[Frontiers in Bioscience 11, 830-837, January 1, 2006]

Platelet-leukocyte aggregates and derived microparticles in inflammation, vascular remodelling and thrombosis

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1. ABSTRACT

As a result of vascular injury, activated platelets will rapidly interact with circulating platelets, via membrane glycoprotein complex alphaIIbbeta3 (GPIIb-IIIa) and fibrinogen, to form a thrombus or a plug preventing fatal bleeding. In addition, platelets interacting with ruptured atherosclerotic plaques or with the surface of diseased vessels can aggregate and induce ischemia that prevents blood flow. However, increasing evidence has also shown that circulating platelets interact with leukocytes and endothelial cells, via specific adhesion molecules, in inflammation, vascular remodelling and thrombosis. The aim of this chapter is to present the importance of cell-cell interactions involving platelets and leukocytes in events related to inflammation, coagulation, vascular remodelling and thrombosis. A key adhesion molecule implicated in platelet interaction with leukocytes is P-selectin, also known as CD62P. It is present on activated platelets and endothelial cells, and its counterpart on leukocytes is known as P-selectin glycoprotein ligand-1 (PSGL-1). A critical co-factor leading to leukocyte activation in platelet-monocyte aggregate formation is the presence of a chemokine known as RANTES. It acts in concert with platelet P-selectin and PSGL-1 in monocyte stimulation.

2. ADHESION MOLECULES

Activated degranulated platelets not only interact with platelets (homotypic cell-cell interactions) but also with circulating leukocytes (heterotypic cell-cell interactions). Adhesion molecules implicated in homotypic interactions are dependent on alphaIIbbeta3 (GPIIb-IIIa) glycoprotein complex and ligands such as fibrinogen. In contract, homotypic interactions are initially mediated by P-selectin (CD62P) expressed by activated platelets and its ligand PSGL-1 present on circulating leukocytes. At a later stage alphaIIbbeta3and fibrinogen will serve as a link between activated platelets and leukocytes via activated leukocyte CD11b/CD18 (Mac-1). Such CD11b/CD18 activation and up-regulation is mediated by the docking of P-selectin to its ligand PSGL-1 (1, 2). P-selectin, a member of the selectin family and adhesion molecules, is present in the 1-granules of resting platelets and expressed on the surface of activated platelets. Other selectin family members are E-selectin and L-selectin. This adhesion molecule is composed of an N-terminal lectin-like domain that is calcium-dependent, an epidermal like-growth factor domain and a series of complement-like repeats domain with a cytoplasmic tail (3). It is of interest to note that P-selectin has also been identified in a soluble form where the

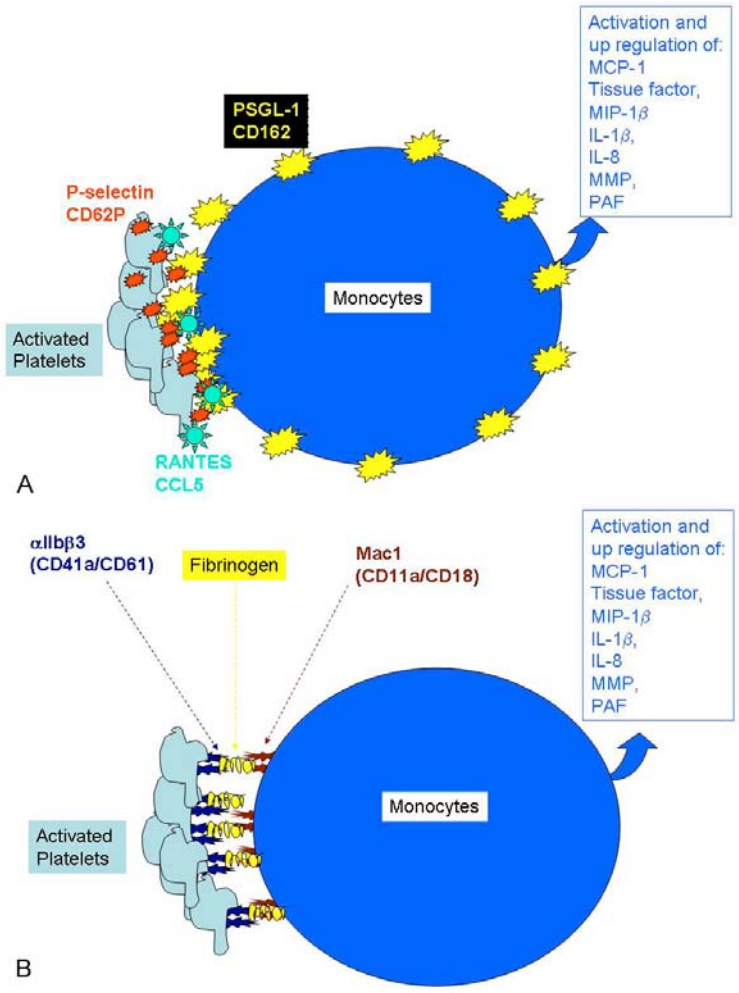

Figure 1. Activated platelet-monocyte aggregate. (1A) Activated platelets interact with monocytes via P-selectin and PSGL-1 (expressed on the surface of circulating monocytes). RANTES, released by platelets, is vital for monocytes activation and cell-cell aggregate formation.(1B) Platelet-monocyte aggregates can be further cemented, at a second stage, by other adhesion molecules such as alphaIIbbeta3 (GPIIb-IIIa) on platelets and CD11b-CD18 on monocytes and a ligand such as fibrinogen. Activated monocytes in platelet-monocyte aggregates will express or release MCP-1, Tissue factor, MIP-1b, IL-1b, IL-8, MMP-9 and PAF.

cytoplasmic tail is absent(4, 5). The role of this soluble form of P-selectin remains to be identified.

3. CHEMOKINES

Stimulated platelets express a higher density of P-selectin on their surface for several hours compared to endothelial cells(6,7). As a result, activated platelet can interact with leukocytes at sites of inflammation and thrombosis for prolonged periods of time. During the process of inflammation, stimulated platelets will express P-selectin and release RANTES (a CC chemokine first detected in T lymphocytes) also known as CCL5 (9). RANTES is found in large amounts in the supernatants from activated platelets, it is a chemoattractant for monocytes and its receptor is expressed on monocytic cells (10, 11, 12). RANTES in concert with platelet P-selectin can induce monocyte chemotactic protein-1 (MCP-1) and IL-8 secretion by monocytes. However, binding of P-selectin to PSGL-1 on monocytes will not induce secretion of monocyte chemokines without RANTES. It has been shown that released RANTES will induce signaling and secretion of monocytes as soon as P-selectin binds to these cells. Platelets can also donate RANTES to endothelial cells for monocyte activation (13). RANTES can be deposited on endothelial cells by a transient adhesion between platelets, their P-selectin and endothelial cells. Thus, RANTES plays a key role in inducing chemokine secretion by monocytes in inflammatory lesions *in vivo*. Moreover, it has been shown that patients with inflammatory bowel disease have an exaggerated platelet activity with increased levels of P-selectin and RANTES (14). In these patients, aspirin medication was found beneficial in lowering their plasma RANTES. Furthermore, RANTES was also found increased in serum and urine of human renal allograft recipients in the early post-transplantation period. This increase of RANTES reflected an early activated immune system (15) (Figure 1 A).

4. INFLAMMATION

P-selectin expressed by activated endothelial cells has been shown to be implicated in the rolling of leukocytes at sites of inflammation. Moreover, the same molecule present on activated platelets also mediates the interactions with leukocytes. Indeed, platelet-leukocyte aggregates appear to play a critical role in inflammation present in vascular diseases (16). In baboons, infused, degranulated, labeled platelets have shown to very rapidly form aggregates with monocytes and neutrophils. Such platelet-monocyte aggregates persist in circulation for a substantial period of time and may potentially induce vascular remodelling. Indeed. Sarma J *et al* suggest that such circulating monocytes binding to activated platelets may potentially have a proatherogenic phenotype (17). Indeed, leukocytes binding to activated platelets have shown increased levels of proinflammatory mediators such as IL-1 beta, IL-8, MCP-1, macrophage inflammatory protein-1ß (MIP-1ß) and matrix metalloproteinases (18). Moreover, activated platelets, via P-selectin, will induce monocytes to express tissue factor , enhance phagocytosis, generate superoxide anion and release PAF (19, 20). Moreover, platelet P-selectin with RANTES can significantly increase cytokine levels in monocytes (21) (Figure 1B).

5. PLATELET-LEUKOCYTE AGGREGATES IN PATIENTS

A high number of platelet-monocyte aggregates were observed in patients following percutaneous coronary intervention or in acute myocardial infarction (22). Platelet-monocyte aggregates are significantly increased in smokers compared to non-smokers (23). Furthermore, increased platelet-monocyte aggregates are also observed in patients with end-stage renal failure, patients with enhanced risk of cardiovascular diseases, such as unstable angina , myocardial infarction or following angioplasty (24, 25, 26). Patients with unstable angina, when compared to patients with stable angina, show a number of markers of inflammation, such as C-reactive protein, P-selectin, Interleukin 6, tumour necrosis factor-alpha, soluble intracellular adhesion molecule-1, as well as platelet-monocyte aggregates (27).

6. THERAPEUTIC TREATMENT TO INHIBIT PLATELET-MONOCYTE AGGREGATES

A number of therapeutic molecules have been used to investigate the inhibition of platelet-monocyte aggregates as they have a potential role in inflammation, vascular remodeling and thrombosis. Clopidogrel has been shown to significantly reduce ex-vivo formation of such aggregates, inhibit P-selectin expression on activated platelets via a thrombin receptor activating peptide or ADP. In contrast, abciximab (a platelet glycoprotein alphaIIbbeta3antagonist) did not significantly reduce the formation of platelet-monocyte aggregates (28). However, *in vitro* platelet-monocyte aggregates formation was inhibited by blocking alphaIIbbeta3 or P-selectin via specific antagonists (29). Clopidogrel, but not aspirin, was observed in patients with atherosclerotic diseases to greatly reduce platelet-monocyte aggregates and P-selectin expression (30). In a very recent publication, Weyrich AS *et al* have investigated the mechanisms by which Dipyridamole inhibits inflammatory gene expression in platelet-monocyte aggregates (31). They show that Dipyridamole, but not aspirin, blocked the transcription of MCP-1 in monocytes aggregated to thrombin-activated platelets. Moreover, it delayed maximal synthesis of IL-8 and regulated matrix metalloproteinase-9 (MMP-9) formation.

7. CIRCULATING MICROPARTICLES IN PATIENTS

Debris of cellular membrane, or membrane vesicles, is released by cells in an apoptotic state or alternatively when cells are activated as observed in blood platelets. Circulating microparticles can be a relatively good marker of programmed cell death, under *in vitro* or *in vivo* conditions, following HIV infection (32). These microparticles may originate from platelets, endothelial cells, monocytes, erythrocytes, lymphocytes and other cells present in circulation or lining the vessel wall. Microparticles express negatively charged phospholipids (aminophospholipid PS), and may also express tissue factor if derived from cells such as monocytes and participate in the generation of fibrin in coagulation. Indeed, these microparticles act a platform that supports the generation of activated clotting enzymes. However, thrombogenicity of microparticles is directly associated with the presence of their tissue factor (33). Increased levels of circulating microparticles are associated with quite a large number of diseases implicating vascular, inflammatory and/or coagulation problems (34). Patients undergoing cardiopulmonary bypass or suffering from rheumatoid arthritis, preeclampsia or multiple organs dysfunction syndrome and sepsis have circulating microparticles that are highly procoagulant (35, 36, 37, 38). Microparticles coagulant activity did not differ between patients with stable angina compared to non coronary patients. However, the number of these procoagulant particles, originating from endothelial cells, was significantly increased in patients with acute coronary syndrome patients compared to other coronary or noncoronary patients (39). The number and coagulability of circulating microparticles in type 1 diabetic patients was observed to be higher than in type 2 diabetic patients. In these patients, increased number of microparticles (originating from platelets and endothelial cells) with enhanced coagulability appears to be implicated in vascular complications (40) (Figure 2A-B).

8. MICROPARTICLES AND FIBRIN FORMATION

Inhibition of P-selectin access to a Dacron graft implanted within an arteriovenous shunt, by antibodies, has prevented the accumulation of fibrin and the adhesion of leukocytes. These first results strongly suggested that P-selectin was not only linked to leukocyte adhesion but also to fibrin deposition (41). This association of P-selectin and fibrin is further confirmed with results showing that deficiency of P-selectin in mice, amputated at the tip of

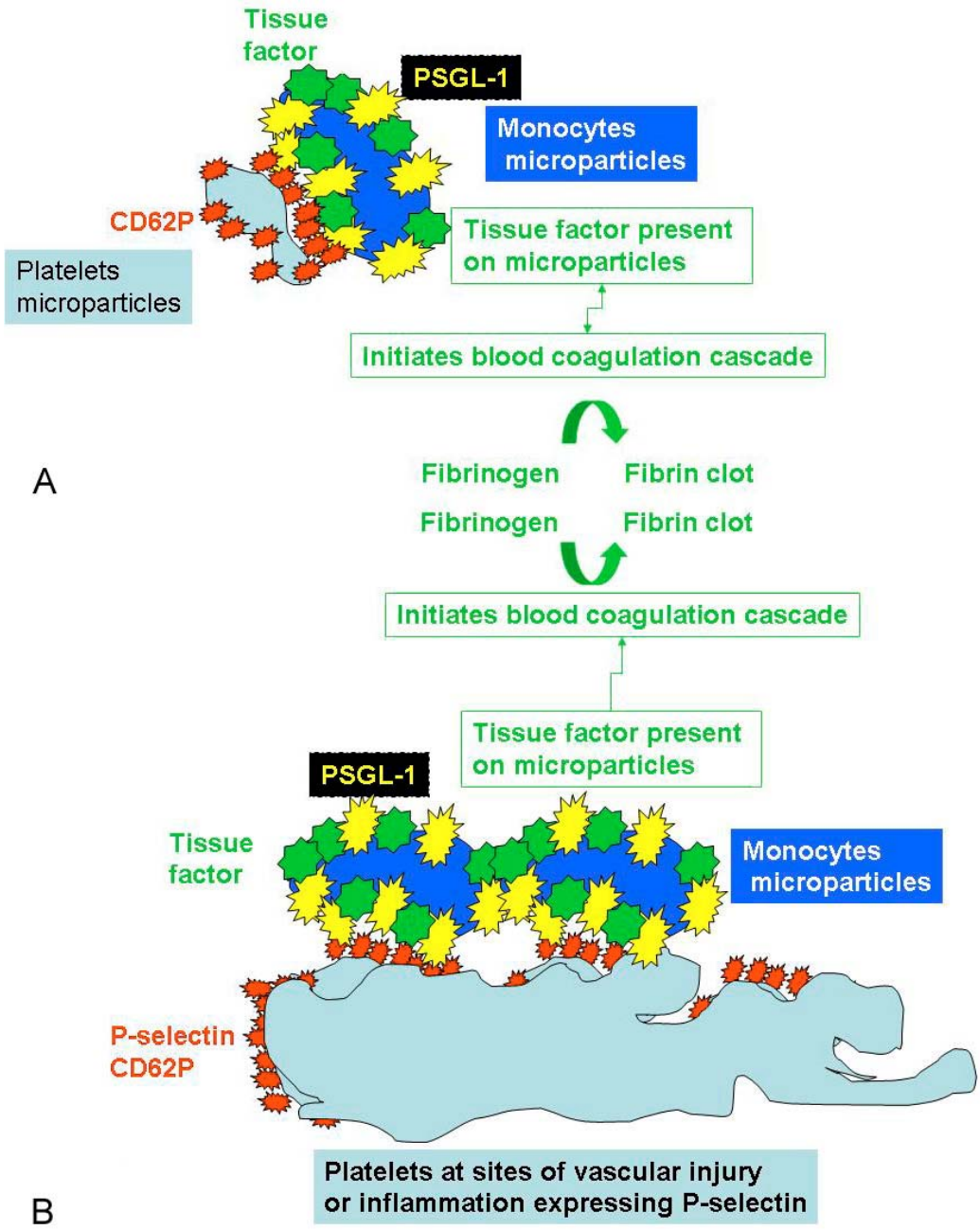

Figure 2. Platelet and monocyte derived microparticles interacting to promote fibrin generation in the coagulation cascade. A: Microparticles derived from monocytes and bearing PSGL-1 and tissue factor will bind to microparticles derived from platelets or endothelial cells expressing P-selectin. Such microparticles aggregates will induce the generation of fibrin. B: Microparticles derived from monocytes and bearing PSGL-1 and tissue factor will bind to activated platelets present at a site of vascular injury or inflammation and induce the generation of fibrin.

heir tail, had a severe prolongation of their bleeding time (42). Moreover, increased soluble P-selectin (lacking the cytoplasmic tail) levels, induced in genetically engineered mice, had a higher capacity for fibrin deposition compared to wild type mice. No fibrin deposition was observed in P-selectin deficient mice (43). These studies showed a definite link between P-selectin and coagulation. The role of P-selectin in coagulation was further investigated in studies of microparticles and tissue factor. Previous work reported the presence of tissue factor in circulation and showed that activated platelets are coated with tissue factor, in the presence of monocyte microparticles, and that this interaction is mediated by CD15 (44). Injecting recombinant P-selectin and immunoglobulin (P-sel-Ig) into mice with haemophilia, has induced a very significant increase in microparticles bearing tissue factor and a

normalized their bleeding time . Presumable, P-sel-Ig bound to circulating monocyte derived microparticles bearing PSGL-1 and tissue factor and such combined P-selectin-PSGL-1 microparticles increased fibrin formation and normalized the bleeding time in these haemophilic mice. Moreover, the role of P-selectin in fibrin formation was further studied by Falati S *et al* (46). These authors used real time *in vivo* microscopy, to demonstrate that platelet P-selectin is critical in the binding of microparticles (released from monocytes) with PSGL-1 and tissue factor. The study showed that P-selectin is co-localized with tissue factor and allows fibrin generation in a thrombus present in the arterioles of wild type mice. In contract, mice deficient in P-selectin showed a minimum amount of tissue factor and fibrin formed at sites of injury.

9. GENETICS OF P-SELECTIN AND PSGL-1

It is conceivable that single nucleotide polymorphisms (SNP) present on P-selectin and/or PSGL-1, in tandem with other SNP's present on other genes, may be associated to exacerbated inflammation and coagulation in certain individuals. Indeed, P-selectin and PSGL-1, were found to be candidates in acute coronary diseases (ACD). P-selectin is coded by a gene of 17 exons localized on chromosome 1q21 to 1q24 (47). Many studies have investigated P-selectin polymorphisms and their association with coronary diseases Indeed, Herrmann *et al* identified 13 polymorphisms linked to atherosclerosis and its complications (48). Thus, a "protective" effect against myocardial infarction (MI) was found associated to the T715P (A/C exon 13) polymorphism of P-selectin. The frequency of Pro715 alleles is less than that of Thr715 in MI patients (48, 49). Moreover, there was no significant difference in the frequency of this "protective" effect between women and men (49). In addition, this polymorphism T715P is localized in the tandem consensus repeat domain. In this domain CR4 plays a functional role in the interactions of P-selectin with leukocytes (50). Thus, T715P polymorphism could be modifying P-selectin-cell interactions but this remains to be investigated. Analysis of T715P polymorphisms in association with 4 other polymorphisms of the coding region (S290N, N562D, V599L, T741T (A/G)) were defined as specific haplotypes. Indeed, the S290N and N562D polymorphisms contribute to the increase of MI's if these three polymorphisms were associated (S290N, N562D, and T715P). S290N and N562D mutations were positioned in a consensus repeat domain which plays a role in the binding of P-selectin ligands on leukocytes. Thus, two asparagines (N290 and N562) in the protein sequence could further increase the recruitment of leukocytes to the endothelium. This would indicate that endothelial cells and leukocytes interaction via P-selectin, contributes to development of coronary heart disease and inflammatory disorders (51). Moreover, the levels of soluble P-selectin protein were associated with other two polymorphism localized in 5'-flanking region of P-selectin gene (C-2123G and A-1969G) in addition to the effect of T715P polymorphism (52). The role of soluble P-selectin levels and P-selectin polymorphisms in atherosclerosis and/or platelet activation, remain to be determined. Recently, another polymorphism in P-selectin gene was found associated to the polymorphisms of interleukin-4 gene (53). The analysis of association between atherosclerosis and polymorphisms of 56 genes related to inflammation and thrombosis has shown that Val640Leu P-selectin and C582T interleukin 4 polymorphisms appear to be as strong independent predictors of stroke. Thus, P-selectin and interleukin 4 genes, known for their inflammatory role in vascular occlusion, seem to be potential targets for the prevention of cardiovascular disease (53). In 2004, a new polymorphism was described for P-selectin gene, V168M SNP was identified in a cohort of patients affected by coronary heart disease but its role remains unclear (54).

P-selectin glycoprotein ligand-1 (PSGL-1) gene codes for the ligand of P-selectin receptor. It was found that 3 alleles (A, B, C) of PSGL-1 gene depended on the configuration of the repeat region forming the VNTR domain (55). The stability of PSGL-1 mRNA was correlated to the length of VNTR. This explains the lower levels of PSGL-1 protein observed in patients carrying alleles B and C (56). However, VNTR haplotype variability was not only associated with coronary artery diseases (CAD) and played a role in neutrophil-platelet adhesion (57). However, M62I polymorphism, located close to P-selectin binding site, was associated with PSGL-1 levels (55) and with the protective effect against CAD risks (54). An association of SNP with the functional role of P-selectin and it ligand, in binding leukocytes, will confirm the results summarized in this review (Barek L *et al*, manuscript in preparation).

10. COAT PLATELETS

The way platelets are activated may alter the expression of adhesion molecules and ligands present on the platelet surface. Such an altered expression may conceivable affect the interaction of platelets with vascular cells. Collagen and thrombin stimulated platelets (COAT) were first described in 2000 (58). It was found that activating platelets by both thrombin and collagen resulted in 30% of a cell population with high levels of procoagulant a-granule proteins on their surface such as factorV, fibrinogen, thrombospondin, fibronectin and von Willebrand factor (59, 60). These proteins are all transglutaminase substrates and are attached to the surface of COAT platelets by a transglutaminase-mediated addition of serotonin (3). Thus, fibrinogen and thrombospondin, by binding to serotonin-conjugated proteins, will provide a link for the stabilization of serotonin-derivatized, procoagulant proteins on COAT platelets. These COAT platelets appear functionally to be very different from other type of activated platelets as monoclonal antibody PAC-1 (an antibody recognizing the activated form of platelet glycoprotein IIb-IIIa) and other GPIIb-IIIa inhibitors did not inhibit their fibrinogen binding (61) . Surprisingly an amplification of COAT-platelet production could be observed in the presence of some of the GPIIb-IIIa antagonists. Finally, a long-term administration of oral GPIIb-IIIa inhibitors could even fail to protect patients and could induce an increase in coronary events (62).

11. REFERENCES

1. Moore KL, Stults NL, Diaz S, Smith DF, Cummings RD, Varki A, McEver RP.Identification of a specific glycoprotein ligand for P-selectin (CD62) on myeloid cells. *J Cell Biol* 118, 445-546 (1992)

2. Sako D, Chang XJ, Barone KM, Vachino G, White HM, Shaw G, Veldman GM, Bean KM, Ahern TJ, Furie B, *et al* Expression cloning of a functional glycoprotein ligand for P-selectin. *Cell* 5, 1179-1186 (1993)

3. Johnston GI, Cook RG, McEver RP Cloning of GMP-140, a granule membrane protein of platelets and endothelium: sequence similarity to proteins involved in cell adhesion and inflammation. *Cell* 56, 1033-1044 (1989)

4. Dunlop LC, Skinner MP, Bendall LJ, Favaloro EJ, Castaldi PA, Gorman JJ, Gamble JR, Vadas MA, Berndt MC.Characterization of GMP-140 (P-selectin) as a circulating plasma protein. *J Exp Med* 175, 1147-1150 (1992)

5. Yang J, Galipeau J, Kozak CA, Furie BC, Furie B.Mouse P-selectin glycoprotein ligand-1: molecular cloning, chromosomal localization, and expression of a functional P-selectin receptor. *Blood* 87, 4176-4186 (1996)

6. George JN, Pickett EB, Saucerman S, McEver RP, Kunicki TJ, Kieffer N, Newman PJ. Platelet surface glycoproteins. Studieson 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* 78, 340-348 (1986)

7. McEver RP, Martin MN. A monoclonal antibody to a membrane glycoprotein binds only to activated platelets. *J Biol Chem* 259, 9799-9804 (1984)

8. Rinder HM, Tracey JL, Rinder CS, Leitenberg D, Smith BR. Neutrophil but not monocyte activation inhibits P-selectin-mediated platelet adhesion. *Thromb Haemost* 72, 750-756 (1994)

9. Schall TJ, Jongstra J, Dyer BJ, Jorgensen J, Clayberger C, Davis MM, Krensky AM. A human T cell-specific molecule is a member of a new gene family. *J Immunol* 141, 1018-1025 (1988)

10. Weyrich AS, Elstad MR, McEver RP, McIntyre TM, Moore KL, Morrissey JH, Prescott SM, Zimmerman GA. Activated platelets signal chemokine synthesis by human monocytes. *J Clin Invest* 97, 1525-1534 (1996)

11. Schall TJ, Bacon K, Toy KJ, Goeddel DV. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. *Nature* 347, 669-671 (1990)

12. Murphy PM. The molecular biology of leukocyte chemoattractant receptors. *Annu Rev Immunol* 12, 593-633 (1994)

13. Schober A, Manka D, von Hundelshausen P, Huo Y, Hanrath P, Sarembock IJ, Ley K, Weber C. Deposition of platelet RANTES triggering monocyte recruitment requires P-selectin and is involved in neointima formation after arterial injury. *Circulation* 106, 1523-1529 (2002)

14. Fagerstam JP, Whiss PA, Strom M, Andersson RG. Expression of platelet P-selectin and detection of soluble P-selectin, NPY and RANTES in patients with inflammatory bowel disease. *Inflamm Res* 49, 466-472 (2000)

15. Baer PC, Koziolek M, Fierlbeck W, Geiger H. CC-Chemokine RANTES Is Increased in Serum and Urine in the Early Post-Transplantation Period of Human Renal Allograft Recipients. *Kidney Blood Press Res* 28, 48-54 (2005)

16. Furman MI, Barnard MR, Krueger LA, Fox ML, Shilale EA, Lessard DM, Marchese P, Frelinger AL 3rd, Goldberg RJ, Michelson AD Circulating monocyte-platelet aggregates are an early marker of acute myocardial infarction. *J Am Coll Cardiol* 38, 1002-1006 (2001)

17. Sarma J, Laan CA, Alam S, Jha A, Fox KA, Dransfield I.Increased platelet binding to circulating monocytes in acute coronary syndromes. *Circulation.* 105, 2166-2171 (2002)

18. Weyrich AS, Elstad MR, McEver RP, McIntyre TM, Moore KL, Morrissey JH, Prescott SM, Zimmerman GA. Activated platelets signal chemokine synthesis by human monocytes. J *Clin Invest* 97,1525-1534 (1996)

19. Celi A, Pellegrini G, Lorenzet R, De Blasi A, Ready N, Furie BC, Furie B P-selectin induces the expression of tissue factor on monocytes. *Proc Natl Acad Sci U S A* 91, 8767-8771 (1994)

20. Pennewitz A, Finkelberg L, Krause S, Losche W Platelets inhibit the activity of platelet-activating factor acetylhydrolase in monocyte-derived macrophages. *Thromb Res* 86, 427-30 (1997)

21. Neumann FJ, Marx N, Gawaz M, Brand K, Ott I, Rokitta C, Sticherling C, Meinl C, May A, Schomig A.Induction of cytokine expression in leukocytes by binding of thrombin-stimulated platelets. Circulation 95, 2387-2394 (1997)

22. Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI Circulating monocyte-platelet aggregates are a more sensitive marker of *in vivo* platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. *Circulation* 104, 1533-1537 (2001)

23. Harding SA, Sarma J, Josephs DH, Cruden NL, Din JN, Twomey PJ, Fox KA, Newby DE.Upregulation of the

CD40/CD40 ligand dyad and platelet-monocyte aggregation in cigarette smokers. *Circulation* 109, 1926-1929 (2004)

24. Ashman N, Macey MG, Fan SL, Azam U, Yaqoob MM. Increased platelet-monocyte aggregates and cardiovascular disease in end-stage renal failure patients. *Nephrol Dial Transplant* 18, 2088-2096 (2003)

25. Ott I, Neumann FJ, Gawaz M, Schmitt M, Schomig A. Increased neutrophil-platelet adhesion in patients with unstable angina. *Circulation* 94, 1239-1246 (1996)

26. Bienvenu JG, Tanguay JF, Theoret JF, Kumar A, Schaub RG, Merhi Y. Recombinant soluble p-selectin glycoprotein ligand-1-Ig reduces restenosis through inhibition of platelet-neutrophil adhesion after double angioplasty in swine. *Circulation* 103, 1128-1134 (2001)

27. Klinkhardt U, Graff J, Harder S.Clopidogrel, but not abciximab, reduces platelet leukocyte conjugates and P-selectin expression in a human *ex vivo in vitro* model. *Clin Pharmacol Ther* 71, 176-185 (2002)

28. Li N, Hu H, Lindqvist M, Wikstrom-Jonsson E, Goodall AH, Hjemdahl P. Platelet-leukocyte cross talk in whole blood. *Arterioscler Thromb Vasc Biol* 20, 2702-2708 (2000)

29. Klinkhardt U, Bauersachs R, Adams J, Graff J, Lindhoff-Last E, Harder S.Clopidogrel but not aspirin reduces P-selectin expression and formation of platelet-leukocyte aggregates in patients with atherosclerotic vascular disease. *Clin Pharmacol Ther* 73, 232-241 (2003)

30. Weyrich AS, Denis MM, Kuhlmann-Eyre JR, Spencer ED, Dixon DA, Marathe GK, McIntyre TM, Zimmerman GA, Prescott SM. Dipyridamole selectively inhibits inflammatory gene expression in platelet-monocyte aggregates. *Circulation* 111, 633-642 (2005)

31. Aupeix K, Hugel B, Martin T, Bischoff P, Lill H, Pasquali JL, Freyssinet JM.The significance of shed membrane particles during programmed cell death *in vitro*, and *in vivo*, in HIV-1 infection. *J Clin Invest* 99, 1546-1554 (1997)

32. Biro E, Sturk-Maquelin KN, Vogel GM, Meuleman DG, Smit MJ, Hack CE, Sturk A, Nieuwland R.Human cell-derived microparticles promote thrombus formation *in vivo* in a tissue factor-dependent manner. *J Thromb Haemost* 1, 2561-2568 (2003)

33. Diamant M, Tushuizen ME, Sturk A, Nieuwland R.Cellular microparticles: new players in the field of vascular disease? *Eur J Clin Invest* 34, 392-401 (2004)

34. Nieuwland R, Berckmans RJ, Rotteveel-Eijkman RC, Maquelin KN, Roozendaal KJ, Jansen PG, ten Have K, Eijsman L, Hack CE, Sturk A.Cell-derived microparticles generated in patients during cardiopulmonary bypass are highly procoagulant. *Circulation* 96, 3534-3541 (1997)

35. VanWijk MJ, Nieuwland R, Boer K, van der Post JA, VanBavel E, Sturk A.Microparticle subpopulations are increased in preeclampsia: possible involvement in vascular dysfunction? *Am J Obstet Gynecol* 187, 450-456 (2002)

36. 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* 46, 1498-503 (2002)

37. 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* 85, 810-820 (2001)

38. 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 10, 841-843 (2000)

39. Sabatier F, Darmon P, Hugel B, Combes V, Sanmarco M, Velut JG, Arnoux D, Charpiot P, Freyssinet JM, Oliver C, Sampol J, Dignat-George F Type 1 and type 2 diabetic patients display different patterns of cellular microparticles. *Diabetes* 51, 2840-2845 (2002)

40. Palabrica T, Lobb R, Furie BC, Aronovitz M, Benjamin C, Hsu YM, Sajer SA, Furie B Leukocyte accumulation promoting fibrin deposition is mediated *in vivo* by P-selectin on adherent platelets. *Nature* 359, 848-851 (1992)

41. Subramaniam M, Frenette PS, Saffaripour S, Johnson RC, Hynes RO, Wagner DD. Defects in hemostasis in P-selectin-deficient mice. *Blood* 87, 1238-1242 (1996)

42. Andre P, Hartwell D, Hrachovinova I, Saffaripour S, Wagner DD Pro-coagulant state resulting from high levels of soluble P-selectin in blood. *Proc Natl Acad Sci U S A* 97, 13835-13840 (2000)

43. Rauch U, Bonderman D, Bohrmann B, Badimon JJ, Himber J, Riederer MA, Nemerson Y.Transfer of tissue factor from leukocytes to platelets is mediated by CD15 and tissue factor. *Blood* 96, 170-175 (2000)

44. Hrachovinova I, Cambien B, Hafezi-Moghadam A, Kappelmayer J, Camphausen RT, Widom A, Xia L, Kazazian HH Jr, Schaub RG, McEver RP, Wagner DD Interaction of P-selectin and PSGL-1 generates microparticles that correct hemostasis in a mouse model of hemophilia A. *Nat Med* 9, 1020-1025 (2003)

45. Falati S, Liu Q, Gross P, Merrill-Skoloff G, Chou J, Vandendries E, Celi A, Croce K, Furie BC, Furie B.Accumulation of tissue factor into developing thrombi *in vivo* is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. *J Exp Med* 197, 1585-1598 (2003)

46. Johnston GI, Bliss GA, Newman PJ, McEver RP. Structure of the human gene encoding granule membrane protein-140, a member of the selectin family of adhesion receptors for leukocytes. *J Biol Chem* 265, 21381-2135 (1990)

47. Herrmann SM, Ricard S, Nicaud V, Mallet C, Evans A, Ruidavets JB, Arveiler D, Luc G, Cambien F. The P-selectin gene is highly polymorphic: reduced frequency of the Pro715 allele carriers in patients with myocardial infarction. Hum Mol Genet. 1998 Aug;7(8):1277-84.

48. Kee F, Morrison C, Evans AE, McCrum E, McMaster D, Dallongeville J, Nicaud V, Poirier O, Cambien F. Polymorphisms of the P-selectin gene and risk of myocardial infarction in men and women in the ECTIM extension study. Etude cas-temoin de l'infarctus myocarde. *Heart* 84, 548-552 (2000)

49. Ruchaud-Sparagano MH, Malaud E, Gayet O, Chignier E, Buckland R, McGregor JL. Mapping the epitope of a functional P-selectin monoclonal antibody (LYP20) to a short complement-like repeat (SCR 4) domain: use of human-mouse chimaera and homologue-replacement mutagenesis. *Biochem J* 332, 309-314 (1998)

50. Tregouet DA, Barbaux S, Escolano S, Tahri N, Golmard JL, Tiret L, Cambien F. Specific haplotypes of the P-selectin gene are associated with myocardial infarction. *Hum Mol Genet* 11, 2015-2023 (2002)

51. Barbaux SC, Blankenberg S, Rupprecht HJ, Francomme C, Bickel C, Hafner G, Nicaud V, Meyer J, Cambien F, Tiret L. Association between P-selectin gene polymorphisms and soluble P-selectin levels and their relation to coronary artery disease. *Arterioscler Thromb Vasc Biol* 21, 1668-73 (2001)

52. Zee RY, Cook NR, Cheng S, Reynolds R, Erlich HA, Lindpaintner K, Ridker PM. Polymorphism in the P-selectin and interleukin-4 genes as determinants of stroke: a population-based, prospective genetic analysis. *Hum Mol Genet* 13, 389-396 (2004)

53. Bugert P, Vosberg M, Entelmann M, Jahn J, Katus HA, Kluter H. Polymorphisms in the P-selectin (CD62P) and P-selectin glycoprotein ligand-1 (PSGL-1) genes and coronary heart disease. *Clin Chem Lab Med* 42, 997-1004 (2004)

54. Afshar-Kharghan V, Diz-Kucukkaya R, Ludwig EH, Marian AJ, Lopez JA. Human polymorphism of P-selectin glycoprotein ligand 1 attributable to variable numbers of tandem decameric repeats in the mucinlike region. *Blood* 97, 3306-3307 (2001)

55. Tregouet DA, Barbaux S, Poirier O, Blankenberg S, Bickel C, Escolano S, Rupprecht HJ, Meyer J, Cambien F, Tiret L. SELPLG gene polymorphisms in relation to plasma SELPLG levels and coronary artery disease. *Ann Hum Genet* 67, 504-511 (2003)

56. Lozano ML, Gonzalez-Conejero R, Corral J, Rivera J, Iniesta JA, Martinez C, Vicente V. Polymorphisms of P-selectin glycoprotein ligand-1 are associated with neutrophil-platelet adhesion and with ischaemic cerebrovascular disease. Br J Haematol 115, 969-976 (2001)

57. AlberioL, Safa O, Clemetson KJ, Esmon CT, Dale GL. Surface expression and functional characterization of alpha-granule factor V in human platelets: effects of ionophore A23187, thrombin, collagen, and convulxin. *Blood* 95, 1694-1702 (2000)

58. Szasz R, Dale GL. Thrombospondin and fibrinogen bind serotonin-derivatized proteins on COAT-platelets. *Blood* 100, 2827-2831 (2002)

59. Dale GL, Friese P, Batar P, Hamilton SF, Reed GL, Jackson KW, Clemetson KJ, Alberio L. Stimulated platelets use serotonin to enhance their retention of procoagulant proteins on the cell surface. *Nature* 415, 175-179 (2002)

60. Hamilton SF, Miller MW, Thompson CA, Dale GL. Glycoprotein IIb/IIIa inhibitors increase COAT-platelet production *in vitro*. *J Lab Clin Med* 143, 320-326 (2004)

61. Szasz R, Dale GL. COAT platelets. *Curr Opin Hematol* 10, 351-355 (2003)

Key Words: Inflammation, Platelet, Leukocyte, Microparticles, Thrombosis, Remodelling, Review

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