Platelet in Atherosclerosis Plaque Formation

Subjects: Others

Contributor: Chaojun Tang

Besides their role in hemostasis and thrombosis, it has become increasingly clear that platelets are also involved in many other pathological processes of the vascular system, such as atherosclerotic plaque formation. Atherosclerosis is a chronic vascular inflammatory disease, which preferentially develops at sites under disturbed blood flow with low speeds and chaotic directions. Hyperglycemia, hyperlipidemia, and hypertension are all risk factors for atherosclerosis. When the vascular microenvironment changes, platelets can respond quickly to interact with endothelial cells and leukocytes, participating in atherosclerosis.

platelet atherogenesis disturbed flow hyperglycemia hyperlipidemia inflammation plate platelet migration

1. Introduction

Atherosclerosis (AS) is a chronic inflammatory disease induced by multiple factors, involving a complex series of circulating blood cells (e.g., platelets and monocytes) and plasma components (e.g., lipoproteins), which interact with vascular cells and initiate atherosclerosis [1][2][3]. AS lesions often occur at the bifurcation or curvature of the large- and medium-sized arteries (e.g., aorta, carotid arteries), where disturbed flow (d-flow) occurs. In addition, a change of the microenvironment in circulation like hypertension, hyperglycemia, or hyperlipidemia can accelerate the formation of atherosclerosis [4][5][6]. When plaques are formed, changes in hemodynamics will exacerbate the tendency to increase plaque in an arterial stenosis environment, making it unstable and causing rupture. Once the plaque ruptures, platelets-rich blood clots are immediately formed to block blood vessels, and ischemic thrombotic events occur.

Platelets are the key mediator of plaque rupture and atherothrombosis. In recent years, many studies have shown that platelets play an inflammatory role as an immune cell and participate in the development of atherosclerosis [7][8]. When the shear stress of the blood flow changes sharply or the vascular microenvironment alters, the platelets circulating in the blood can quickly perceive these signals and respond. Subsequently, they are activated rapidly during endothelial dysfunction and then they adhere to damaged blood vessels to maintain blood vessel integrity [1][9]. Further, activated platelets recruit immune cells, promote their transmigration across the intima, and accelerate the process of atherosclerosis by the engagement of surface receptors or the release of inflammatory factors [10]. Several reviews about platelets in atherosclerosis are available, each with a different emphasis and perspective [1][3][11][12]. Nording et al. [12] summarized the important role of platelets in atherosclerosis and atherothrombosis, and concluded that antiplatelet therapy is not suitable for primary prevention treatment due to its bleeding risk in the

treatment of clinical cardiovascular disease. In this review, we try to focus on those basic studies that are related to how platelets, as inflammatory mediators, respond to atherosclerosis risk factors and regulate atherosclerotic plaque formation. Specifically, we illustrate the important roles of molecules derived from platelets in atherogenesis. Finally, we discuss whether platelet migration is involved in the process. We also raise the unsolved questions of platelets in atherogenesis as well as the highlights and perspectives in future research.

2. Platelet-Derived Proinflammatory Mediators in Atherogenesis

After platelet activation, a large amount of adhesive and proinflammatory substances stored in α granules and dense tubular systems are released. These substances interact with circulating leukocytes and blood vessel wall cells to induce a strong inflammatory response [1]. Proinflammatory substances released by platelets usually include various cytokines (IL1β, CD40L), chemokines (CCL5, CXCL4, CXCL7, CXCL12), growth factors (PDGF), and damage-related molecular model molecules DAMPs (HMGB, cyclophilin A) (Figure 3) [13][14]. The complex functional relationships of different platelet-derived mediators provide a mechanistic framework for the insight into the mechanisms by which platelets promote atherosclerosis.

2.1. Cytokines

IL-1β and CD40 ligand (CD40L, CD154) are important proinflammatory cytokines. IL-1β is synthesized and released after platelet activation and has become a target for coronary heart disease treatment. Activated platelet-synthesized interleukin (IL)-1β can induce the inflammatory response of ECs and promote the adhesion of platelets to leukocytes under the synergistic effect of e-selectin, ICAM-1, and chemokines [14]. In patients prone to development of atherosclerosis, polymorphism of the IL-1β gene cluster is associated with the extent of coronary arteries lesions, especially IL1B: −511 and −31 C/T polymorphism [15][16]. CD40L is stored in the cytoplasm of resting platelets, and is rapidly released to the membrane surface after platelet activation, and then it is cleaved to form a soluble functional fragment, sCD40L [1]. Platelets are the main source of circulating sCD40L, and elevated levels of circulating sCD40L have been reported in patients with hypercholesterolemia and diabetes [17][18][19]. This suggests that platelet-derived sCD40L can be an indicator for predicting postoperative risk of cardiovascular disease. IL-1β and CD40L on platelets induces ECs to express adhesion molecules (ICAM-1/VCAM-1) and secrete inflammatory factors (IL-8 and MCP-1), and promote the recruitment and extravasation of leukocytes at the site of injury, directly triggering an inflammatory response in the vessel wall [20]. Interrupting CD40 signal transduction with CD40L antibodies or CD40L deficiency in ApoE$^{-/-}$ mice can improve atherosclerotic lesions, reducing the infiltration of intermediate macrophages and T lymphocytes [21][22]. In clinical studies, the upregulation of platelet CD40L indicates a poor prognosis in stroke patients and is associated with increased platelet-mononuclear aggregate formation [23]. These results indicate that CD40L plays an important role in AS. However, Bavendiek et al. [24] showed that a CD40L deficiency on bone marrow-derived cells does not alter diet-induced atherosclerosis in hypercholesterolemia mice. This suggests that CD40L mainly regulates the occurrence of atherosclerosis through its expression on non-hematopoietic cell types, and platelet CD40L may not participate in AS. Moreover, the lack of CD40L will affect the stability of arterial thrombosis and delay arterial occlusion in vivo [25]. Therefore, simply

blocking CD40L may not be feasible in the clinical treatment of atherosclerosis and other cardiovascular diseases, because long-term inhibition will increase the risk of thromboembolic events. Conceivably, more targeted intervention strategies in CD40 signaling will have less deleterious side effects.

Meanwhile, platelets also express substantial levels of CD40, which is the alleged counter receptor for CD40L [26]. CD40 is different from CD40L, and the role of the receptor CD40 in the development of atherosclerosis remains disputable. Zirlik [27] reported that CD40 deficiency in $Ldlr^{-/-}$ mice does not ameliorate atherosclerosis, although the endothelial CD40-TNF receptor-related factor (TRAF) signaling pathways have been proven to promote atherosclerosis. Lutgens [28] also reported that deficiency in hematopoietic CD40 in $Ldlr^{-/-}$ mice or genetic interruption of CD40-TRAF6 signaling in $ApoE^{-/-}$ mice reduces atherosclerosis and increases plaque fibrosis. Gerdes [29] further reported that platelet CD40 promotes atherogenesis by stimulating endothelial cell activation and recruiting leukocytes. In summary, the current findings suggest that platelet CD40 and CD40L can serve as a key interface between inflammation, thrombosis, and atherosclerosis and are attractive potential therapeutic targets for cardiovascular disease.

2.2. Chemokines

2.2.1. CXCL4

Platelet factor 4 (PF-4 or CXCL4), a member of the C-X-C subfamily of chemokines, is stored in the α-particles of platelet, and is extremely abundant in platelets. It is quickly mobilized and released into plasma when platelets are activated, and is the first type of medium for early thrombosis or plaque formation [30]. The presence of PF4 in atherosclerotic lesions correlates with clinical parameters in patients with atherosclerosis [31]. Additionally, co-localization of PF4 and ox-LDL can be observed in human atherosclerotic lesions, especially in macrophage-derived foam cells [30][32]. In vitro experiments found that PF4 not only induces the differentiation of monocytes into macrophages, named “M4” [33][34], but also promoted the binding of ox-LDL to vascular cells and macrophages and the accumulation of cholesterol esters [31]. These observations suggest that platelet activation may promote the accumulation of harmful lipoproteins and thus promote atherosclerosis. The more direct evidence is that $PF4^{-/-}$; $ApoE^{-/-}$ mice have a strong decrease in atherosclerotic lesion formation compared to $ApoE^{-/-}$ mice [35]. The reason for the reduction in plaque is most likely that PF4 inhibits the expression of the hemoglobin scavenger receptor (CD163) in proinflammatory macrophages, and CD163 has anti-lipid peroxidation and anti-inflammatory effects [36]. Normally, PF4 is more likely to form heterodimers, dimers, and oligomers with CCL5, inducing the binding of monocytes to ECs, thereby promoting the transmigration of monocyte into the subendothelial space.

2.2.2. CCL5

CCL5 (RANTES) is the most expressed chemokine during platelet transcription, and mainly activates CCR5 and CCR1 receptors. The microparticles released after platelet activation promotes the delivery of RANTES to the surface of monocytes in atherosclerotic arteries [37]. RANTES deposition enhances the recruitment of monocytes by inflammatory microvascular or aortic ECs, a process that depends on P-selectin expression [38][39]. Treatment with the RANTES antagonist Met-RANTES in $Ldlr^{-/-}$ mice reduces leukocyte infiltration and diet-induced

atherosclerosis [40][41]. Clinical studies in patients with acute myocardial infarction and stable angina pectoris indicate that RANTES is more likely to be a biomarker for the presence of chronic coronary artery disease and is critical for the initial stage of atherosclerotic plaque formation [42]. Although RANTEs can be used as an indicator of AS inflammation, the specific role of platelet-derived RANTEs in AS needs more evidence. In fact, platelet-derived RANTES, in most cases, together with other mediators, induce monocyte inflammatory factor secretion (McP-1, McP-4, and IL-8) and accelerate AS [3]. One of the most common combinations is that CXCL4 and CCL5 interact to form heterodimers, then synergistically recruit monocytes to inflammatory vascular ECs [37] and induce the release of neutrophil extracellular traps (NETs) [43]. Disrupting the synergistic effect of CXCL4/CCL5 with peptide inhibitors MKEY will reduce leukocyte recruitment, NETosis formation, and ultimately reduce infarction size [44][45][46]. In conclusion, compared with CCL5 deficiency [47], blocking chemokine heterodimers can reduce inflammatory side effects and maintain normal immune defense.

2.2.3. CXCL7

CXCL7 is abundant in platelets; it is divided into several variants by pre-platelet basic protein (pre-PBP), including platelet basic protein (PBP), connective tissue-activating peptide III (CTAP-III), β thrombin (β-TG), and neutrophil-activating peptide 2 (NAP-2) [48]. NAP-2 is considered to be the only variant with chemotactic activity. Platelet-derived NAP-2 (CXCL7) deficiency or blockade of its receptor CXCR1/2 can significantly reduce thrombosis-induced neutrophil migration [49]. Patients with acute myocardial infarction were treated with PCI and found that plasma CXCL7 levels were negatively correlated with myocardial dysfunction [50]. However, there is no more relevant research reported in cardiovascular, especially atherosclerosis, patients. To the best of our knowledge, there are few studies on CXCL7 in the progress of AS. Additionally, there are few reports related to mononuclear/macrophages either. Further research is still needed on the role of platelet-derived CXCL7 in atherosclerosis.

2.2.4. CXCL12

The chemokine CXCL12, also known as stromal cell-derived factor-1 (SDF-1), is stored in platelet α granules. Early research found that high expression of CXCL12 was detected in SMCs, ECs, and macrophages of human atherosclerotic plaques from human carotid arteries [51]. However, recent studies by Merckelbach et al. [52] on human carotid atherosclerotic plaques have shown that CXCL12 is expressed only on the macrodissected areas of macrophages. The CXCR12 receptor CXCR4 is expressed on plaque macrophages, SMCs, and leukocytes. The reason for this difference may be related to the disease status and complications of patients with different plaque samples. In addition, the lower sensitivity of IHC staining and the shorter half-life of CXCL12 may also lead to poor observation of CXCL12 staining. In vitro experimental studies have found that platelet-derived CXCL12 can participate in the regulation of monocyte function, and induce the differentiation of monocytes into macrophages and foam cells through the receptors CXCR4-CXCR7 [53]. Therefore, CXCL12 may participate in AS through mononuclear differentiation. Animal studies further found that systemic treatment of CXCL12 in ApoE$^{-/-}$ mice promoted the mobilization and accumulation of smooth muscle progenitor cells at the site of vascular injury, thereby increasing plaque stability [54]. However, there is no evidence to show whether platelet-derived CXCL12 is

playing a role here, thus further studies are necessary to elucidate the exact role of the platelet-derived CXCL12 in atherosclerotic plaques and potential impact on plaque vulnerability.

2.3. Other Platelet-Derived Inflammatory Mediators

The number of inflammatory mediators released from activated platelets is rapidly increasing. Platelet-derived growth factor (PDGF) is stored and released by α granules from activated platelets but can be widely secreted by macrophages, VSMCs, and endothelial cells [55]. The presence of PDGF was detected in the atherosclerotic vessel wall, especially PDGF-A and PDGF-B [56]. Simultaneously, PDGF receptors (PDGFR), PDGFR-α and PDGFR-β, expressed by macrophages and SMCs, are also significantly upregulated [57]. Multiple studies have shown that PDGF and PDGFR mainly regulate the development of atherosclerotic lesions by inducing the migration and proliferation of SMCs [58][59][60]. In ApoE$^{-/-}$ mice, blocking the PDGF-PDGFR-β pathway by neutralizing antibodies or chemical inhibitors prevented vascular smooth muscle cell accumulation and delayed fibrous cap formation [61][62]. In addition, enhancement of PDGF signaling led to severer inflammation, and promoted the progression of atherosclerosis in ApoE$^{-/-}$ mice [63]. However, at present, all studies have not pointed out how PDGF derived from platelets affects the expression of PDGFR in ECs, VSMCs, and infiltrating macrophages participating in vascular inflammation and remodeling.

Amphoterin (HMG1) is an endogenous protein in human platelets, which is exposed on the surface during platelet activation [64]. HMGB1 expression in human atherosclerotic plaques and coronary artery thrombi was identified. Therapeutic blockade of HMGB1 reduced the development of diet-induced atherosclerosis in ApoE$^{-/-}$ mice [65]. Furthermore, platelet-derived HMGB1 induces NETs formation and thrombosis [66][67]. This suggests that platelet-derived HMGB1 may be involved in the progression of both atherosclerosis and atherosclerotic thrombosis. Cyclophilin A (CyPA), a protein released when platelets are activated, is found in atherosclerotic plaques [68]. CyPA stimulates migration and proliferation of VSMCs, expression of adhesion molecules in endothelial cells, and inflammatory cell chemotaxis, and promotes atherosclerosis in ApoE$^{-/-}$ mice [69][70]. In addition, platelets also release several other proteins that may be related to atherosclerosis, such as granule protein III, histamine, etc. At present, we have less research on these “new” platelet-derived mediators, and future research should be an attempt to determine the role of these inflammatory factors in the formation of plaque.

3. Concluding Remarks and Future Perspectives

Platelets are important blood cells in the body. The initial physiological functions of platelets are mainly to stop the bleeding and keep the vascular endothelium intact. However, now, accumulating evidence indicates that platelets, as a novel immune and inflammatory cell, can modulate the inflammatory response of neighboring cells, such as leukocytes, ECs, and vascular SMCs. Simultaneously, platelets also receive the inflammatory signals produced by their neighboring cells. This double effect between platelets and neighboring cells plays roles in both the early and late stage during atherosclerosis. Although a variety of antiplatelet drugs have been widely used in patients with atherosclerotic diseases, platelet-mediated inflammation (complications due to side effects of the drug) appears to be operating. Therefore, the latest advances in understanding how platelets participate in the formation of

atherosclerotic plaques will provide important clues for antiplatelet drugs in the prevention and treatment of cardiovascular diseases, especially atherosclerosis. Several issues need to be addressed in future studies: (1) whether and how platelets regulate atherosclerotic plaque formation under disturbance flow; (2) how platelets foster monocyte recruitment to atherosclerotic lesions and how they reprogram macrophages; (3) the association of platelet-derived inflammation mediators with atherogenesis should be explored in detail, such as using platelet-specific knockout mice; (4) further study is required to confirm whether platelet heterogeneity has been involved by using single-cell sequencing technology; and (5) whether platelets actively migrate to the subintima to participate in atherogenesis.

References

1. Huo, Y.; Ley, K.F. Role of platelets in the development of atherosclerosis. Trends Cardiovasc. Med. 2004, 14, 18–22.
2. Lievens, D.; von Hundelshausen, P. Platelets in atherosclerosis. Thromb. Haemost. 2011, 106, 827–838.
3. Aukrust, P.; Halvorsen, B.; Ueland, T.; Michelsen, A.E.; Skjelland, M.; Gullestad, L.; Yndestad, A.; Otterdal, K. Activated platelets and atherosclerosis. Expert Rev. Cardiovasc. Ther. 2010, 8, 1297–1307.
4. Kobiyama, K.; Ley, K. Atherosclerosis. Circ. Res. 2018, 123, 1118–1120.
5. Zhao, Y.; Yang, Y.; Xing, R.; Cui, X.; Xiao, Y.; Xie, L.; You, P.; Wang, T.; Zeng, L.; Peng, W.; et al. Hyperlipidemia induces typical atherosclerosis development in Ldlr and Apoe deficient rats. Atherosclerosis 2018, 271, 26–35.
6. Aronson, D.; Rayfield, E.J. How hyperglycemia promotes atherosclerosis: Molecular mechanisms. Cardiovasc. Diabetol. 2002, 1, 1–10.
7. Von Hundelshausen, P.; Weber, C. Platelets as Immune Cells Bridging Inflammation and Cardiovascular Disease. Circ. Res. 2007, 100, 27–40.
8. Siegel-Axel, D.; Daub, K.; Seizer, P.; Lindemann, S.; Gawaz, M. Platelet lipoprotein interplay: Trigger of foam cell formation and driver of atherosclerosis. Cardiovasc. Res. 2008, 78, 8–17.
9. Hartwig, J.H. The Platelet: Form and Function. Semin. Hematol. 2006, 43, S94–S100.
10. Li, Z.; Yang, F.; Dunn, S.; Gross, A.K.; Smyth, S.S. Platelets as immune mediators: Their role in host defense responses and sepsis. Thromb. Res. 2011, 127, 184–188.
11. Von Hundelshausen, P.; Duchene, J. Platelet-derived chemokines in atherosclerosis. Hämostaseologie 2017, 35, 137–141.

12. Nording, H.; Baron, L.; Langer, H.F. Platelets as therapeutic Targets to prevent Atherosclerosis. Atherosclerosis 2020, 307, 97–108.
13. Massberg, S.; Brand, K.; Gruner, S.; Page, S.; Muller, E.; Muller, I.; Bergmeier, W.; Richter, T.; Lorenz, M.; Konrad, I.; et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. J. Exp. Med. 2002, 196, 887–896.
14. Lindemann, S.; Tolley, N.D.; Dixon, D.A.; Mcintyre, T.M.; Prescott, S.M.; Zimmerman, G.A.; Weyrich, A.S. Activated platelets mediate inflammatory signaling by regulated interleukin 1β synthesis. J. Cell Biol. 2001, 154, 485–490.
15. Rechciński, T.; Grębowska, A.; Kurpesa, M.; Sztybrych, M.; Peruga, J.Z.; Trzos, E.; Rudnicka, W.; Krzemińskapakuła, M.; Chmiela, M. Interleukin-1b and interleukin-1 receptor inhibitor gene cluster polymorphisms in patients with coronary artery disease after percutaneous angioplasty or coronary artery bypass grafting. Kardiologia Polska 2009, 67, 601.
16. Gorący, I.; Kaczmarczyk, M.; Ciechanowicz, A.; Lewandowska, K.; Jakubiszyn, P.; Bodnar, O.; Kopijek, B.; Brodkiewicz, A.; Cyrylowski, L. Polymorphism of Interleukin 1B May Modulate the Risk of Ischemic Stroke in Polish Patients. Med.-Buenos Aires 2019, 55, 558.
17. Garlichs, C.D.; Kozina, S.; Fatehmoghadam, S.; Tomandl, B.; Stumpf, C.; Eskafi, S.; Raaz, D.; Schmeiser, A.; Yilmaz, A.; Ludwig, J. Upregulation of CD40-CD40 Ligand (CD154) in Patients with Acute Cerebral Ischemia. Stroke 2003, 34, 1412–1418.
18. Varo, N.; De Lemos, J.A.; Libby, P.; Morrow, D.A.; Murphy, S.A.; Nuzzo, R.; Gibson, C.M.; Cannon, C.P.; Braunwald, E.; Schonbeck, U. Soluble CD40L: Risk prediction after acute coronary syndromes. Circulation 2003, 108, 1049–1052.
19. Sanguigni, V.; Pignatelli, P.; Lenti, L.; Ferro, D.; Bellia, A.; Carnevale, R.; Tesauro, M.; Sorge, R.; Lauro, R.; Violi, F. Short-term treatment with atorvastatin reduces platelet CD40 ligand and thrombin generation in hypercholesterolemic patients. Circulation 2005, 111, 412–419.
20. Henn, V.; Slupsky, J.R.; Grafe, M.; Anagnostopoulos, I.; Forster, R.; Mullerberghaus, G.; Kroczek, R.A. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature 1998, 391, 591–594.
21. Mach, F.; Schonbeck, U.; Sukhova, G.K.; Atkinson, E.; Libby, P. Reduction of atherosclerosis in mice by inhibition of CD40 signalling. Nature 1998, 394, 200–203.
22. Lutgens, E.; Gorelik, L.; Daemen, M.J.A.P.; Ed, D.M.; Grewal, I.S.; Koteliansky, V.; Flavell, R.A. Requirement for CD154 in the progression of atherosclerosis. Nat. Med. 1999, 5, 1313–1316.
23. Lukasik, M.; Dworacki, G.; Kufelgrabowska, J.; Watala, C.; Kozubski, W. Upregulation of CD40 ligand and enhanced monocyte-platelet aggregate formation are associated with worse clinical outcome after ischaemic stroke. Thromb. Haemost. 2012, 107, 346–355.

24. Bavendiek, U.; Zirlik, A.; Laclair, S.; Macfarlane, L.A.; Libby, P.; Schonbeck, U. Atherogenesis in Mice Does Not Require CD40 Ligand From Bone Marrow–Derived Cells. Arterioscler. Thromb. Vasc. Biol. 2005, 25, 1244–1249.

25. Andre, P.; Prasad, K.; Denis, C.; Papalia, J.; Wagner, D. CD40L stabilizes arterial thrombi by a 3 integrin-dependent mechanism. Nat. Med. 2001, 8, 247–252.

26. Henn, V.; Steinbach, S.; Buchner, K.; Presek, P.; Kroczek, R.A. The inflammatory action of CD40 ligand (CD154) expressed on activated human platelets is temporally limited by coexpressed CD40. Blood 2001, 98, 1047–1054.

27. Zirlik, A.; Bavendiek, U.; Libby, P.; Macfarlane, L.A.; Gerdes, N.; Jagielska, J.; Ernst, S.; Aikawa, M.; Nakano, H.; Tsitsikov, E. TRAF-1, -2, -3, -5, and -6 Are Induced in Atherosclerotic Plaques and Differentially Mediate Proinflammatory Functions of CD40L in Endothelial Cells. Arterioscler. Thromb. Vasc. Biol. 2007, 27, 1101–1107.

28. Lutgens, E.; Lievens, D.; Beckers, L.; Wijnands, E.; Soehnlein, O.; Zernecke, A.; Seijkens, T.; Engel, D.; Cleutjens, J.P.M.; Keller, A.M. Deficient CD40-TRAF6 signaling in leukocytes prevents atherosclerosis by skewing the immune response toward an antiinflammatory profile. J. Exp. Med. 2010, 207, 391–404.

29. Gerdes, N.; Seijkens, T.; Lievens, D.; Kuijpers, M.J.; Winkels, H.; Projahn, D.; Hartwig, H.; Beckers, L.; Megens, R.T.; Boon, L.; et al. Platelet CD40 Exacerbates Atherosclerosis by Transcellular Activation of Endothelial Cells and Leukocytes. Arterioscler. Thromb. Vasc. Biol. 2016, 36, 482–490.

30. Nassar, T.; Sachais, B.S.; Akkawi, S.; Kowalska, M.A.; Bdeir, K.; Leitersdorf, E.; Hiss, E.; Ziporen, L.; Aviram, M.; Cines, D.B. Platelet Factor 4 Enhances the Binding of Oxidized Low-density Lipoprotein to Vascular Wall Cells. J. Biol. Chem. 2003, 278, 6187–6193.

31. Pitsilos, S.; Hunt, J.L.; Mohler, E.R.; Prabhakar, A.M.; Poncz, M.; Dawicki, J.; Khalapyan, T.Z.; Wolfe, M.L.; Fairman, R.M.; Mitchell, M.E. Platelet factor 4 localization in carotid atherosclerotic plaques: Correlation with clinical parameters. Thromb. Haemost. 2003, 90, 1112–1120.

32. O'brien, J.R.; Etherington, M.D.; Pashley, M.A. Intra-Platelet Platelet Factor 4 (IP.PF4) and the Heparin-Mobilisable Pool of PF4 in Health and Atherosclerosis. Thromb. Haemost. 1984, 51, 354–357.

33. Scheuerer, B.; Ernst, M.; Durrbaumlandmann, I.; Fleischer, J.; Gragegriebenow, E.; Brandt, E.; Flad, H.D.; Petersen, F. The CXC-chemokine platelet factor 4 promotes monocyte survival and induces monocyte differentiation into macrophages. Blood 2000, 95, 1158–1166.

34. Erbel, C.; Tyka, M.; Helmes, C.M.; Akhavanpoor, M.; Rupp, G.; Domschke, G.; Linden, F.; Wolf, A.; Doesch, A.O.; Lasitschka, F. CXCL4-induced plaque macrophages can be specifically

identified by co-expression of MMP7+S100A8+in vitro and in vivo. Innate Immun. 2015, 21, 255–265.

35. Sachais, B.S.; Turrentine, T.; Mckenna, J.M.D.; Rux, A.H.; Rader, D.J.; Kowalska, M.A. Elimination of platelet factor 4 (PF4) from platelets reduces atherosclerosis in C57Bl/6 and apoE-/- mice. Thromb. Haemost. 2007, 98, 1108–1113.
36. Gleissner, C.A.; Shaked, I.; Erbel, C.; Bockler, D.; Katus, H.A.; Ley, K. CXCL4 Downregulates the Atheroprotective Hemoglobin Receptor CD163 in Human Macrophages. Circ. Res. 2010, 106, 203–211.
37. Von Hundelshausen, P.; Koenen, R.R.; Sack, M.; Mause, S.F.; Adriaens, W.; Proudfoot, A.E.I.; Hackeng, T.M.; Weber, C. Heterophilic interactions of platelet factor 4 and RANTES promote monocyte arrest on endothelium. Blood 2005, 105, 924–930.
38. Von Hundelshausen, P.; Weber, K.S.C.; Huo, Y.; Proudfoot, A.E.I.; Nelson, P.J.; Ley, K.; Weber, C. RANTES Deposition by Platelets Triggers Monocyte Arrest on Inflamed and Atherosclerotic Endothelium. Circulation 2001, 103, 1772–1777.
39. Schober, A.; Manka, D.; Von Hundelshausen, P.; Huo, Y.; Hanrath, P.; Sarembock, I.J.; Ley, K.; Weber, C. Deposition of Platelet RANTES Triggering Monocyte Recruitment Requires P-Selectin and Is Involved in Neointima Formation After Arterial Injury. Circulation 2002, 106, 1523–1529.
40. Veillard, N.R.; Kwak, B.R.; Pelli, G.; Mulhaupt, F.; James, R.W.; Proudfoot, A.E.I.; Mach, F. Antagonism of RANTES Receptors Reduces Atherosclerotic Plaque Formation in Mice. Circ. Res. 2004, 94, 253–261.
41. Braunersreuther, V.; Steffens, S.; Arnaud, C.; Pelli, G.; Burger, F.; Proudfoot, A.E.I.; Mach, F. A Novel RANTES Antagonist Prevents Progression of Established Atherosclerotic Lesions in Mice. Arterioscler. Thromb. Vasc. Biol. 2008, 28, 1090–1096.
42. Koperlenkiewicz, O.M.; Kaminska, J.; Lisowska, A.; Milewska, A.J.; Hirnle, T.; Dymickapiekarska, V. Factors Associated with RANTES Concentration in Cardiovascular Disease Patients. BioMed Res. Int. 2019, 2019, 3026453.
43. Rossaint, J.; Herter, J.M.; Van Aken, H.; Napirei, M.; Doring, Y.; Weber, C.; Soehnlein, O.; Zarbock, A. Synchronized integrin engagement and chemokine activation is crucial in neutrophil extracellular trap–mediated sterile inflammation. Blood 2014, 123, 2573–2584.
44. Carlson, J.; Baxter, S.A.; Dreau, D.; Nesmelova, I.V. The heterodimerization of platelet-derived chemokines. Biochim. Biophys. Acta 2013, 1834, 158–168.
45. Koenen, R.R.; Von Hundelshausen, P.; Nesmelova, I.V.; Zernecke, A.; Liehn, E.A.; Sarabi, A.; Kramp, B.; Piccinini, A.M.; Paludan, S.R.; Kowalska, M.A. Disrupting functional interactions between platelet chemokines inhibits atherosclerosis in hyperlipidemic mice. Nat. Med. 2009, 15, 97–103.

46. Vajen, T.; Koenen, R.R.; Werner, I.; Staudt, M.; Projahn, D.; Curaj, A.; Sonmez, T.T.; Simsekyilmaz, S.; Schumacher, D.; Mollmann, J. Blocking CCL5-CXCL4 heteromerization preserves heart function after myocardial infarction by attenuating leukocyte recruitment and NETosis. Sci. Rep. 2018, 8, 10647.

47. Tyner, J.W.; Uchida, O.; Kajiwara, N.; Kim, E.Y.; Patel, A.C.; Osullivan, M.P.; Walter, M.J.; Schwendener, R.A.; Cook, D.N.; Danoff, T.M. CCL5-CCR5 interaction provides antiapoptotic signals for macrophage survival during viral infection. Nat. Med. 2005, 11, 1180–1187.

48. Gleissner, C.A.; Von Hundelshausen, P.; Ley, K. Platelet Chemokines in Vascular Disease. Arterioscler. Thromb. Vasc. Biol. 2008, 28, 1920–1927.

49. Ghasemzadeh, M.; Kaplan, Z.S.; Alwis, I.; Schoenwaelder, S.M.; Ashworth, K.J.; Westein, E.; Hosseini, E.; Salem, H.H.; Slattery, R.; McColl, S.R.; et al. The CXCR1/2 ligand NAP-2 promotes directed intravascular leukocyte migration through platelet thrombi. Blood 2013, 121, 4555–4566.

50. Orn, S.; Breland, U.M.; Mollnes, T.E.; Manhenke, C.; Dickstein, K.; Aukrust, P.; Ueland, T. The Chemokine Network in Relation to Infarct Size and Left Ventricular Remodeling Following Acute Myocardial Infarction. Am. J. Cardiol. 2009, 104, 1179–1183.

51. Abiyounes, S.; Sauty, A.; Mach, F.; Sukhova, G.K.; Libby, P.; Luster, A.D. The Stromal Cell–Derived Factor-1 Chemokine Is a Potent Platelet Agonist Highly Expressed in Atherosclerotic Plaques. Circ. Res. 2000, 86, 131–138.

52. Merckelbach, S.; Der Vorst, E.P.C.V.; Kallmayer, M.; Rischpler, C.; Burgkart, R.; Doring, Y.; De Borst, G.; Schwaiger, M.; Eckstein, H.H.; Weber, C. Expression and Cellular Localization of CXCR4 and CXCL12 in Human Carotid Atherosclerotic Plaques. Thromb. Haemost. 2018, 118, 195–206.

53. Chatterjee, M.; Von Ungernsternberg, S.N.I.; Seizer, P.; Schlegel, F.; Buttcher, M.; Sindhu, N.A.; Muller, S.; Mack, A.F.; Gawaz, M. Platelet-derived CXCL12 regulates monocyte function, survival, differentiation into macrophages and foam cells through differential involvement of CXCR4–CXCR7. Cell Death Dis. 2015, 6, e1989.

54. Akhtar, S.; Gremse, F.; Kiessling, F.; Weber, C.; Schober, A. CXCL12 Promotes the Stabilization of Atherosclerotic Lesions Mediated by Smooth Muscle Progenitor Cells in Apoe -Deficient Mice. Arterioscler. Thromb. Vasc. Biol. 2013, 33, 679–686.

55. Andrae, J.; Gallini, R.; Betsholtz, C. Role of platelet-derived growth factors in physiology and medicine. Genes Dev. 2008, 22, 1276–1312.

56. Raines, E.W. PDGF and cardiovascular disease. Cytokine Growth Factor Rev. 2004, 15, 237–254.

57. Raica, M.; Cimpean, A.M. Platelet-Derived Growth Factor (PDGF)/PDGF Receptors (PDGFR) Axis as Target for Antitumor and Antiangiogenic Therapy. Pharmaceuticals 2010, 3, 572–599.

58. Folestad, E.; Kunath, A.; Wågsäter, D. PDGF-C and PDGF-D signaling in vascular diseases and animal models. Mol. Asp. Med. 2018, 62, 1–11.
59. Ricci, C.; Ferri, N. Naturally occurring PDGF receptor inhibitors with potential anti-atherosclerotic properties. Vasc. Pharmacol. 2015, 70, 1–7.
60. Hu, W.; Huang, Y. Targeting the platelet-derived growth factor signalling in cardiovascular disease. Clin. Exp. Pharmacol. Physiol. 2015, 42, 1221–1224.
61. Sano, H.; Sudo, T.; Yokode, M.; Murayama, T.; Kataoka, H.; Takakura, N.; Nishikawa, S.; Nishikawa, S.; Kita, T. Functional Blockade of Platelet-Derived Growth Factor Receptor-β but Not of Receptor-α Prevents Vascular Smooth Muscle Cell Accumulation in Fibrous Cap Lesions in Apolipoprotein E–Deficient Mice. Circulation 2001, 103, 2955–2960.
62. Kozaki, K.; Kaminski, W.E.; Tang, J.; Hollenbach, S.; Lindahl, P.; Sullivan, C.M.; Yu, J.C.; Abe, K.; Martin, P.J.; Ross, R. Blockade of Platelet-Derived Growth Factor or Its Receptors Transiently Delays but Does Not Prevent Fibrous Cap Formation in ApoE Null Mice. Am. J. Pathol. 2002, 161, 1395–1407.
63. He, C.; Medley, S.C.; Hu, T.; Hinsdale, M.E.; Lupu, F.; Virmani, R.; Olson, L.E. PDGFRβ signalling regulates local inflammation and synergizes with hypercholesterolaemia to promote atherosclerosis. Nat. Commun. 2015, 6, 7770.
64. Rouhiainen, A.; Imai, S.; Rauvala, H.; Parkkinen, J. Occurrence of Amphoterin (HMG1) as an Endogenous Protein of Human Platelets that Is Exported to the Cell Surface upon Platelet Activation. Thromb. Haemost. 2000, 84, 1087–1094.
65. Ahrens, I.; Chen, Y.; Topcic, D.; Bode, M.; Haenel, D.; Hagemeyer, C.E.; Seeba, H.; Duerschmied, D.; Bassler, N.; Jandeleitdahm, K. HMGB1 binds to activated platelets via the receptor for advanced glycation end products and is present in platelet rich human coronary artery thrombi. Thromb. Haemost. 2015, 114, 994–1003.
66. Vogel, S.; Bodenstein, R.; Chen, Q.; Feil, S.; Feil, R.; Rheinlaender, J.; Schaffer, T.E.; Bohn, E.; Frick, J.; Borst, O. Platelet-derived HMGB1 is a critical mediator of thrombosis. J. Clin. Investig. 2015, 125, 4638–4654.
67. Maugeri, N.; Campana, L.; Gavina, M.; Covino, C.; De Metrio, M.; Panciroli, C.; Maiuri, L.; Maseri, A.; Dangelo, A.; Bianchi, M. Activated platelets present high mobility group box 1 to neutrophils, inducing autophagy and promoting the extrusion of neutrophil extracellular traps. J. Thromb. Haemost. 2014, 12, 2074–2088.
68. Coppinger, J.A.; Cagney, G.; Toomey, S.; Kislinger, T.; Belton, O.; Mcredmond, J.P.; Cahill, D.J.; Emili, A.; Fitzgerald, D.J.; Maguire, P.B. Characterization of the proteins released from activated platelets leads to localization of novel platelet proteins in human atherosclerotic lesions. Blood 2004, 103, 2096–2104.

69. Nigro, P.; Satoh, K.; Odell, M.R.; Soe, N.N.; Cui, Z.; Mohan, A.; Abe, J.I.; Alexis, J.D.; Sparks, J.D.; Berk, B.C. Cyclophilin A is an inflammatory mediator that promotes atherosclerosis in apolipoprotein E–deficient mice. J. Exp. Med. 2011, 208, 53–66.
70. Seizer, P.; Von Ungernsternberg, S.N.I.; Schonberger, T.; Borst, O.; Munzer, P.; Schmidt, E.; Mack, A.F.; Heinzmann, D.; Chatterjee, M.; Langer, H.F. Extracellular Cyclophilin A Activates Platelets Via EMMPRIN (CD147) and PI3K/Akt Signaling, Which Promotes Platelet Adhesion and Thrombus Formation In Vitro and In Vivo. Arterioscler. Thromb. Vasc. Biol. 2015, 35, 655–663.