

Platelet-Leukocyte Interaction in Atherosclerosis and Atherothrombosis: What We Have Learnt From Human Studies and Animal Models

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ABSTRACT

Platelets, the cellular fragments of their precursor megakaryocytes, play an important role in hemostasis and other pathophysiological circumstances including atherothrombosis and atherosclerosis. Activated platelet not only can aggregate with each other to form the thrombi, but also can interact with leukocytes in the circulation to form the platelet-leukocyte aggregates (PLAs). Increased number of PLAs has been reported in patients with atherosclerosis or atherothrombosis, and animal models using different knock-out mice have unraveled the molecular mechanisms underlying the platelet leukocyte interaction. This review describes the platelet leukocyte interaction in atherosclerosis and atherothrombosis by integrating our knowledge of basic mechanisms of platelet leukocyte interaction from animal studies with the clinical studies illustrating the platelet leukocyte interaction in patients with atherosclerosis.

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Key words: Platelets; Platelet leukocyte interaction; Platelet leukocyte aggregation; Atherosclerosis; Atherothrombosis

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INTRODUCTION

Platelets, the cellular fragment of their precursor megakaryocytes, play an important role not only in hemostasis and thrombosis^[1], but also in others physiological and pathophysiological conditions including inflammation, tumor metastasis, fibrosis and atherosclerosis^[1-4]. Atherosclerosis is a chronic inflammatory process in which endothelial cells dysfunction, mononuclear cell recruitment and intrusion, and lipid dysfunction are all involved^[5-7]. Activated platelets are present in circulation of patients with unstable and stable coronary artery disease^[8,9] and has been reported to be actively involved in the pathogenesis of atherosclerosis through the inflammatory processes^[10]. Depleting platelets in circulation was able to dramatically decrease the atherosclerotic lesion size in mouse model through the mechanism of interrupting the adhesion of activated platelets to endothelium^[11]. When the atherosclerotic plaque ruptures, exposed collagen under the injured endothelium initiates the activation and aggregation of platelets to the injured endothelial cells and facilitates the thrombus formation^[10]. Therefore, platelet activation is one of the major characteristics present throughout the atherosclerotic and atherothrombosis processes.

Leukocytes, another important cellular type in circulation, are also actively involved in the pathogenesis of atherosclerosis through mechanisms including leukocytosis, leukocyte infiltration and foam cell formation^[12]. Blocking leukocyte recruitment or depleting leukocytes from circulation, particularly monocytes and neutrophils infiltration, could also successfully protect atherosclerotic lesion formation in mouse model^[13,14]. However, platelets and leukocytes do not act separately in atherogenesis. Activate platelets are not only able to conjugate with leukocytes but also able to further regulate the function of these leukocytes to facilitate atherogenesis. This review will describe the platelet leukocyte interaction in atherosclerosis and atherothrombosis by integrating our knowledge of basic mechanisms of platelet leukocyte interaction from animal studies with the clinical studies illustrating the platelet leukocyte interaction in patients with atherosclerosis.

PLATELETS ARE ACTIVATED IN ATHEROSCLEROSIS AND ATHEROTHROMBOSIS

Platelets remain in their original, inactivated state when circulating in vessels with intact endothelium, while being activated when these anucleate cells encountered with the exposed collagen under the injured endothelium. Study has shown that platelets adhere to the injured vascular endothelium before the development of atherosclerotic lesion in mice and inhibiting platelet adhesion reduced atherosclerotic lesion formation, indicating an important role of platelets in atherogenesis^[10,11]. Once the vulnerable atherosclerotic plaque ruptures, platelets are immediately activated at the site of the injured vessels and form the thrombi. Platelets from patients with hypercholesterolemic or diabetic conditions are hyper-reactive and much easier to be activated and faster in forming the thrombosis, leading to the much more severe symptoms and diffused atherothrombosis under such circumstances compared with those patients without these risk factors^[9,15-17].

PLATELET-LEUKOCYTE INTERACTION IN PATHOGENESIS OF ATHEROSCLEROSIS AND ATHEROTHROMBOSIS

Apart from aggregating with each other which is regarded as homogenous aggregation, platelets had also been reported to conjugate with leukocytes in circulation once activated, defined as a heterogenous aggregation^[18]. However, the role of these aggregates in cardiovascular diseases especially atherosclerosis was not fully understood until more than ten years after the report of increased platelet-leukocyte aggregates in circulation of patients with stroke, cardiopulmonary bypass and hemodialysis from several studies^[19-21]. The first study linking PLAs to atherosclerosis was reported by Ott *et al* showing that patients of unstable angina had increased number of PLAs in circulation compared with patients with stable angina, indicating platelet activation under the condition of a potential acute cardiovascular event^[22]. Afterward, several other studies added more evidence showing the association of the propensity of thrombosis with increased platelet leukocyte interaction in cardiovascular diseases including stable angina, unstable angina and myocardial infarction (Table 1)^[23-28]. In animal models, depleting neutrophils or platelets have been able to reduce the atherosclerotic lesion size in atherosclerosis-prone mice^[11,14], and the adhesion and infiltration of

these neutrophils are mediated by the platelet-derived chemokines CCL5^[14]. Therefore, both the human and mice data indicated an important but not fully unraveled role of platelet leukocyte interaction in atherosclerosis and atherothrombosis. PLAs are also proposed as a reliable and sensitive marker of a pro-thrombotic condition in patients with stable angina or atherosclerosis.

MOLECULES UNDERLYING PLATELET-LEUKOCYTE INTERACTION

Thanks to the studies of platelet-leukocyte interaction in other diseases using different animal models, the molecular mechanism mediating platelet leukocyte interaction has been illustrated. Platelet-leukocyte interaction can be briefly divided into three stages, namely initiation of the interaction, stabilization of the aggregates and amplification of leukocyte activation. Binding of P-selectin (CD62P) on the membrane of activated platelets to the P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes is believed to be the most important molecule to initiate the platelet leukocyte interaction^[10,29-33]. P-selectin is a protein stored in α -granules of inactivated platelets and quickly translocates to membrane from granules upon platelet activation. Cross-linking of PSGL-1 by P-selectin also primes leukocytes intracellularly for cytokine and chemoattractant-induced integrin β 2 activation to firm adhesion of leukocytes through regulating the Src family tyrosine kinase activity^[30]. The increased integrin β 2 further facilitates the interaction between leukocyte surface Mac-1 (also known as integrin α M β 2 and CD11b/CD18) and platelet surface glycoprotein Ib (GPIb)^[30,34], which is an important step to stabilize the initial PLAs formation. The initial interaction between α M β 2 and GPIb led to the generation of an outside-in, SFK-Pyk2-mediated signal to stabilize the integrin-ligand binding or induce the activity of Src family tyrosine kinase Lyn^[14,17]. The PLAs will be further stabilized through several other pairs of ligand-receptor interaction including the binding of leukocyte surface Mac-1 to the platelet surface integrin α Ib β 3 and/or to platelet surface adhesion molecule 3 (JAM-3)^[35-38], the binding of ligand on platelet surface to the triggering receptor expressed on myeloid cells 1 (TREM-1)^[39], binding of ICAM-2 on platelets to LFA-1 (CD11a/CD18) on neutrophils^[40,41], interaction between CD40L on platelets and CD40

Table 1 Current clinical studies demonstrating platelet leukocyte interaction in cardiovascular diseases.

Cardiovascular diseases or related risk factors	Type of interaction	Findings	Year	Ref
Unstable angina (UA)	PNA	(1) 4.4-fold increase of PNA in unstable angina compared with stable angina (2) PNAs have increased CD11b, an indicator of neutrophil activation.	1996	[22]
patients with CAD experiencing progression of angina	PLA	Patients with increased PLA after angiography experience late clinical events including AMI, UA, restenosis and requirement of coronary artery bypass grafting.	1996	[26]
Stable coronary artery disease (CAD)	PMA	(1) ~2.5-fold \uparrow of PMA compared with control (2) PMA further increase upon platelet activation by ADP or TRAP	1998	[27]
AMI	PMA	GpIIb/IIIa agonist Abciximab could reduce the platelet monocyte interaction and decreased the Mac-1 expression level on monocytes	1999	[38]
Acute myocardial infarction (AMI)	PMA	(1) ~ 1-fold \uparrow in AMI compared with control or unstable angina; (2) could be detected as early as 4 hours after AMI; (3) could be detected in AMI patients with normal creatine kinase levels.	2001	[28]
Diabetic woman with CAD	PNA	(1) ~50% \uparrow of PNA in diabetic woman with CAD than without CAD (2) diabetic women has significantly increased PNA after platelet activation than diabetic men	2003	[25]
Acute coronary syndromes (ACS)	PMA, PNA	(1) ~ 1-fold \uparrow of PMA and PNA in ACS compared with healthy control (2) PMA and PNA interaction could be attenuated by Clopidogrel	2005	[24]
Hyperlipidemia	PMA, PNA	PMA is 4 times higher and PNA is 2.5 times higher in patients with hyperlipidemia than normal control.	2005	[16]

PMA: platelet-monocyte aggregate; ADP: adenosine diphosphate ; TRAP: thrombin receptor agonist peptide; CAD: coronary artery disease; UA: unstable angina; AMI: acute myocardial infarction; ACS: acute coronary syndromes; ADP: adenosine diphosphate; TRAP: Thrombin receptor-activating peptide.

on monocytes^[42], and also between GpIb α on platelet and ICAM-1 on neutrophils^[43,44]. Detailed molecules mediating the platelet leukocyte interaction and their role in atherosclerosis and atherothrombosis are summarized in table 2.

CONSEQUENCES OF PLATELET-LEUKOCYTE INTERACTION IN ATHEROSCLEROSIS AND ATHEROTHROMBOSIS

About 5-10% of leukocytes are aggregated with platelets in human whole blood reported in healthy controls^[45,46] and PLAs number in animal models varies from different studies and models, ranging from 5% to 60%^[29,47]. However, platelet leukocyte interaction is a transient process which would be dissociated by various anti-platelet activation molecules in circulation like nitro oxidase (NO), matrix metalloproteinases and prostaglandin^[48], and are highly regulated by shear stress^[49]. On the other side, those leukocytes firmly aggregated with platelets will gain a more active phenotype in being recruited to the injured endothelium, and contribute to the atherosclerotic plaque formation. This step is defined as the amplification of leukocyte activation. There are many studies have demonstrated the mechanisms of how the behavior of leukocytes change once aggregated with platelets and how the activated leukocyte further activate and recruit platelets in circulation.

In human studies, platelet monocyte interaction could induce an expansion of the pool of circulating CD14^{high}CD16⁺ monocytes, a subpopulation of monocytes in human which is similar to Ly-6C^{high} monocytes in mouse^[50]. These monocytes are the major leukocyte population that enters the atherosclerotic lesion and forms foam cells. The most important evidence from mice model supporting the role of platelet leukocyte interaction in atherogenesis was provided the study carried out by Huo *et al.*^[32]. The study showed that following

a single injection of activated platelets, monocytes disappear from the circulation and increased monocyte adhesion was observed on atherosclerotic lesions in carotid arteries, indicating that platelet-monocyte aggregation is one of the ways in which circulating activated platelets may participate in the formation of atherosclerotic lesions^[32].

Besides regulating monocytoysis and the interaction with monocyte to facilitate the atherosclerosis development, platelets could also reprogram the leukocytes to gain pro-atherosclerotic phenotype. For example, monocytes attached with platelets have increased cell surface CD11b or vascular cell adhesion molecule-1 (VCAM-1) levels. These adhesions molecule facilitate the monocyte interaction with injured endothelium and further infiltration^[32,37]. Activated platelets can also deliver the chemokines or pro-inflammatory factors including RANTES (CCL5) and PF4 (CXC motif ligand 4, CXCL4) to leukocytes/monocytes which further facilitates the adhesion of leukocytes to atherosclerotic lesions^[32]. Platelet-monocyte aggregates could further support the monocyte adhesion to endothelium by enhancing the secondary tethering and cluster formation through up-regulating L-selectin and P-selectin^[51]. When platelets are aggregated with neutrophils, these neutrophils could gain enhanced ability of transmigration in response to oxidized low-density lipoprotein (oxLDL) in a PI3K-dependent manner^[52]. As an important factor in atherosclerosis, oxLDL could also increase the interaction between platelet and monocyte, in a process that involved platelet CD36-OxLDL interaction, release of chemokines such as CXCL4, direct platelet-monocyte interaction, and phagocytosis of platelets^[53]. In summary, the aggregation between platelets and monocyte promotes leukocytosis, leukocyte infiltration and extravasation, and enhanced foam cell formation, indicate a role of PLA in atherosclerotic plaque development and plaque destabilization^[53].

As the plaque ruptures, not only platelets are recruited to the injured endothelium to form the thrombi, leukocytes also actively participate in the thrombi formation through their interaction with

Table 2 Important molecules mediating platelet leukocyte interaction and platelet leukocyte interaction in atherosclerosis and atherothrombosis.

Surface molecule of platelet	Surface molecule of leukocytes	Type of leukocytes	Role in atherosclerosis and atherothrombosis	Ref
P-selectin	PSGL-1	Neutrophils, Monocytes, Th1 cells	(1) platelet P-selectin-mediated delivery of platelet-derived proinflammatory factors (CXCL4, CCR5) to monocytes/leukocytes and the vessel wall to facilitate atherosclerosis lesion development. (2) induce the expression of tissue factor on monocytes and promotes fibrin deposition within a growing thrombus at sites of vascular injury.	[31-33]
ICAM-2	LFA-1 (CD11a/CD18)	Neutrophils	(1) Induce neutrophil transendothelial migration (2) stabilize the platelet neutrophil interaction	[40,41]
CD40L	CD40	Monocytes	(1) CD40L plays a role in accelerating plaque development and early stages of atherosclerosis, and promoting progression toward advanced atherosclerosis. (2) CD40L facilitates platelet-leukocyte interactions and mediates leukocyte recruitment via CCL2 (MCP-1). (3) Induce monocyte activation which consequently produce of cytokines, induce chemokine secretion, and up-regulate adhesion molecules, leading to recruitment to and extravasation of leukocytes at the site of injury	[40-42]
GpIb α / fibrin	Mac-1 (CD11b/CD18)	Neutrophils, Monocytes	(1) through "outside-in" signaling by Src-family tyrosine kinases (SFKs) to stabilize the of PLA (2) facilitate the interaction between PLA and injured endothelium	[14,17,30,34]
GpIb α	ICAM-1	Neutrophils	(1) Firm adhesion and migration of leukocytes (2) stabilize the platelet neutrophil interaction	[43,44]
GpIIb/IIIa	Mac-1	Neutrophils	(1) mediating and stabilize platelet neutrophil interaction (2) GpIIb/IIIa receptor blocks platelet-leukocyte interaction and decreased surface expression of the leukocyte integrin Mac-1 in acute myocardial infarction	[36-38]
TREM-1L	TREM-1	Neutrophils	TREM-1/ligand interaction contributes to the amplification of leukocytes	[39]
JAM-3	Mac-1	Neutrophils, Lymphocytes	Mediating platelet neutrophil interaction which could be blocked by purified JAM-3 or antibodies	[35]

PMA: platelet-monocyte aggregate; ADP: adenosine diphosphate ; TRAP: thrombin receptor agonist peptide; CAD: coronary artery disease; UA: unstable angina; AMI: acute myocardial infarction; ACS: acute coronary syndromes; ADP: adenosine diphosphate; TRAP: Thrombin receptor-activating peptide.

platelets. Activated leukocytes attached with platelets can recruit circulating activated platelets through P-selectin/PSGL-1 interactions and contribute to further platelet activation through cathepsin G and fibrin deposition, which helps to form the firm atherosclerotic thrombi^[44]. On the other side, activated platelets which have already attached on the injured endothelial will attract more leukocytes and the fibrin-collagen matrix would trap red blood cells from circulation to completely block the vessel lumen^[11,14].

CHALLENGES IN EVALUATING PLAS IN CIRCULATION OF PATIENTS WITH CORONARY ARTERY DISEASES

Based on the observation of increased PLAs in circulation of patients with cardiovascular diseases including stable angina, unstable angina and myocardial infarction, as well as the supporting mechanistic studies from animal models, PLAs could be a promising and sensitive marker to screen the patients with potential risk of developing atherothrombosis. Therefore, accurately measuring the PLAs in circulation is of vital importance which requires careful sample handling and standard protocols since factors including how long the blood sample is process after collection, the anticoagulant used, the way to lyse and fix the samples, the flow cytometer used all contribute to the final result of the PLAs^[45,46]. Compared with EDTA, sodium citrate and PPACK, heparin has the least artificial effect on the PLAs^[46]. Lysing red blood cell and fixation do not impact the final PLAs count in circulation a lot^[45,46]. However, samples should be processed as soon as possible, preferentially within 12-24 hour after the collection since the longer the sample is sitting on the bench, the higher the PLAs number would be^[45]. Sample processing should be always kept on ice or centrifuged at 4°C to avoid the unnecessary activation of leukocytes.

Cost-effectiveness of this flow cytometry-based test including the cost of experimental reagent, the flow cytometer and operator training, might be another challenge. However, with careful experimental design to titrate the concentration of commercial available antibodies, the cost of reagent especially antibodies could be reduced by more than 50% according to our experience. The cost of flow cytometer which was earlier an experimental device in general might be another consideration of clinicians to choose such a test or not. However, technological advances have enabled widespread applications for the use of flow cytometer in clinical purposes especially in large hospital and institutes with basic research facilities. With sharing the instrumental and expertise sources, the cost-effectiveness of such a test could be dramatically decreased and more practical to be performed.

SUMMARY

As two major cell types that contribute to the pathogenesis of atherosclerosis and atherothrombosis, interaction between platelets and leukocytes are of great interesting not only because that increased number of PLAs have been found in circulation of patients with atherosclerosis and its complications, but also because of the increased number of animal studies unraveling the mechanisms underlying the interaction. Although a great effort is still needed to clarify the mechanisms of PLAs in atherosclerosis and atherothrombosis, using PLA as a marker for platelet and/or leukocyte activation and for evaluating the risk of developing atherothrombosis holds great promise. Since several small scale human studies have pointed out a potential benefit of using commercially available anti-platelet medicines in reducing platelet leukocyte interaction^[24, 38] and

the role of PLA in atherosclerosis development and atherothrombosis has been gradually unraveled, PLA would also be an interesting therapeutic target to reduce the atherosclerosis development and atherothrombosis.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

REFERENCES

- 1 Wagner DD, Burger PC. Platelets in inflammation and thrombosis. *Arterioscler Thromb Vasc Biol* 2003; **23**: 2131-2137
- 2 Meyer A, Wang W, Qu J, Croft L, Degen JL, Collier BS, Ahamed J. Platelet tgf-beta1 contributions to plasma tgf-beta1, cardiac fibrosis, and systolic dysfunction in a mouse model of pressure overload. *Blood* 2012; **119**: 1064-1074
- 3 von Hundelshausen P, Weber C. Platelets as immune cells: Bridging inflammation and cardiovascular disease. *Circ Res* 2007; **100**: 27-40
- 4 Karpatkin S, Pearlstein E. Role of platelets in tumor cell metastases. *Ann Intern Med* 1981; **95**: 636-641
- 5 Wang W, Peng W, Zhang X, Lu L, Zhang R, Zhang Q, Wang L, Chen Q, Shen W. Chromosome 9p21.3 polymorphism in a chinese han population is associated with angiographic coronary plaque progression in non-diabetic but not in type 2 diabetic patients. *Cardiovasc Diabetol* 2010; **9**: 33
- 6 Wang Y, Wang GZ, Rabinovitch PS, Tabas I. Macrophage mitochondrial oxidative stress promotes atherosclerosis and nuclear factor-kappab-mediated inflammation in macrophages. *Circ Res* 2014; **114**: 421-433
- 7 Liao X, Sluimer JC, Wang Y, Subramanian M, Brown K, Pattison JS, Robbins J, Martinez J, Tabas I. Macrophage autophagy plays a protective role in advanced atherosclerosis. *Cell Metab* 2012; **15**: 545-553
- 8 Fitzgerald DJ, Roy L, Catella F, FitzGerald GA. Platelet activation in unstable coronary disease. *N Engl J Med* 1986; **315**: 983-989
- 9 Ferroni P, Basili S, Falco A, Davi G. Platelet activation in type 2 diabetes mellitus. *J Thromb Haemost* 2004; **2**: 1282-1291
- 10 Davi G, Patrono C. Platelet activation and atherothrombosis. *N Engl J Med* 2007; **357**: 2482-2494
- 11 Massberg S, Brand K, Gruner S, Page S, Muller E, Muller I, Bergmeier W, Richter T, Lorenz M, Konrad I, Nieswandt B, Gawaz M. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. *J Exp Med* 2002; **196**: 887-896
- 12 Westerterp M, Bochem AE, Yvan-Charvet L, Murphy AJ, Wang N, Tall AR. Atp-binding cassette transporters, atherosclerosis, and inflammation. *Circ Res* 2014; **114**: 157-170
- 13 Yvan-Charvet L, Pagler T, Gautier EL, Avagyan S, Siry RL, Han S, Welch CL, Wang N, Randolph GJ, Snoch HW, Tall AR. Atp-binding cassette transporters and hdl suppress hematopoietic stem cell proliferation. *Science* 2010; **328**: 1689-1693
- 14 Drechsler M, Megens RT, van Zandvoort M, Weber C, Soehnlein O. Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. *Circulation* 2010; **122**: 1837-1845
- 15 Broijersens A, Hamsten A, Eriksson M, Angelin B, Hjendahl P. Platelet activity in vivo in hyperlipoproteinemia--importance of combined hyperlipidemia. *Thromb Haemost* 1998; **79**: 268-275
- 16 Sener A, Ozsavci D, Oba R, Demirel GY, Uras F, Yardimci KT. Do platelet apoptosis, activation, aggregation, lipid peroxidation and platelet-leukocyte aggregate formation occur simultaneously in hyperlipidemia? *Clin Biochem* 2005; **38**: 1081-1087
- 17 Bornfeldt KE. 2013 russell ross memorial lecture in vascular biology: Cellular and molecular mechanisms of diabetes mellitus-accelerated atherosclerosis. *Arterioscler Thromb Vasc Biol* 2014; **34**: 705-714

- 18 Jungi TW, Spycher MO, Nydegger UE, Barandun S. Platelet-leukocyte interaction: Selective binding of thrombin-stimulated platelets to human monocytes, polymorphonuclear leukocytes, and related cell lines. *Blood* 1986; **67**: 629-636
- 19 Marquardt L, Anders C, Buggle F, Palm F, Hellstern P, Grau AJ. Leukocyte-platelet aggregates in acute and subacute ischemic stroke. *Cerebrovasc Dis* 2009; **28**: 276-282
- 20 Gawaz MP, Mujais SK, Schmidt B, Gurland HJ. Platelet-leukocyte aggregation during hemodialysis. *Kidney Int* 1994; **46**: 489-495
- 21 Rinder CS, Bonan JL, Rinder HM, Mathew J, Hines R, Smith BR. Cardiopulmonary bypass induces leukocyte-platelet adhesion. *Blood* 1992; **79**: 1201-1205
- 22 Ott I, Neumann FJ, Gawaz M, Schmitt M, Schomig A. Increased neutrophil-platelet adhesion in patients with unstable angina. *Circulation* 1996; **94**: 1239-1246
- 23 Jensen MK, de Nully Brown P, Lund BV, Nielsen OJ, Hasselbalch HC. Increased circulating platelet-leukocyte aggregates in myeloproliferative disorders is correlated to previous thrombosis, platelet activation and platelet count. *Eur J Haematol* 2001; **66**: 143-151
- 24 Xiao Z, Theroux P. Clopidogrel inhibits platelet-leukocyte interactions and thrombin receptor agonist peptide-induced platelet activation in patients with an acute coronary syndrome. *J Am Coll Cardiol* 2004; **43**: 1982-1988
- 25 Tuttle HA, Davis-Gorman G, Goldman S, Copeland JG, McDonagh PF. Platelet-neutrophil conjugate formation is increased in diabetic women with cardiovascular disease. *Cardiovasc Diabetol* 2003; **2**: 12
- 26 Mickelson JK, Lakkis NM, Villarreal-Levy G, Hughes BJ, Smith CW. Leukocyte activation with platelet adhesion after coronary angioplasty: A mechanism for recurrent disease? *J Am Coll Cardiol*. 1996; **28**: 345-353
- 27 Furman MI, Benoit SE, Barnard MR, Valeri CR, Borbone ML, Becker RC, Hechtman HB, Michelson AD. Increased platelet reactivity and circulating monocyte-platelet aggregates in patients with stable coronary artery disease. *J Am Coll Cardiol* 1998; **31**: 352-358
- 28 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* 2001; **38**: 1002-1006
- 29 Yokoyama S, Ikeda H, Haramaki N, Yasukawa H, Murohara T, Imaizumi T. Platelet p-selectin plays an important role in arterial thrombogenesis by forming large stable platelet-leukocyte aggregates. *J Am Coll Cardiol* 2005; **45**: 1280-1286
- 30 Evangelista V, Manarini S, Collier BS, Smyth SS. Role of p-selectin, beta2-integrins, and src tyrosine kinases in mouse neutrophil-platelet adhesion. *J Thromb Haemost* 2003; **1**: 1048-1054
- 31 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* 1992; **359**: 848-851
- 32 Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, Littman DR, Weber C, Ley K. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein e. *Nat Med* 2003; **9**: 61-67
- 33 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* 1994; **91**: 8767-8771
- 34 Simon DI, Chen Z, Xu H, Li CQ, Dong J, McIntire LV, Ballantyne CM, Zhang L, Furman MI, Berndt MC, Lopez JA. Platelet glycoprotein Iba1 is a counterreceptor for the leukocyte integrin mac-1 (cd11b/cd18). *J Exp Med* 2000; **192**: 193-204
- 35 Santoso S, Sachs UJ, Kroll H, Linder M, Ruf A, Preissner KT, Chavakis T. The junctional adhesion molecule 3 (jam-3) on human platelets is a counterreceptor for the leukocyte integrin mac-1. *J Exp Med* 2002; **196**: 679-691
- 36 Simon DI, Xu H, Ortlepp S, Rogers C, Rao NK. 7e3 monoclonal antibody directed against the platelet glycoprotein iib/iiia cross-reacts with the leukocyte integrin mac-1 and blocks adhesion to fibrinogen and icam-1. *Arterioscler Thromb Vasc Biol* 1997; **17**: 528-535
- 37 Patko Z, Csanasz A, Acsady G, Peter K, Schwarz M. Roles of mac-1 and glycoprotein iib/iiia integrins in leukocyte-platelet aggregate formation: Stabilization by mac-1 and inhibition by gpiib/iiia blockers. *Platelets* 2012; **23**: 368-375
- 38 Neumann FJ, Zohlnhofer D, Fakhoury L, Ott I, Gawaz M, Schomig A. Effect of glycoprotein iib/iiia receptor blockade on platelet-leukocyte interaction and surface expression of the leukocyte integrin mac-1 in acute myocardial infarction. *J Am Coll Cardiol* 1999; **34**: 1420-1426
- 39 Haselmayer P, Grosse-Hovest L, von Landenberg P, Schild H, Radsak MP. Trem-1 ligand expression on platelets enhances neutrophil activation. *Blood* 2007; **110**: 1029-1035
- 40 Diacovo TG, deFougerolles AR, Bainton DF, Springer TA. A functional integrin ligand on the surface of platelets: Intercellular adhesion molecule-2. *J Clin Invest* 1994; **94**: 1243-1251
- 41 Issekutz AC, Rowter D, Springer TA. Role of icam-1 and icam-2 and alternate cd11/cd18 ligands in neutrophil transendothelial migration. *J Leukoc Biol* 1999; **65**: 117-126
- 42 Lievens D, Zerneck A, Seijkens T, Soehnlein O, Beckers L, Munnix IC, Wijnands E, Goossens P, van Kruchten R, Thevissen L, Boon L, Flavell RA, Noelle RJ, Gerdes N, Biessen EA, Daemen MJ, Heemskerk JW, Weber C, Lutgens E. Platelet cd40l mediates thrombotic and inflammatory processes in atherosclerosis. *Blood* 2010; **116**: 4317-4327
- 43 Cerletti C, Evangelista V, de Gaetano G. P-selectin-beta 2-integrin cross-talk: A molecular mechanism for polymorphonuclear leukocyte recruitment at the site of vascular damage. *Thromb Haemost* 1999; **82**: 787-793
- 44 Cerletti C, Evangelista V, de Gaetano G. Platelet-polymorphonuclear leukocyte functional interactions: Role of adhesive molecules. *Haemostasis* 1996; **26 Suppl 4**: 20-27
- 45 Li N, Goodall AH, Hjerdahl P. Efficient flow cytometric assay for platelet-leukocyte aggregates in whole blood using fluorescence signal triggering. *Cytometry* 1999; **35**: 154-161
- 46 Harding SA, Din JN, Sarma J, Jessop A, Weatherall M, Fox KA, Newby DE. Flow cytometric analysis of circulating platelet-monocyte aggregates in whole blood: Methodological considerations. *Thromb Haemost* 2007; **98**: 451-456
- 47 Polanowska-Grabowska R, Wallace K, Field JJ, Chen L, Marshall MA, Figler R, Gear AR, Linden J. P-selectin-mediated platelet-neutrophil aggregate formation activates neutrophils in mouse and human sickle cell disease. *Arterioscler Thromb Vasc Biol* 2010; **30**: 2392-2399
- 48 Chung AW, Radomski A, Alonso-Escolano D, Jurasz P, Stewart MW, Malinski T, Radomski MW. Platelet-leukocyte aggregation induced by par agonists: Regulation by nitric oxide and matrix metalloproteinases. *Br J Pharmacol* 2004; **143**: 845-855
- 49 Hu H, Varon D, Hjerdahl P, Savion N, Schulman S, Li N. Platelet-leukocyte aggregation under shear stress: Differential involvement of selectins and integrins. *Thromb Haemost* 2003; **90**: 679-687
- 50 Passacuale G, Vamadevan P, Pereira L, Hamid C, Corrigan V, Ferro A. Monocyte-platelet interaction induces a pro-inflammatory phenotype in circulating monocytes. *PLoS One* 2011; **6**: e25595
- 51 da Costa Martins P, van den Berk N, Ulfman LH, Koenderman L, Hordijk PL, Zwaginga JJ. Platelet-monocyte complexes support monocyte adhesion to endothelium by enhancing secondary tethering and cluster formation. *Arterioscler Thromb Vasc Biol* 2004; **24**: 193-199
- 52 Badnya S, Butler LM, Soderberg-Naucler C, Volf I, Assinger A.

Platelets directly enhance neutrophil transmigration in response to oxidised low-density lipoprotein. *Thromb Haemost* 2012; **108**: 719-729

- 53 Badnaya S, Schrottmaier WC, Kral JB, Yaiw KC, Volf I, Schab-bauer G, Soderberg-Naucler C, Assinger A. Platelets mediate oxidized low-density lipoprotein-induced monocyte extravasation

and foam cell formation. *Arterioscler Thromb Vasc Biol* 2014; **34**: 571-580

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