Review

Platelet-Derived Microparticles

and the Potential of Glycoprotein IIb/IIIa Antagonists in Treating Acute Coronary Syndrome

Ximing Li, PhD, MD
Hongliang Cong, PhD, MD

Platelet glycoprotein IIb/IIIa receptors are major platelet membrane constituents. They are integral to the formation of the surface fibrinogen receptor on activated platelets, in which 73% of platelet-derived microparticles are positive for the glycoprotein IIa/IIIb receptor. Activated platelets can shed platelet-derived microparticles, especially during the course of an acute coronary syndrome. Data have shown that platelet-derived microparticles can bind to the endothelium, to leukocytes, and to the submatrix of vascular walls, and launch some signal-transduction pathways, such as the pertussis-toxin-sensitive G protein, extracellular signal-regulated kinase, and phosphoinositide 3-kinase pathways. One research group found that platelet-derived microparticles transfer glycoprotein IIb/IIIa receptors to isolated and whole-blood neutrophils. The receptors can co-localize with β_2-integrins and cooperate in the activation of nuclear factor κB (NF-κB), which can be inhibited by glycoprotein IIb/IIIa receptor antagonists. Accordingly, it is possible that glycoprotein IIb/IIIa receptor antagonists produce a direct and marked effect on endothelial cells, smooth-muscle cells, and leukocytes through a platelet-derived microparticle pathway that will lead to a potential treatment for acute coronary syndrome.

Herein, we review the medical literature and discuss the potential application of platelet-derived microparticles toward the treatment of acute coronary syndrome. ***(Tex Heart Inst J 2009;36(2):134-9)***

Key words: *Binding sites, antibody; biological markers/blood; blood platelets/chemistry/pathology/physiology/ultrastructure; cardiovascular diseases/blood; cell communication/analysis/physiology; cell membrane/metabolism/physiology; platelet activation/adhesiveness/physiology; platelet glycoprotein GPIIb/IIIa complex/analysis/biosynthesis/metabolism/physiology; platelet membrane glycoproteins/analysis; receptors, cell surface/physiology; signal transduction/physiology*

From: *Department of Cardiology, Tianjin Chest Hospital, Tianjin 300051, People's Republic of China*

Address for reprints: *Hongliang Cong, PhD, MD, Department of Cardiology, Tianjin Chest Hospital, Xian Road 93#, Heping District, Tianjin 300051, PRC*

E-mail: *HongliangCong@126.com*

Platelet glycoprotein (GP) IIb/IIIa receptors, which are major constituents of platelet membranes, are integral to the formation of the surface fibrinogen receptor on activated platelets. The GP IIb/IIIa receptors are present in a preponderance of platelet-derived microparticles (PMPs). Activated platelets can shed PMPs, especially during an acute coronary syndrome. Platelet-derived microparticles can bind to vessel walls and launch signal-transduction pathways, such as the pertussis-toxin-sensitive G protein, extracellular signal-regulated kinase, and the phosphoinositide 3-kinase (PI3-kinase) pathways. Here, we review the medical literature and discuss how GP IIb/IIIa receptor antagonists, acting through a PMP pathway, suggest a research focus toward the treatment of acute coronary syndrome.

The Character and Function of Platelet-Derived Microparticles

The term microparticles usually refers to particles larger than 100 nm in diameter that are derived from the plasma membrane among the various membrane vesicles that cells release. Smaller vesicles (40–100 nm) that originate from endoplasmic membranes are referred to as exosomes, and larger particles (>1.5 μm) that contain nuclear material are known as apoptotic bodies.[1]

In 1967, Wolf[2] described the membrane fragments that are shed from activated platelets as "platelet dust," or "platelet vesicles." After having been observed in electron micrographs, the particles were characterized as procoagulative in 1985.[3] These are the particles now widely referred to as PMPs.

All microparticles harbor cell-surface proteins and contain cytoplasmic components of their original cells. They exhibit negatively charged phospholipids, chiefly phosphatidylserine (PS), at their surface, which accounts for the procoagulative character and proinflammatory properties of microparticles, including the alteration of vascular function. The membranes of PMPs contain platelet GP Ib, IIb, IIIa, P-selectin, and thrombospondin,[4,5] in addition to other platelet membrane receptors, such as chemokine (C-X-C motif) receptor 4 and protease-activated receptor 1.[6,7] It has been reported that arachidonic acid released from PMPs directly activates GP Mac-1 and the intercellular

adhesion molecule-1 on monocytes and the P- and E-selectins on endothelial cells.[6,7] Bode and colleagues[8] found that 73% of PMPs were positive for the GP IIb/IIIa receptor, which is a Ca^{2+}-dependent heterodimer on activated platelets that can bind 1 of 4 different adhesive proteins (fibrinogen, fibronectin, von Willebrand factor, and vitronectin). The binding of fibrinogen primarily enables platelet aggregation; fibronectin and the von Willebrand factor may also enable adhesion and aggregation on the subendothelium.[9]

Platelet-derived microparticles have been observed in vivo in clinical conditions that are associated with platelet activation, including idiopathic thrombocytopenia purpura, transient ischemic attacks, and during cardiopulmonary bypass. Increased concentrations of circulating PMPs are also found during aging, and further increases are encountered in peripheral arterial disease and myocardial infarction.[10] The biological function of PMPs remains speculative, but the tenase and prothrombinase activity that includes factor Va, high-affinity-factor Xa, and factor-VIII activity[11] is concentrated on these particles. In addition, PMPs display anticoagulant activity, since they inactivate prothrombinase by means of activated protein C. These observations suggest that PMPs play a role in modulating hemostasis and thrombosis.[12]

The Increase of Platelet-Derived Microparticles in Acute Coronary Syndrome

The erosion, fissure, or rupture of an atherosclerotic plaque is the signaling event in acute coronary syndrome, and rupture can also occur during percutaneous coronary intervention. When plaque rupture occurs, the subendothelial protein matrix is immediately disrupted, which allows platelet-adhesion molecules such as von Willebrand factor and collagen to interact with circulating platelets. Platelets adhere to collagen and von Willebrand factor at the site of injury by means of specific GP receptors. This results in platelet activation, with a change in the platelets' shape, the release of storage granules that contain platelet agonists such as adenosine diphosphate and thromboxane A_2, and a conformational change in the platelet fibrinogen receptor GP IIb/IIIa. Although platelet deposition is restricted by circulating blood, already-activated platelets (with PMPs released) provide a new prothrombotic interface for fibrin, circulating blood, and a growing thrombus. This results in the growth of thrombus and narrowing of the vessel. Increases in shear stress, associated with vascular narrowing, favor this process by further promoting new platelet activation and the release of PMPs. An occlusive thrombus forms, and patients experience catastrophic events.

When platelets are activated by agonists such as collagen or thrombin, several responses occur: shape change, secretion, aggregation, phosphorylation of specific platelet proteins,[13] exposure of anionic phospholipid on the extracellular face of the platelet membrane,[14] and release of microparticles that are rich in procoagulant activity.[15] These microparticles possess platelet–subendothelium attachment receptors (GP IIb/IIIa, Ib, Ia, and IIa),[4,16,17] and P-selectin,[16] a receptor that is involved in platelet–leukocyte interactions[18-20] and in inflammatory response.[20]

Siljander and colleagues[21] found that PMPs are associated with developed fibrin fibrils. Moreover, the investigators showed in vitro that PMPs, when separated from platelet remnants, did bind to fibrin, where they were able to act as procoagulants in the presence of plasma and tissue factor. Finally, granular GP IIb/IIIa and P-selectin-positive material were seen to decorate fresh, embolectomized thromboemboli in a fibrin-strand-like pattern. The PMPs were shown to bind to the forming thrombus, and specifically to fibrin.

Glycoprotein IIb/IIIa antagonists cannot only inhibit the GP IIb/IIIa receptors on platelets; they also have an effect on PMPs.[22] However, few studies have probed this effect.

Different biological effects have been attributed to PMPs, including their possible participation in the pathogenesis of atherosclerosis and vascular injury during inflammation,[14] and in the promotion of bone-cell proliferation.[23] The attachment of isolated PMPs on subendothelia[24] has suggested a hemostatic function for PMPs. Glycoprotein IIb/IIIa-positive PMPs appear to be promising prognostic indicators in patients who have chest pain, but whose cardiac troponin levels are within normal range and whose electrocardiograms are nondiagnostic.[25]

Acquisition of Glycoprotein IIb/IIIa Receptors via Platelet-Derived Microparticles

Platelet glycoprotein IIb/IIIa receptors are major platelet-membrane constituents that are integral to the formation of the surface fibrinogen receptor on activated platelets. Approaches to achieve more profound platelet inhibition at the site of injured coronary plaque have focused on the integrin GP IIb/III receptor on the platelet surface membrane, which binds circulating fibrinogen or von Willebrand factor and cross-link platelets as the final common pathway to platelet aggregation.

The GP IIb/IIIa receptor is largely confined to platelets and megakaryocytes. It is also found on some melanoma cells,[26] where, by linking the stromal connective tissue to the M3Dau melanoma cells, the receptor may enable the stromal matrix to regulate tumor growth and differentiation in vivo.[26,27] However, recent studies have shown that some phagocytes may acquire the GP IIb/IIIa receptor from PMPs. Salanova and coworkers[28] found that GP IIb/IIIa receptors are transferred to isolated and whole-blood neutrophils via PMPs. Using specific antibodies in neutrophils that were treated with granulocyte macrophage colony-stimulating fac-

tor (GM-CSF), the investigators observed that acquired GP IIb/IIIa receptors co-localized with β_2-integrins and cooperated in the activation of nuclear factor κB (NF-κB). The Src and Syk nonreceptor tyrosine kinases, in addition to the actin cytoskeleton, controlled NF-κB activation. These acquired receptors are functional, and they enable NF-κB activation in GM-CSF–stimulated neutrophils that interact with fibronectin.[28] It was not determined exactly how acquired GP IIb/IIIa receptors link to the neutrophil-signaling machinery. However, similar to GP IIb/IIIa receptor signaling in platelets, Src and Syk kinases (and the actin cytoskeleton) seem to be involved.[28]

A large body of data suggests that macrophages can recognize PS specifically. Several research groups have found that human and rodent macrophages, and insect phagocytes, preferentially take up negatively charged liposomes, particularly those that contain PS.[29-34] In addition, human and rodent macrophages (including freshly isolated human alveolar and splenic macrophages, human bone-marrow–derived macrophages cultured for 10 ± 14 days, cultured human monocytes, and resident and thioglycolate-elicited mouse peritoneal macrophages) can bind to and engulf symmetric red-cell ghosts, red cells with PS inserted externally, oxidized red cells, or sickled red cells, all of which express PS externally.[35-41] Accordingly, PS plays a key role in signaling phagocytes to perform. These phagocytes can be professional (the leukocytes) or amateur (including fibroblasts, epithelial cells, and vascular smooth-muscle cells). Exposure of PS on the external leaflet of the plasma cell membrane appears to be common to many apoptotic cells,[42-50] and this phospholipid appears to be recognized in a stereospecific fashion by subsets of macrophages,[42,43,51] by melanoma cells,[52] by vascular smooth-muscle cells,[47] and by Sertoli cells.[50] As mentioned above, on the surface of PMPs there is plenty of PS, which serves as a cofactor for the coagulation cascade. Therefore, PMPs can probably be engulfed by the phagocytes, suggesting also that GP IIb/IIIa antagonists have an effect on the phagocytes through their acquired GP IIb/IIIa receptors via phagocytosis. However, other than the report by Salanova and coworkers,[28] studies are few.

The Effects of Platelet-Derived Microparticles on Cells

The binding of PMPs to cells can modify the cells' functional properties. The PMPs can bind hematopoietic progenitors and stimulate their engraftment.[53] The binding of PMPs to neutrophils induces a significant increase in both CD11b expression and phagocytic activity in a concentration-dependent manner. These findings suggest a possible role for PMPs in addition to providing platelet factors: specifically, as an activator and mediator of neutrophils in ischemic injury, thrombosis, and inflammation.[54] Janowska-Wieczorek and associates[53] found, rather surprisingly, that mobilized-peripheral-blood (mPB) CD341 cells expressed a significantly higher level of GP IIb/IIIa(CD41 antigen) than did CD341 cells that were isolated from either non-mPB or bone marrow. Hence, the investigators hypothesized that the presence of the CD41 antigen on mPB CD341 cells results from the binding of PMPs to their surfaces.[53]

Platelet microparticles bind to the subendothelial matrix in vitro and in vivo and can act as a substrate for further platelet binding. This interaction may play a substantial role in the adhesion of platelets to the site of endothelial injury.[55] Platelet-derived microparticles provide a catalytic surface that accelerates coagulation: they can bind to neutrophils[54] to mediate leukocyte–leukocyte interaction, and elevated levels of PMPs may amplify leukocyte-mediated tissue injury in thrombotic and inflammatory disorders.[56] Therefore, PMPs can bind neutrophils, mediate their aggregation, and activate their phagocytic properties.[54]

Some data provide evidence that PMPs can transfer biological information between cells, acting as veritable vectors of signal molecules. Even though PMPs can act on hematopoietic and circulating cells, most of the exchange of information from PMPs takes place at the level of the endothelium and contributes to the physiologic and pathophysiologic role of microparticles. Accordingly, PMPs can affect vasodilation and the antithrombotic and antiadhesive properties of the vascular wall. Also, they may be involved in the regulation of vascular permeability and the proliferation of smooth muscle cells. In addition to their role in the regulation of hemostasis and thrombosis, PMPs evoke monocyte adhesion to endothelial cells (ECs) by inducing adhesion-molecule exposure, stimulating the proliferation, survival, adhesion, and chemotaxis of hematopoietic cells, and increasing the engraftment of hematopoietic stem cells.[53] Also, PMPs induce angiogenesis in vitro,[57] probably through activation of ECs.[58,59]

How do PMPs work to cause the effects? Nomura and co-authors[60] thought that cytoskeleton served as a bridge to signal paths so that the GP IIb/IIIa complex could perform its function. Platelet-derived microparticles can be viewed as a pathway that can be used by cells to exchange information in addition to the transduction linked to the activation of classically known receptors or transporters. Platelet-derived microparticles taken from patients who were experiencing acute myocardial infarction caused severe endothelial dysfunction in rat aortas by affecting the endothelial nitric oxide transduction pathway, but not the endothelial nitric oxide synthase expression.[61] Paradoxically, it has been observed that PMPs affect ECs by protecting them from apoptosis and by inducing the proliferation and formation of tubule-like structures.[57] On the other hand, PMPs can

inflict damage on ECs by inducing an inflammatory response and diminishing endothelium-dependent vessel dilation.[62]

The PMP-stimulated proliferation, chemotaxis, and tube formation of ECs has been mediated via the pertussis toxin-sensitive G protein, extracellular signal-regulated kinase, and the PI3-kinase pathway.[57] Pertussis toxin, a G-protein inhibitor, blocks the effects of PMPs on GP IIb/IIIa.[63] Therefore, the G proteins, which regulate (for example) the activity of adrenergic receptors, may be involved in coupling agonist interaction to the receptor function of GP IIb/IIIa.[9] The PI3-kinase plays a pivotal role in mediating EC survival, proliferation, cytoskeletal reorganization, and cellular motility, which are all crucially important for vessel growth.[64] The PI3-kinase is activated by angiogenesis-related cytokines, such as vascular endothelial growth factor and basic fibroblast growth factor.[65]

As stated above, PMPs can bind to at least the endothelium, the leukocyte, and the submatrix of a vascular wall, and probably be swallowed by leukocytic and smooth-cell phagocytes to pass the GP IIb/IIIa receptors to them. Salanova and coworkers[28] also found that therapeutic GP IIb/IIIa inhibitory compounds such as abciximab, eptifibatide, and tirofiban prevent NF-κB activation through acquired GP IIb/IIIa receptors and may have novel implications in anti-inflammatory treatment protocols. This suggests that the advantageous effect of GP IIb/IIIa antagonists results not only from its platelet inhibition, but partly and probably from its influence on PMPs through GP IIb/IIIa receptors that have originated from platelets.

It is a novel and exciting finding that PMPs can transfer GP IIb/IIIa receptors to other cells, and the presence and consequential effect of PMPs and their receptors in human cells invite further investigation. If GP IIb/IIIa receptor antagonists indeed produce a direct and marked effect on ECs, smooth-muscle cells, and leukocytes through a PMP pathway, investigators have a potential focal point for treatment of acute coronary syndrome.

References

1. Boulanger CM, Amabile N, Tedgui A. Circulating microparticles: a potential prognostic marker for atherosclerotic vascular disease. Hypertension 2006;48(2):180-6.
2. Wolf P. The nature and significance of platelet products in human plasma. Br J Haematol 1967;13(3):269-88.
3. Sandberg H, Bode AP, Dombrose FA, Hoechli M, Lentz BR. Expression of coagulant activity in human platelets: release of membranous vesicles providing platelet factor 1 and platelet factor 3. Thromb Res 1985;39(1):63-79.
4. 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(2):340-8.
5. Gawaz M, Ott I, Reininger AJ, Heinzmann U, Neumann FJ. Agglutination of isolated platelet membranes. Arterioscler Thromb Vasc Biol 1996;16(5):621-7.
6. Barry OP, FitzGerald GA. Mechanisms of cellular activation by platelet microparticles. Thromb Haemost 1999;82(2):794-800.
7. Barry OP, Pratico D, Savani RC, FitzGerald GA. Modulation of monocyte-endothelial cell interactions by platelet microparticles. J Clin Invest 1998;102(1):136-44.
8. Bode AP, Orton SM, Frye MJ, Udis BJ. Vesiculation of platelets during in vitro aging. Blood 1991;77(4):887-95.
9. Phillips DR, Charo IF, Parise LV, Fitzgerald LA. The platelet membrane glycoprotein IIb-IIIa complex. Blood 1988;71(4):831-43.
10. van der Zee PM, Biro E, Ko Y, de Winter RJ, Hack CE, Sturk A, Nieuwland R. P-selectin- and CD63-exposing platelet microparticles reflect platelet activation in peripheral arterial disease and myocardial infarction. Clin Chem 2006;52(4):657-64.
11. Gilbert GE, Sims PJ, Wiedmer T, Furie B, Furie BC, Shattil SJ. Platelet-derived microparticles express high affinity receptors for factor VIII. J Biol Chem 1991;266(26):17261-8.
12. Gemmell CH, Sefton MV, Yeo EL. Platelet-derived microparticle formation involves glycoprotein IIb-IIIa. Inhibition by RGDS and a Glanzmann's thrombasthenia defect. J Biol Chem 1993;268(20):14586-9.
13. Siess W. Molecular mechanisms of platelet activation. Physiol Rev 1989;69(1):58-178.
14. 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(6):1498-503.
15. Thiagarajan P, Tait JF. Collagen-induced exposure of anionic phospholipid in platelets and platelet-derived microparticles. J Biol Chem 1991;266(36):24302-7.
16. Abrams CS, Ellison N, Budzynski AZ, Shattil SJ. Direct detection of activated platelets and platelet-derived microparticles in humans. Blood 1990;75(1):128-38.
17. Wencel-Drake JD, Dieter MG, Lam SC. Immunolocalization of beta 1 integrins in platelets and platelet-derived microvesicles. Blood 1993;82(4):1197-203.
18. de Bruijne-Admiraal LG, Modderman PW, Von dem Borne AE, Sonnenberg A. P-selectin mediates Ca(2+)-dependent adhesion of activated platelets to many different types of leukocytes: detection by flow cytometry. Blood 1992;80(1):134-42.
19. Larsen E, Celi A, Gilbert GE, Furie BC, Erban JK, Bonfanti R, et al. PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell 1989;59(2):305-12.
20. Mayadas TN, Johnson RC, Rayburn H, Hynes RO, Wagner DD. Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. Cell 1993;74(3):541-54.
21. Siljander P, Carpen O, Lassila R. Platelet-derived microparticles associate with fibrin during thrombosis. Blood 1996;87(11):4651-63.
22. Haluska CK, Riske KA, Marchi-Artzner V, Lehn JM, Lipowsky R, Dimova R. Time scales of membrane fusion revealed by direct imaging of vesicle fusion with high temporal resolution. Proc Natl Acad Sci USA 2006;103(43):15841-6.
23. Gruber R, Varga F, Fischer MB, Watzek G. Platelets stimulate proliferation of bone cells: involvement of platelet-derived growth factor, microparticles and membranes. Clin Oral Implants Res 2002;13(5):529-35.
24. Owens MR, Holme S, Cardinali S. Platelet microvesicles adhere to subendothelium and promote adhesion of platelets. Thromb Res 1992;66(2-3):247-58.

25. Chirinos JA, Velazquez H, Canoniero M, Soriano AO, Bernal-Mizrachi L, Jy W, et al. Levels of GP-IIb/IIIa positive platelet microparticles (PMP) but not CD31+/CD42+ PMP predict adverse outcomes in troponin-negative patients with chest pain. Blood 2004;104(11 Pt 1):2583.
26. Boukerche H, Berthier-Vergnes O, Bailly M, Dore JF, Leung LL, McGregor JL. A monoclonal antibody (LYP18) directed against the blood platelet glycoprotein IIb/IIIa complex inhibits human melanoma growth in vivo. Blood 1989;74(3): 909-12.
27. Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. Science 1987;238(4826):491-7.
28. Salanova B, Choi M, Rolle S, Wellner M, Luft FC, Kettritz R. Beta2-integrins and acquired glycoprotein IIb/IIIa (GPIIb/IIIa) receptors cooperate in NF-kappaB activation of human neutrophils. J Biol Chem 2007;282(38):27960-9.
29. Fidler IJ, Raz A, Fogler WE, Kirsh R, Bugelski P, Poste G. Design of liposomes to improve delivery of macrophage-augmenting agents to alveolar macrophages. Cancer Res 1980; 40(12):4460-6.
30. Schroit AJ, Fidler IJ. Effects of liposome structure and lipid composition on the activation of the tumoricidal properties of macrophages by liposomes containing muramyl dipeptide. Cancer Res 1982;42(1):161-7.
31. Mehta K, Lopez-Berestein G, Hersh EM, Juliano RL. Uptake of liposomes and liposome-encapsulated muramyl dipeptide by human peripheral blood monocytes. J Reticuloendothel Soc 1982;32(2):155-64.
32. Ratner S, Schroit AJ, Vinson SB, Fidler IJ. Analogous recognition of phospholipids by insect phagocytes and mammalian macrophages. Proc Soc Exp Biol Med 1986;182(2):272-6.
33. Allen TM, Williamson P, Schlegel RA. Phosphatidylserine as a determinant of reticuloendothelial recognition of liposome models of the erythrocyte surface. Proc Natl Acad Sci USA 1988;85(21):8067-71.
34. Mietto L, Boarato E, Toffano G, Bruni A. Internalization of phosphatidylserine by adherent and non-adherent rat mononuclear cells. Biochim Biophys Acta 1989;1013(1):1-6.
35. Tanaka Y, Schroit AJ. Insertion of fluorescent phosphatidylserine into the plasma membrane of red blood cells. Recognition by autologous macrophages. J Biol Chem 1983;258(18): 11335-43.
36. Hebbel RP, Miller WJ. Phagocytosis of sickle erythrocytes: immunologic and oxidative determinants of hemolytic anemia. Blood 1984;64(3):733-41.
37. Schwartz RS, Tanaka Y, Fidler IJ, Chiu DT, Lubin B, Schroit AJ. Increased adherence of sickled and phosphatidylserine-enriched human erythrocytes to cultured human peripheral blood monocytes. J Clin Invest 1985;75(6):1965-72.
38. Connor J, Pak CC, Schroit AJ. Exposure of phosphatidylserine in the outer leaflet of human red blood cells. Relationship to cell density, cell age, and clearance by mononuclear cells. J Biol Chem 1994;269(4):2399-404.
39. Pradhan D, Williamson P, Schlegel RA. Phosphatidylserine vesicles inhibit phagocytosis of erythrocytes with a symmetric transbilayer distribution of phospholipids. Mol Membr Biol 1994;11(3):181-7.
40. Sambrano GR, Parthasarathy S, Steinberg D. Recognition of oxidatively damaged erythrocytes by a macrophage receptor with specificity for oxidized low density lipoprotein. Proc Natl Acad Sci USA 1994;91(8):3265-9.
41. Sambrano GR, Steinberg D. Recognition of oxidatively damaged and apoptotic cells by an oxidized low density lipoprotein receptor on mouse peritoneal macrophages: role of membrane phosphatidylserine. Proc Natl Acad Sci USA 1995;92(5): 1396-400.
42. Fadok VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, Henson PM. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. J Immunol 1992;148(7):2207-16.
43. Fadok VA, Savill JS, Haslett C, Bratton DL, Doherty DE, Campbell PA, Henson PM. Different populations of macrophages use either the vitronectin receptor or the phosphatidylserine receptor to recognize and remove apoptotic cells. J Immunol 1992;149(12):4029-35.
44. Schlegel RA, Stevens M, Lumley-Sapanski K, Williamson P. Altered lipid packing identifies apoptotic thymocytes. Immunol Lett 1993;36(3):283-8.
45. Mower DA Jr, Peckham DW, Illera VA, Fishbaugh JK, Stunz LL, Ashman RF. Decreased membrane phospholipid packing and decreased cell size precede DNA cleavage in mature mouse B cell apoptosis. J Immunol 1994;152(10):4832-42.
46. Koopman G, Reutelingsperger CP, Kuijten GA, Keehnen RM, Pals ST, van Oers MH. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood 1994;84(5):1415-20.
47. Bennett MR, Gibson DF, Schwartz SM, Tait JF. Binding and phagocytosis of apoptotic vascular smooth muscle cells is mediated in part by exposure of phosphatidylserine. Circ Res 1995;77(6):1136-42.
48. Homburg CH, de Haas M, von dem Borne AE, Verhoeven AJ, Reutelingsperger CP, Roos D. Human neutrophils lose their surface Fc gamma RIII and acquire Annexin V binding sites during apoptosis in vitro. Blood 1995;85(2):532-40.
49. Martin SJ, Reutelingsperger CP, McGahon AJ, Rader JA, van Schie RC, LaFace DM, Green DR. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. J Exp Med 1995;182(5): 1545-56.
50. Shiratsuchi A, Umeda M, Ohba Y, Nakanishi Y. Recognition of phosphatidylserine on the surface of apoptotic spermatogenic cells and subsequent phagocytosis by Sertoli cells of the rat. J Biol Chem 1997;272(4):2354-8.
51. Pradhan D, Krahling S, Williamson P, Schlegel RA. Multiple systems for recognition of apoptotic lymphocytes by macrophages. Mol Biol Cell 1997;8(5):767-78.
52. Fadok VA, Xue D, Henson P. If phosphatidylserine is the death knell, a new phosphatidylserine-specific receptor is the bellringer. Cell Death Differ 2001;8(6):582-7.
53. Janowska-Wieczorek A, Majka M, Kijowski J, Baj-Krzyworzeka M, Reca R, Turner AR, et al. Platelet-derived microparticles bind to hematopoietic stem/progenitor cells and enhance their engraftment. Blood 2001;98(10):3143-9.
54. Jy W, Mao WW, Horstman L, Tao J, Ahn YS. Platelet microparticles bind, activate and aggregate neutrophils in vitro. Blood Cells Mol Dis 1995;21(3):217-31.
55. Merten M, Pakala R, Thiagarajan P, Benedict CR. Platelet microparticles promote platelet interaction with subendothelial matrix in a glycoprotein IIb/IIIa-dependent mechanism. Circulation 1999;99(19):2577-82.
56. Forlow SB, McEver RP, Nollert MU. Leukocyte-leukocyte interactions mediated by platelet microparticles under flow. Blood 2000;95(4):1317-23.
57. Kim HK, Song KS, Chung JH, Lee KR, Lee SN. Platelet microparticles induce angiogenesis in vitro. Br J Haematol 2004; 124(3):376-84.
58. 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(9): 2118-27.
59. Martinez MC, Tesse A, Zobairi F, Andriantsitohaina R. Shed membrane microparticles from circulating and vascular cells

in regulating vascular function. Am J Physiol Heart Circ Physiol 2005;288(3):H1004-9.
60. Nomura S, Suzuki M, Kido H, Yamaguchi K, Fukuroi T, Yanabu M, et al. Differences between platelet and microparticle glycoprotein IIb/IIIa. Cytometry 1992;13(6):621-9.
61. Boulanger CM, Scoazec A, Ebrahimian T, Henry P, Mathieu E, Tedgui A, Mallat Z. Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. Circulation 2001;104(22):2649-52.
62. VanWijk MJ, VanBavel E, Sturk A, Nieuwland R. Microparticles in cardiovascular diseases. Cardiovasc Res 2003;59(2): 277-87.
63. Shattil SJ, Brass LF. Induction of the fibrinogen receptor on human platelets by intracellular mediators. J Biol Chem 1987; 262(3):992-1000.
64. Brader S, Eccles SA. Phosphoinositide 3-kinase signalling pathways in tumor progression, invasion and angiogenesis. Tumori 2004;90(1):2-8.
65. Qi JH, Matsumoto T, Huang K, Olausson K, Christofferson R, Claesson-Welsh L. Phosphoinositide 3 kinase is critical for survival, mitogenesis and migration but not for differentiation of endothelial cells. Angiogenesis 1999;3(4):371-80.