that PMP stimulation of HUVECs increased levels of intracellular adhesion molecule-1 (ICAM-1), and stimulation of monocyte and U-937 cells increased expression of lymphocyte function-associated antigen-1 (CD11a/CD18), macrophage antigen-1 (CD11b/CD18), and CD14. Nomura et al [65] also reported that PMPs induced by high shear stress enhance expression of cell adhesion molecules by THP-1 and endothelial cells. They noted that these PMPs may contribute to the development of adhesion and participate in the vascular damage observed in inflammatory disorders. Forlow et al [66] reported that P-selectin–expressing PMPs bind to leukocytes that express P-selectin glycoprotein ligand-1 (PSGL-1), suggesting that PMPs may enhance leukocyte aggregation and leukocyte accumulation on substrates that express selectin, especially in diseases in which the number of PMPs is increased.

4.5. PMPs Activate Platelets, Monocytes, and Endothelial Cells Through a Mitogen-Activated Protein Kinase–Dependent Pathway

The interaction and functional cross-talk between leukocytes and endothelial cells is essential for vascular homeostasis [67]. Most MPs appear to induce endothelial cell activation [65,68,69]. The signaling pathway involved in PMP-induced cellular activation has been studied by Barry et al [59-61]. Both PMPs and arachidonic acid from PMPs induce platelet activation in a PKC-dependent, PKA-independent manner [59]. A role of PKC in both PMP- and

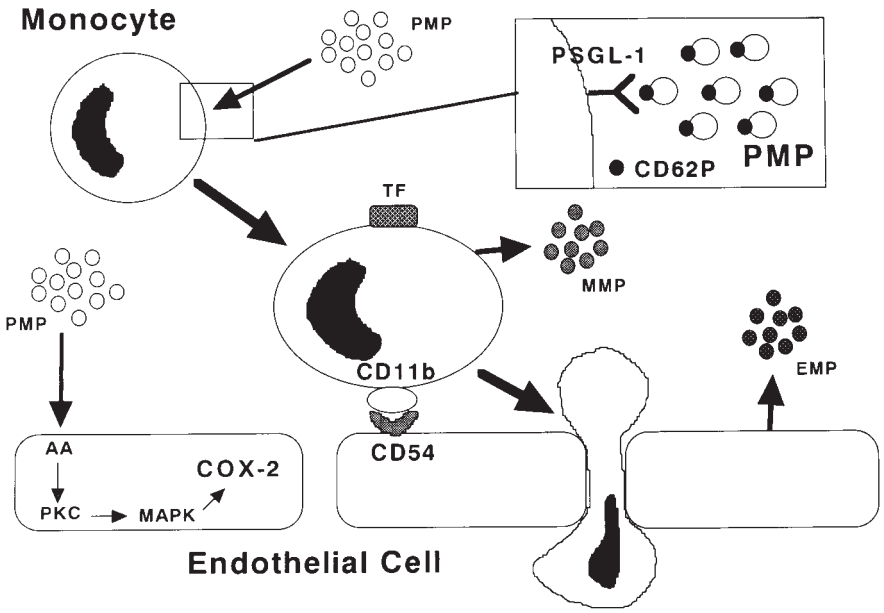

Figure 1. Mechanism of vascular changes by platelet-derived microparticles (PMPs). PMPs activate monocytes by a reaction between P-selectin and PSGL-1 (P-selectin glycoprotein ligand-1). Activated monocytes induce expression on the cell surface of tissue factor (TF) and CD11b. Activated monocytes also induce release of monocyte-derived microparticles (MMP). PMPs induce COX-2 production in endothelial cells. PMPs enhance expression of CD54 (ICAM-1) on the endothelial surface. Activated endothelial cells also induce release of endothelial cell–derived microparticles (EMP), enhancing adhesion between endothelial cells and monocytes. Finally, monocytes induce migration of endothelial cells, resulting in vascular changes. AA indicates arachidonic acid; PKC, protein kinase C; MAPK, mitogen-activated protein kinase.

arachidonic acid from PMP–induced cell-to-cell adhesion and chemotaxis has been suggested [60]. Similarly, PMPs and arachidonic acid from PMPs induce monocyte COX-2 expression in a PKC-dependent fashion [61]. PMP stimulation of U-937 cells resulted in the translocation of PKC from the cytosol to the cell membrane with concomitant activation of downstream mitogen-activated protein (MAP) kinases [61]. In particular, PMP induced activation of ERK1/2, JNK1, and p38 kinase in U-937 cells [61]. Although PKC activation is required for PMP-induced activation of ERK1/2 MAPK, activation of stress kinases, p38 kinase, and JNK1 is PKC-independent. Furthermore, PMP-induced phosphorylation of ERK1/2 MAPK, p38 kinase, and JNK1 is PI-3-kinase dependent. PMP-induced activation of U-937 COX-2 expression is also dependent upon activation of ERK1/2, p38 kinase, and PI-3-kinase. PMPs also induce transcriptional activation of COX-2 and transcription factors c-Jun and Elk-1 but not cyclic AMP (adenosine monophosphate) response element (CRE) [61]. The novel functions of PMPs are summarized in Figure 1.

5. Clinical Significance of PMP

Many clinical disorders are associated with elevated PMP levels (Table 1). However, the significance of PMPs in various clinical conditions remains controversial. Recently, assays for measurement of PMPs have improved [69-71] and their clinical significance is gradually being clarified.

5.1. Scott Syndrome

Scott syndrome, a rare bleeding disorder, was first described in 1979 [72]. It was reported in 1989 that platelets from patients with Scott syndrome were "markedly impaired in their ability to generate PMPs in response to all platelet activators, and this is accompanied by a comparable decrease in the number and function of inducible factor Va receptors" [1]. Scott syndrome was reviewed by Weiss in 1994 [73], and it was reported in 1996 that Scott syndrome "appears to be transmitted as an autosomal recessive trait reflecting the deletion or mutation of a putative phosphatidylserine

Table 1.
Clinical Disorders Associated With Elevated PMP Levels

Immune thrombocytopenic purpura (ITP)
Thrombotic thrombocytopenic purpura (TTP)
Heparin-induced thrombocytopenia (HIT)
Drug-induced thrombocytopenia
Transient ischemic attack (TIA)
Multiple sclerosis (MS)
Alzheimer disease (AZ)
Acute coronary syndrome (ACS)
Cardiopulmonary bypass
Scott syndrome
Diabetes mellitus
Uremia
Antiphospholipid antibody syndrome (APS)
Systemic lupus erythematosus (SLE)

translocase" [74,75]. Further studies found that this translocase (scramblase) is present but inactive in persons with Scott syndrome [76].

5.2. Immune Thrombocytopenic Purpura

The presence of PMPs in a clinical disorder was first described for immune thrombocytopenic purpura (ITP) [77,78]. In 1992 it was reported that patients with ITP have significantly elevated levels of PMPs as measured by flow cytometry [6]. Of greater interest, the same study showed that ITP patients who had thrombocytopenia but were asymptomatic had approximately twice the level of PMPs found in those patients with symptoms, suggesting that the PMPs in ITP patients are functional and protect these patients from thrombocytopenic bleeding [79,80]. Generation of PMPs was correlated with concentrations of antiplatelet antibodies and was largely abolished by preheating plasma to inactivate complement, indicating the involvement of complement in antibody-mediated PMP generation [81-83]. These findings suggested that at least some anti-platelet antibodies can induce complement-mediated formation of PMP and initiate platelet destruction.

5.3. Transient Ischemic Attacks

On the basis of having observed small-vessel transient ischemic attacks (TIA) in patients with ITP and high PMP levels, the same researchers performed PMP assays on non-ITP patients with cerebrovascular accidents or other neurologic disorders. Four groups of patients were studied: those with large-vessel strokes, small-vessel strokes, multiinfarct dementias, or Alzheimer disease. All patients except those with Alzheimer disease had significantly elevated PMP levels [84]. PMP levels were higher in patients with small-vessel strokes (small-vessel TIA, lacunar infarcts) than in those with large-vessel strokes [85]. It thus appears that elevated PMP levels are associated with small-vessel thrombosis with or without ITP. Elevated PMP levels are associated chiefly with small-vessel occlusions, which manifest as small-vessel TIA, mini-strokes, or progressive cognitive dysfunction and lead to dementias. Nomura et al [27] reported that PMPs rich in β-amyloid precursor proteins were strictly segregated between healthy controls and patients with cerebral infarcts, diabetes ($P < .001$), or uremia ($P < .01$).

5.4. Acute Coronary Syndrome

Platelets play a key role in arterial thrombosis and acute coronary syndrome (ACS) [86-88]. Katopodis et al [89] evaluated platelet calcium homeostasis and activation markers in 2 groups of patients undergoing coronary angiography for suspected ACS: those with recent myocardial infarction and those with unstable angina, and a control group. PMP levels were significantly higher in ACS patients than in control subjects. Direct percutaneous transluminal coronary angioplasty (PTCA) is more effective than thrombolysis in restoring patency and preventing reocclusion of infarct-related arteries [90]. Gawaz et al [91] examined various aspects of platelet function in patients with acute myocardial infarction undergoing direct PTCA. They concluded that platelet activation is significantly enhanced soon after direct PTCA as reflected by increased platelet consumption and PMP formation. There is some evidence that PMPs participate in atherosclerosis and reccurrent stenosis [62,92-94]. Merten et al [93] reported that PMPs bind to subendothelial matrices in vitro and in vivo and can act as substrate for further platelet binding. This interaction may play a significant role in platelet adhesion to sites of endothelial injury. Weber et al [94] reported that PMPs might directly affect proliferation of vascular smooth muscle cells.

5.5. Other Thrombotic Disorders

Kelton et al [28] found high calpain levels in the sera of patients with active thrombotic thrombocytopenic purpura (TTP) and that this activity was found on PMPs. This was not observed after patient recovery. More recently, Galli et al [95] performed a careful study of PMPs in TTP and found a rise and fall in PMP levels with the course of disease, suggesting that PMPs may be clinically relevant.

Anti-heparin antibodies in heparin-induced thrombocytopenia (HIT) are potent activators of platelets and cause copious shedding of procoagulant PMPs [96], indicating that PMPs cause the thrombotic complications in HIT-with-thrombosis and that they might serve as a diagnostic criterion for it [97]. Recently, Hughes et al [98] conducted a morphological analysis of PMPs to document the presence of PMPs in HIT.

Patients with diabetes mellitus develop hypercoagulability and platelet hyperaggregation; premature atherosclerosis also occurs with this disease [99]. A few studies on the potential role of PMPs in diabetic complications can been reported [7,27,62,100-103].

Some patients undergoing cardiopulmonary bypass (CPB) surgery experience neurological complications manifesting as mild cognitive and somatic changes [104]. George et al [18] reported elevated PMP levels during CPB. They reported a drop in expression of GPIb and -IIb on platelets following CPB. In contrast, Nieuwland et al [105] demonstrated that PMPs generated in vivo can stimulate coagulation in CPB patients.

Elevated PMP levels have been detected in other clinical conditions including uremia [106], infectious diseases [107,108], peripheral blood stem cell harvest [109], collagen diseases [110,111], and arteriosclerosis obliterans (ASO) [112]. In addition, PMPs are used as indicators for antithrombotic therapy [113,114] or side effects of blood transfusion [115].

6. Conclusion

We have summarized the literature to date relevant to PMPs, including the growing list of clinical disorders associated with elevated PMP levels. PMPs were initially thought to be small particles with procoagulant activity, however, the possibility that PMPs evoke cellular responses in the immediate microenvironments where they are formed is now under investigation.

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