

Theme Issue Article

Platelet-vessel wall interactions in atherosclerotic disease

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Summary

During the prolonged course of atherosclerotic disease, platelets are of central importance as they contribute to the initiation of the disease, to its progression and acute exacerbation but also provide potential regenerative mechanisms. Platelets secrete chemokines and cytokines that mediate vascular inflammation and are in turn activated by substances released from cells of the vascular wall. These interactions represent positive and negative feedback loops, which in case of dysregulation may lead to development and progression of disease. Furthermore, platelet adhesion to the endothelium is critical for the initiation of atherosclerotic lesion formation *in vivo*. Even prior to endothelial denudation, platelet adhesion governed by disturbed flow at predilection sites for atherosclerosis induces recruitment of proathe-

rosclerotic cells and release of proinflammatory mediators from all involved cell types. Finally, the pathogenetic role of platelets for late atherosclerotic events including plaque rupture, microembolism or spasms within the microcirculation is well established. However, increasing evidence indicates that platelets mediate on the other hand potential regenerative mechanisms. Platelets recruit circulating progenitor cells to sites of vascular injury. Furthermore, they influence their biological activity and maturation. Therefore, platelets contribute at all stages of vascular disease by interfering with highly dynamic processes. Understanding interactions of platelets with other circulating cells and the vascular wall is a prerequisite to understand cardiovascular disease and to identify potential therapeutic targets.

Keywords

Atherothrombosis, platelet physiology, stem cells

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Platelet secretion and generation of cytokines

The pathogenesis of atherosclerosis contains a strong pro-inflammatory component, which involves diverse cell types and mediating signals. Between platelets and chemokines an intricate functional relationship exists, which provides a framework of mechanisms for synergistic functions and allows insights into the deleterious basis for proatherogenic, proinflammatory or thrombogenic effects (1). Activated platelets can release chemokines and can induce the generation and secretion of chemokines in various cells of the vascular wall including CCL3, RANTES (CCL5), CCL7, CCL17, CXCL1, CXCL5, or CXCL8, as well as precursors for CXCL7, such as β -thromboglobulin (1, 2) (see Table 1).

In turn, certain chemokines can enhance platelet aggregation and adhesion in combination with primary agonists and can trigger monocyte recruitment (3). Platelet factor 4 (PF4; CXCL4) is the most abundant CXC chemokine released from platelet alpha-granules. PF4 acts as a chemoattractant for monocytes promoting their differentiation into macrophages (4). Furthermore, PF4 may directly promote atherosclerosis by the inhibition of LDL catabolism, which is mediated in part by competing for binding to the LDL receptor, by promoting interactions with cell-associated chondroitin sulfate proteoglycans and by disrupting the normal endocytic trafficking of LDL/LDL-R complexes (5). In addition, PF4 markedly enhances the esterification and uptake of oxidized LDL by macrophages (6). Among the wide range of chemokines found to be expressed in

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Table 1: Platelet-derived chemokines.

Reference	Chemokine	Counter-receptor	Species
Boehlen et al. (67)	CXCL1/ GRO- α	CXCR2 and CXCR1	human
Brandt et al. (68)	CXCL4/ PF4	CXCR3B, PG	human, murine
Struyf et al. (69)	CXCL4L1/ PF4	?	human
Klinger et al. (70)	CXCL5 ENA-78	CXCR2	human
Brandt et al. (68)	CXCL7 and precursors PBP, β -TG, CTAPIII, NAP-2	CXCR2	human
Gear et al. (71)	CXCL8/ IL-8	CXCR1, CXCR2	human
Massberg et al. (23)	CXCL12/ SDF-1	CXCR4	human, murine
Power et al. (72)	CCL1/ I-309	CCR8	human
Schober et al. (73)	CCL2/ MCP-1	CCR2	human, murine
Klinger et al. (74)	CCL3/ MIP-1 α	CCR1, CCR2, CCR3	human
Kameyoshi et al., Klinger et al. (74, 75)	CCL5/ RANTES	CCR1, CCR3, CCR5	human, murine
Power et al. (72)	CCL7/ MCP-3	CCR1, CCR2, CCR3	human
Fujisawa et al. (76)	CCL17/ TARC	CCR4, CCR8	human

atherosclerotic lesions, the deposition of PF4 has been correlated with lesion severity and symptomatic atherosclerosis, suggesting that persistent platelet activation may contribute to the evolution of vascular lesions. Given that PF4 and oxidized LDL colocalize in macrophage-derived foam cells of atherosclerotic lesions, this mechanism likely promotes vascular lipid accumulation (7).

Elevated serum levels of CD40 ligand (CD40L, CD154) indicate an acute risk for a coronary event (8). Release of platelet-derived CD40L, which is stored in high amounts in platelet granules and released within seconds after activation *in vitro*, induces inflammatory responses in endothelium (9). Ligation of CD40 on endothelial cells (ECs) by CD40L expressed on the surface of activated platelets increased the release of interleukin (IL)-8 and monocyte chemoattractant protein 1 (MCP-1), major chemoattractants for neutrophils and monocytes (9). Only recently, the expression of LIGHT (TNFSF 14, a protein, which belongs to the tumor necrosis factor super-family and causes pro-inflammatory and pro-thrombotic changes qualitatively comparable to effects of CD40 ligand) was identified on platelets upon exposure to ADP or TRAP-1 (10, 11). Platelet-associated LIGHT influenced adhesion of platelets to the endothelium, while soluble LIGHT induced a pro-inflammatory state in vascular ECs, rendering this molecule a potential therapeutic or diagnostic target in atherosclerosis (11). As patients with myocardial infarction revealed increased levels of soluble LIGHT, this molecule may be of clinical importance for the pathogenesis of coronary artery disease (11). Platelet-induced alterations like the induction of endothelial MCP-1 secretion by stimulated platelets has been attributed to an activation of nuclear factor κ B (NF- κ B)-dependent transcriptional mechanisms, possibly involving platelet-derived IL-1 β (12). Furthermore, transient adhesion of platelets to the endothelium initiates degradation of κ B and supports activation of NF- κ B in ECs, thereby inducing NF- κ B-dependent chemokine gene transcription (Fig. 1A). In line with these findings, the

disruption of CD40 ligand-induced signaling by antibody inhibition or genetic deletion has been shown to reduce the formation and progression of atherosclerotic lesions in mice (13, 14). Similarly to IL-1 β , CD40L expressed on platelets induces release of chemokines from endothelial cells and expression of adhesion molecules, thereby generating signals for the recruitment of leukocytes. CD40 ligation on ECs, smooth muscle cells and macrophages initiates the expression and release of matrix-degrading enzymes, the MMPs, which significantly contribute to destruction and remodeling of inflamed tissue. Activated platelets release MMP-2 during aggregation (15). Furthermore, adhesion of activated platelets to ECs results in generation and secretion of MMP-9 and of the protease receptor urokinase-type plasminogen activator receptor (uPAR) on cultured endothelium (16). The endothelial release of MMP-9 is dependent on both the fibrinogen receptor GPIIb/IIIa and CD40L, since inhibition of either mechanism resulted in reduction of platelet-induced matrix degradation activity of ECs. Moreover, GPIIb/IIIa ligation results in substantial release of CD40L in the absence of any further platelet agonist (16). These results suggest that the release of platelet-derived proinflammatory mediators like CD40L is dependent on GPIIb/IIIa-mediated adhesion. However, secretion of platelet-derived chemokines can be stimulated by classical primary agonists such as thrombin or by oxidized low-density lipoprotein (LDL), as shown for the release of CXC chemokines in patients with coronary artery disease (17). Another chemokine released by stimulated platelets is the CC chemokine RANTES, which can be immobilized on the surface of activated microvascular or aortic endothelial cells (3, 18). Platelet-monocyte interaction can result in the deposition of the platelet-derived chemokines RANTES to the monocyte surface and early atherosclerotic endothelium (18, 19). This appears to be particularly effective under flow conditions supporting platelet rolling and it triggers subsequent recruitment of proinflammatory cells including monocytes and T cells (3, 18). The specific role of this

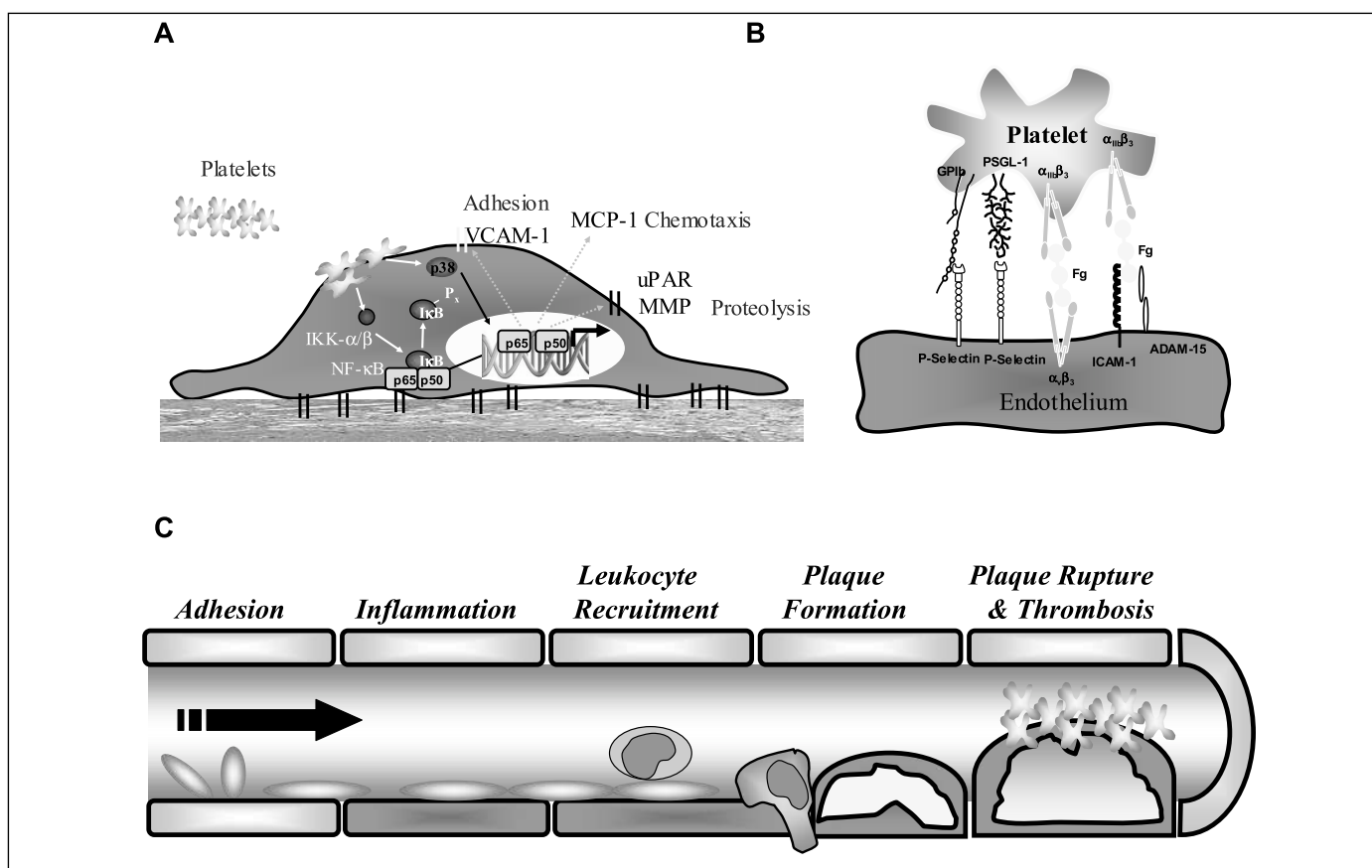


Figure 1: Platelets alter endothelial properties, promote inflammation and induce atherosclerosis. A) By adhesion to endothelial cells platelets induce release of chemoattractants, upregulation of endothelial adhesion molecules and secretion of metalloproteinases. This is mediated by interference with the I κ B and NF- κ B pathway in ECs. B) Distinct receptor-ligand pairs mediate the interaction of platelets

with endothelial cells and, thus, represent potential diagnostic and therapeutic targets in vascular disease. C) Platelets contribute to the pathogenesis of atherosclerosis from its initiation until plaque rupture, a process that involves complex and interacting steps, diverse cell types and mediating signals.

chemokine for atherogenesis is further strengthened by the fact that treatment of atherosclerosis-prone mice with inhibiting Met-RANTES resulted in altered lesion formation (3, 18). Thus, it has become evident that the deposition of RANTES is a principle mechanism by which activated platelets can support and sustain atherogenic monocyte recruitment. Another central proinflammatory substance released by platelets is IL-1 β . IL-1 β is of particular interest as it has been recently shown to be one of the proteins actively generated by platelets themselves (19). The generation of IL-1 β is stepwise regulated by a complex mechanism. The pre-mRNA for IL-1 β , which is present in quiescent platelets, needs to be spliced. This splicing is dependent on platelet activation and GPIIb/IIIa engagement (20). Besides platelets being on the effector face of endothelial inflammation, they themselves can be activated by chemokines. Endothelial bound CX3CL1 (Fractalkine), which has been shown to be over-expressed in atherosclerotic lesions and vascular injury, contributes to platelet activation and adhesion (21). Recently, it has become evident that CX3CL1 induced leukocyte adhesion is dependent on the presence of platelets and correspondingly specific inhibition of platelet adhesion to inflamed endothelial cells significantly reduced leukocyte recruitment to atherosclerotic

lesions *in vivo* (22). Interestingly, platelets not only recruit pro-inflammatory cells like monocytes to atherosclerotic lesions, but also express and release chemokines, for instance SDF-1, a process potentially inducing progenitor cell mediated regenerative mechanisms (see below) (23, 24). Moreover, platelets are capable of mediating arrest of immuno-modulatory dendritic cells *in vitro* as well as *in vivo* (25).

Taken together platelets and chemokines can act as mutual and bidirectional effectors most likely regulated by positive and negative feedback loops, which in case of dysregulation may lead to development of disease (1).

Platelet adhesion initiates atherosclerosis – a model of platelet-induced disease

Not long ago the concept of platelets in atherosclerosis was that of a cell belonging to the coagulation cascade and participating in the final step of atherosclerosis, the rupture of the plaque followed by thrombotic narrowing or occlusion of a vessel. However, abundant new insights into the pathogenesis of atherosclerosis and atherothrombosis have changed this dogma. Pla-

platelets are more and more recognized to be involved in the earliest processes of atherogenesis (Fig. 1C). Governed by disturbed flow platelets adhere to the arterial wall *in vivo*, even in the absence of endothelial cell denudation, thereby initiating lesion formation (26–27). The intact, non-activated endothelium normally prevents platelet adhesion to the extracellular matrix. Under inflammatory conditions platelets can adhere to the intact but activated endothelial cell monolayer (28–30). Even under high shear stress platelet adhesion to the intact endothelium occurs *in vivo* and is coordinated in a multistep process that involves platelet tethering, followed by rolling and subsequent firm adhesion to the vascular wall (31, 32). These processes are dependent on receptor interactions via selectins, integrins and immunoglobulin-like receptors, which induce receptor-specific activation signals in both platelets and the respective partner in cell-cell adhesion, for instance endothelial cells (Fig. 1B).

The initial loose contact between circulating platelets and vascular endothelium (“platelet rolling”) is mediated by selectins, present on both endothelial cells and platelets (33–35). P-selectin is rapidly expressed on the endothelial surface in response to inflammatory stimuli by translocating from membranes of storage granules (Weibel-Palade bodies) to the plasma membrane within seconds. Endothelial P-selectin has been demonstrated to mediate platelet rolling in both arterioles and venules in acute inflammatory processes (36). E-selectin, which is also expressed on inflamed endothelial cells, allows a loose contact between platelets and endothelium *in vivo*, as well (36). A study with P-selectin (encoded by the *Selp* gene)-deficient platelets demonstrated that platelets interact with inflamed endothelium through the binding of platelet P-selectin with an endothelial ligand (37). Moreover, bone marrow transplantation experiments showed that mice receiving *Selp*^{-/-} platelets (lacking P-selectin) developed smaller lesions than those receiving wild-type platelets (38). Even more dramatic findings were obtained in a model of wire-induced artery injury in *Selp*^{-/-} and *ApoE*^{-/-} double knockout mice (39). The strongest effects in the inhibition of atherosclerosis, however, seem to be obtainable with combined deficiency of E-selectins (the endothelial expressed selectin) and P-selectins, showing 80% and 40% protection in the early and advanced stages of the disease (40).

GPIIb-IIIa ($\alpha_{IIb}\beta_3$) is the major integrin on platelets and plays a key role in platelet accumulation on activated endothelium. In the presence of soluble fibrinogen, $\alpha_{IIb}\beta_3$ mediates heterotypic cell adhesion to $\alpha_v\beta_3$ -expressing cells including endothelial cells (30, 41). Moreover, platelets firmly adhere to activated endothelial cells via $\alpha_{IIb}\beta_3$, a mechanism, which can be blocked by antagonists of β_3 -integrins (30). *In vivo*, firm platelet adhesion to the endothelium can be inhibited by mAb anti- $\alpha_{IIb}\beta_3$, and platelets defective in $\alpha_{IIb}\beta_3$ do not firmly adhere to activated endothelial cells (42). Very recently, the role of $\alpha_{IIb}\beta_3$ for atherosclerosis related cerebral injury was further elucidated (43). To address the contribution of platelet GPIIb to atheroprotection atherosclerotic lesion formation in GPIIb(+/+) -ApoE(-/-) or GPIIb(-/-)ApoE(-/-) mice in the carotid artery and aortic arch was determined (43). Interestingly, the absence of GPIIb attenuated lesion formation in both vascular locations, indicating that platelets contribute substantially to atherosclerosis via GPIIb (43). Moreover, after occlusion of the middle cerebral artery, the cer-

bral infarct size was drastically reduced in mice lacking GP IIb compared with wild-types (43). In another report, however, blockade of platelet aggregation with anti-GPIIb/IIIa F(ab)2 fragments had no positive effect on stroke size but increased the incidence of intracerebral haemorrhage and mortality after transient middle cerebral artery occlusion in a dose-dependent manner (44). Further studies will have to clarify this issue. Among the integrins expressed on the luminal side of endothelial cells, the vitronectin receptor ($\alpha_v\beta_3$) furthermore appears to play a crucial role in promoting platelet adhesion. The vitronectin receptor is upregulated in response to endothelial cell activation, e.g. by IL-1 β or thrombin (30, 45). Inhibition of $\alpha_v\beta_3$ attenuates platelet-endothelial cell interaction (30). Hence, both platelet $\alpha_{IIb}\beta_3$ and endothelial $\alpha_v\beta_3$ are involved in mediating firm platelet adhesion to endothelial cells. In fact, heterotypic cell adhesion through $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ requires the presence of fibrinogen, which bridges the platelet fibrinogen receptor to the endothelial vitronectin receptor (Fig. 1B) (41). A central adhesion molecule for the contact of platelets with the vascular wall is GPIb-IX. The importance of this adhesion receptor for platelet-mediated vascular inflammation is demonstrated by the fact that inhibition of platelet adhesion with a blocking mAb to GPIb reduced atherosclerosis *in vivo* (46). In summary, platelet-endothelial cell interactions represent a multistep process, in which selectins, integrins and immunoglobulin-like adhesion receptors play a predominant role. These receptor-dependent platelet-endothelial cell interactions allow transcellular communication via soluble mediators leading to a proinflammatory status of both endothelial cells and further cells subsequently recruited to the scenario and, thus, contribute to vascular inflammation and atherosclerosis.

Impact of platelets on progenitor cells

Progenitor cells and vascular repair

The structural and functional integrity of the endothelium is determined by the balance between endothelial injury and repair. Prolonged exposure to cardiovascular risk factors leads to oxidative stress, elevated EC turnover and ultimately EC death. Vascular progenitor cells derived from different tissues have an ability to repair damaged vessel, in which the local micro-environment of the progenitors plays a crucial role in orchestrating cell homing and differentiation (47). Studies have demonstrated that atherosclerosis is a pathophysiological process initiated by EC death in specific areas such as bifurcation regions, which is followed by subsequent replacement by endothelial progenitor cells (EPCs) (48). Indeed, increasing evidence indicates that circulating EPCs contribute to re-endothelialization (49–51).

Progenitor cell recruitment by platelets

At sites of vascular injury or rupture of an atherosclerotic plaque various components of the subendothelial matrix are exposed including collagen or von Willebrand factor (52). Discontinuity of the vascular endothelium results in rapid platelet adhesion and thrombus formation (52, 53). As discussed in the previous sections, platelets are strong attractants for circulating pro-inflammatory cells like monocytes or neutrophils. Thus, it is likely that platelets can also provide a bridging mechanism for other circu-

lating cells like EPCs. Indeed, the first proof of this principle was delivered recently as isolated platelets were able to mediate adhesion of human CD34⁺ stem cells or mouse embryonal EPCs under arterial shear conditions (54, 55). Speaking of the mouse cells this adhesion was dependent on the P-selectin/PSGL-1 axis and β_1 -integrin (54, 55). Meanwhile other reports have confirmed the finding that progenitor cells can be recruited by platelets (56, 57). Using intravital microscopy, we could furthermore show that platelets provide the critical signal, which recruits murine CD34⁺ bone marrow cells and c-Kit⁺ Sca-1⁺ Lin⁻ bone marrow-derived progenitor cells to sites of vascular injury *in vivo* (23). Binding of bone marrow cells to platelets in this setting involved both P-selectin and GPIIb integrin on platelets. In the mouse system, moreover β_2 -integrins seem to play a pivotal role for the homing of circulating EPCs. In a murine model of hind limb ischemia, Sca-1⁺/Lin⁻ hematopoietic progenitor cells from β_2 -integrin-deficient mice are less capable of homing to sites of ischemia and of improving neovascularization (58). A relevance of β_2 -integrins for a platelet-EPC interaction has not been shown so far.

Influence of platelets on progenitor cell biology

The question arises whether the impact of platelets on progenitor cells is restricted to the recruitment of cells from the circulation. Once adherent or activated, platelets surface-upregulate or re-

lease an arsenal of highly active substances including potent cytokines, chemokines (see above) or mediators of proliferation (59). Therefore it seems likely that platelets are capable of modifying progenitor cell biology. Indeed, when exposed to isolated platelets murine embryonal EPCs or human CD34⁺ stem cells can differentiate towards mature endothelial cells (54, 60). Furthermore, platelets seem to enhance the biological power of EPCs by increasing their chemotaxis and migratory potential (54). The simple picture of platelets mediating regenerative effects through progenitor cells is very tempting. Like in most biological systems the picture is, however, more complex. Recently, we characterized another fate, which progenitor cells undergo after incubation with platelets. CD34⁺ human stem cells exposed to platelets under distinct conditions *in vitro* resulted in platelet uptake by the progenitor cells and finally in the development of foam cell-like cells (60–62). Therefore also contraproductive effects are mediated by platelet-EPC interaction, which can be controlled *in vitro* by the application of statins (60, 63). A major molecule involved in platelet-mediated effects on progenitor cells seems to be the chemokine SDF-1, as platelet-derived SDF-1 regulates recruitment, proliferation and differentiation of progenitor cells, a mechanism most likely contributing to vascular repair and regeneration of ischemic myocardium (23, 24, 64). Moreover, inhibition of the SDF-1/CXCR4 axis prevented recruitment of progenitor cells to vascular lesions *in vivo* (64, 65). For instance, pla-

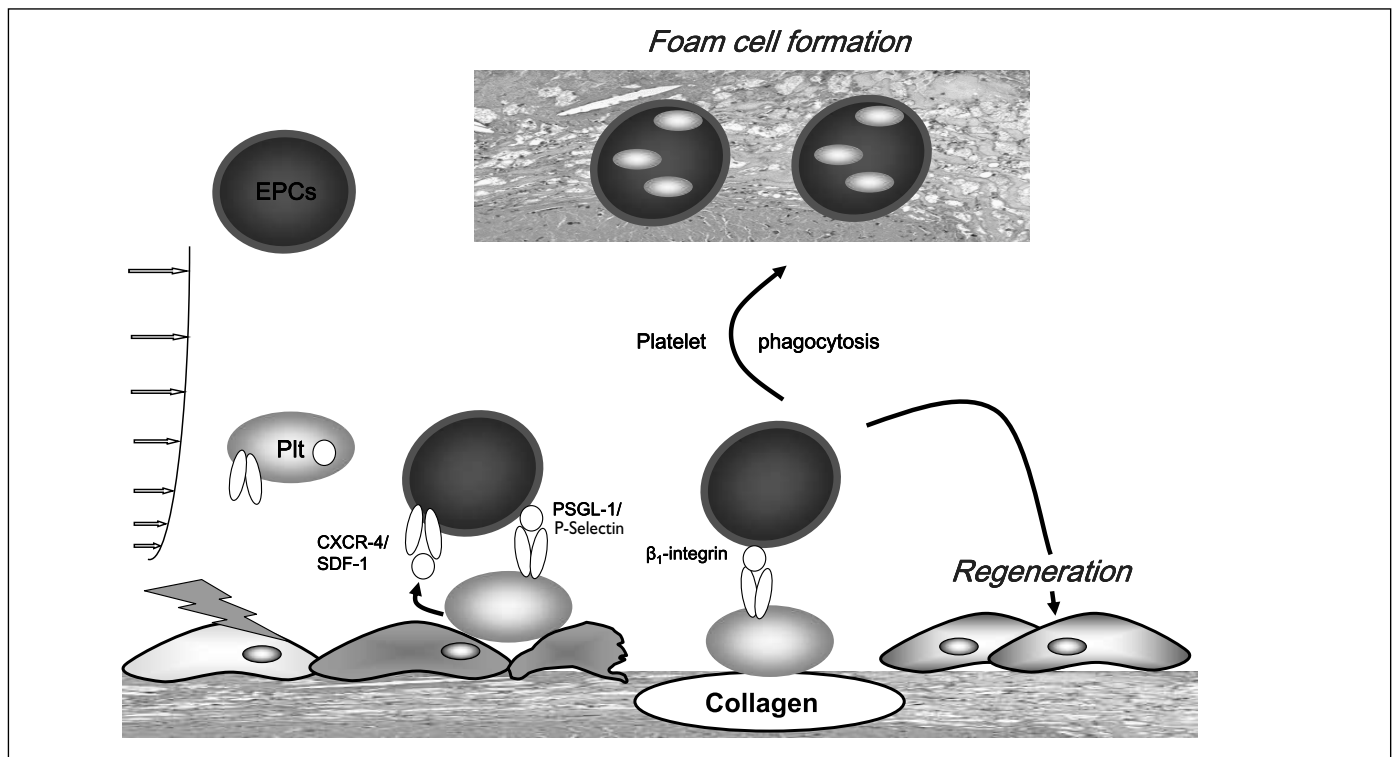


Figure 2: Platelets serve as a bridging mechanism for circulating endothelial progenitor cells and thereby can contribute to atheroprotection and vascular regeneration. Platelets accumulate at sites of dysfunctional endothelium and disrupted atherosclerotic lesions. By release of pro-inflammatory molecules they themselves can induce or aggravate endothelial dysfunction. Armed with po-

tent adhesion molecules for circulating cells, platelets are capable of recruiting circulating endothelial progenitor cells (EPCs). Depending on the surrounding microenvironment, pro-atherogenic (for instance the development of foam cells) or vascular reparatory mechanisms (differentiation of progenitor cells towards mature endothelial cells) can be promoted by interaction of platelets with progenitor cells.

platelet-derived SDF-1 binding to its receptor CXCR4 favours platelet-induced differentiation of CD34⁺ progenitor cells into mature endothelial cells *in vitro* (24, 64). Interestingly, the central angiogenic molecule VEGF or the structurally related molecule VEGF-C have been shown to be present in and released from activated platelets, implying a potential role of platelets in angiogenesis (66). Although platelets seem to represent a central regulatory element for progenitor cell fate in tissue regeneration (Fig. 2), the exact mechanisms mediating the differentiation of pro-

genitor cells towards the one or the other cell type and the relevance of these findings *in vivo* still have to be determined.

Altogether, platelets may have undesired effects mediating early and late mechanisms that promote atherosclerosis. However, they also execute positive tasks like the induction of repair mechanisms. Thus, understanding platelet interactions with further circulating cells, the vessel wall and its components is fundamental to understand vascular disease, to prevent its initiation and to invent powerful means to treat it.

References

- Weber C. Platelets and chemokines in atherosclerosis: partners in crime. *Circ Res* 2005; 96: 612–616.
- von Hundelshausen P, Petersen F, Brandt E. Platelet-derived chemokines in vascular biology. *Thromb Haemost* 2007; 97: 704–713.
- von Hundelshausen P, Weber KS, Huo Y, et al. RANTES deposition by platelets triggers monocyte arrest on inflamed and atherosclerotic endothelium. *Circulation* 2001; 103: 1772–1777.
- Scheuerer B, Ernst M, Durrbaum-Landmann I, et al. The CXC-chemokine platelet factor 4 promotes monocyte survival and induces monocyte differentiation into macrophages. *Blood* 2000; 95: 1158–1166.
- Sachais BS, Kuo A, Nassar T, et al. Platelet factor 4 binds to low-density lipoprotein receptors and disrupts the endocytic machinery, resulting in retention of low-density lipoprotein on the cell surface. *Blood* 2002; 99: 3613–3622.
- Nassar T, Sachais BS, Akkawi S, et al. Platelet factor 4 enhances the binding of oxidized low-density lipoprotein to vascular wall cells. *J Biol Chem* 2003; 278: 6187–6193.
- von Hundelshausen P, Weber C. Platelets as immune cells: bridging inflammation and cardiovascular disease. *Circ Res* 2007; 100: 27–40.
- Heeschen C, Dimmeler S, Hamm CW, et al. Soluble CD40 ligand in acute coronary syndromes. *N Engl J Med* 2003; 348: 1104–1111.
- Henn V, Slupsky JR, Grafe M, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature* 1998F; 391: 591–594.
- Otterdal K, Smith C, Oie E, et al. Platelet-derived LIGHT induces inflammatory responses in endothelial cells and monocytes. *Blood* 2006; 108: 928–935.
- Celik S, Langer H, Stellos K, et al. Platelet-associated LIGHT (TNFSF14) mediates adhesion of platelets to human vascular endothelium. *Thromb Haemost* 2007; 98: 798–805.
- Gawaz M, Neumann FJ, Dickfeld T, et al. Activated platelets induce monocyte chemotactic protein-1 secretion and surface expression of intercellular adhesion molecule-1 on endothelial cells. *Circulation* 1998; 98: 1164–1171.
- Mach F, Schonbeck U, Sukhova GK, et al. Reduction of atherosclerosis in mice by inhibition of CD40 signalling. *Nature* 1998; 394: 200–203.
- Lutgens E, Gorelik L, Daemen MJ, et al. Requirement for CD154 in the progression of atherosclerosis. *Nat Med* 1999; 5: 1313–1316.
- Sawicki G, Salas E, Murat J, et al. Release of gelatinase A during platelet activation mediates aggregation. *Nature* 1997; 386: 616–619.
- May AE, Kalsch T, Massberg S, et al. Engagement of glycoprotein IIb/IIIa (alphaIIb)beta3 on platelets upregulates CD40L and triggers CD40L-dependent matrix degradation by endothelial cells. *Circulation* 2002; 106: 2111–2117.
- Holm T, Damas JK, Holven K, et al. CXC-chemokines in coronary artery disease: possible pathogenic role of interactions between oxidized low-density lipoprotein, platelets and peripheral blood mononuclear cells. *J Thromb Haemost* 2003; 1: 257–262.
- Schober A, Manka D, von Hundelshausen P, et al. Deposition of platelet RANTES triggering monocyte recruitment requires P-selectin and is involved in neointima formation after arterial injury. *Circulation* 2002; 106: 1523–1529.
- Lindemann S, Tolley ND, Dixon DA, et al. Activated platelets mediate inflammatory signaling by regulated interleukin 1beta synthesis. *J Cell Biol* 2001; 154: 485–490.
- Denis MM, Tolley ND, Bunting M, et al. Escaping the nuclear confines: signal-dependent pre-mRNA splicing in anucleate platelets. *Cell* 2005; 122: 379–391.
- Schafer A, Schulz C, Eigenthaler M, et al. Novel role of the membrane-bound chemokine fractalkine in platelet activation and adhesion. *Blood* 2004; 103: 407–412.
- Schulz C, Schafer A, Stolla M, et al. Chemokine fractalkine mediates leukocyte recruitment to inflammatory endothelial cells in flowing whole blood: a critical role for P-selectin expressed on activated platelets. *Circulation* 2007; 116: 764–773.
- Massberg S, Konrad I, Schurzinger K, et al. Platelets secrete stromal cell-derived factor 1{alpha} and recruit bone marrow-derived progenitor cells to arterial thrombi *in vivo*. *J Exp Med* 2006; 203: 1221–1233.
- Stellos K, Gawaz M. Platelets and stromal cell-derived factor-1 in progenitor cell recruitment. *Semin Thromb Hemost* 2007; 33: 159–164.
- Langer HF, Daub K, Braun G, et al. Platelets recruit human dendritic cells via Mac-1/JAM-C interaction and modulate dendritic cell function *in vitro*. *Arterioscler Thromb Vasc Biol* 2007; 27: 1463–1470.
- Jorgensen L. ADP-induced platelet aggregation in the microcirculation of pig myocardial infarction and rabbit kidneys. *J Thromb Haemost* 2005; 3: 1119–1124.
- Theilmeier G, Michiels C, Spaepen E, et al. Endothelial von Willebrand factor recruits platelets to atherosclerosis-prone sites in response to hypercholesterolemia. *Blood* 2002; 99: 4486–4493.
- Bombeli T, Schwartz BR, Harlan JM. Adhesion of activated platelets to endothelial cells: evidence for a GPIIb/IIIa-dependent bridging mechanism and novel roles for endothelial intercellular adhesion molecule 1 (ICAM-1), alpha5beta3 integrin, and GPIIb/IIIa. *J Exp Med* 1998; 187: 329–339.
- Gawaz M, Neumann FJ, Ott I, et al. Platelet function in acute myocardial infarction treated with direct angioplasty. *Circulation* 1996; 93: 229–237.
- Gawaz M, Neumann FJ, Dickfeld T, et al. Vitronectin receptor (alpha(v)beta3) mediates platelet adhesion to the luminal aspect of endothelial cells: implications for reperfusion in acute myocardial infarction. *Circulation* 1997; 96: 1809–1818.
- Frenette PS, Johnson RC, Hynes RO, et al. Platelets roll on stimulated endothelium *in vivo*: an interaction mediated by endothelial P-selectin. *Proc Natl Acad Sci USA* 1995; 92: 7450–7454.
- Massberg S, Enders G, Leiderer R, et al. Platelet-endothelial cell interactions during ischemia/reperfusion: the role of P-selectin. *Blood* 1998; 92: 507–515.
- Frenette PS, Denis CV, Weiss L, et al. P-Selectin glycoprotein ligand 1 (PSGL-1) is expressed on platelets and can mediate platelet-endothelial interactions *in vivo*. *J Exp Med* 2000; 191: 1413–1422.
- Johnson RC, Mayadas TN, Frenette PS, et al. Blood cell dynamics in P-selectin-deficient mice. *Blood* 1995; 86: 1106–1114.
- Subramaniam M, Frenette PS, Saffaripour S, et al. Defects in hemostasis in P-selectin-deficient mice. *Blood* 1996; 87: 1238–1242.
- Frenette PS, Moyna C, Hartwell DW, et al. Platelet-endothelial interactions in inflamed mesenteric venules. *Blood* 1998; 87: 1238–1324.
- Huo Y, Schober A, Forlow SB, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med* 2003; 9: 61–67.
- Burger PC, Wagner DD. Platelet P-selectin facilitates atherosclerotic lesion development. *Blood* 2003; 101: 2661–2666.
- Manka D, Forlow SB, Sanders JM, et al. Critical role of platelet P-selectin in the response to arterial injury in apolipoprotein-E-deficient mice. *Arterioscler Thromb Vasc Biol* 2004; 24: 1124–1129.
- Dong ZM, Chapman SM, Brown AA, et al. The combined role of P- and E-selectins in atherosclerosis. *J Clin Invest* 1998; 102: 145–152.
- Gawaz MP, Loftus JC, Bajt ML, et al. Ligand bridging mediates integrin alpha IIb beta 3 (platelet GPIIb-IIIa) dependent homotypic and heterotypic cell-cell interactions. *J Clin Invest* 1991; 88: 1128–1134.
- Massberg S, Enders G, Matos FC, et al. Fibrinogen deposition at the postischemic vessel wall promotes platelet adhesion during ischemia-reperfusion *in vivo*. *Blood* 1999; 94: 3829–3838.
- Massberg S, Schurzinger K, Lorenz M, et al. Platelet adhesion via glycoprotein IIb integrin is critical for atheroprotection and focal cerebral ischemia: an *in vivo* study in mice lacking glycoprotein IIb. *Circulation* 2005; 112: 1180–1188.
- Kleinschnitz C, Pozgajova M, Pham M, et al. Targeting platelets in acute experimental stroke: impact of glycoprotein Ib, VI, and IIb/IIIa blockade on infarct size, functional outcome, and intracranial bleeding. *Circulation* 2007; 115: 2323–2330.
- Gawaz M, Brand K, Dickfeld T, et al. Platelets induce alterations of chemotactic and adhesive properties of endothelial cells mediated through an interleukin-1-dependent mechanism. Implications for atherogenesis. *Atherosclerosis* 2000; 148: 75–85.

46. Massberg S, Brand K, Gruner S, et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. *J Exp Med* 2002; 196: 887–896.
47. Xu Q. Progenitor cells in vascular repair. *Curr Opin Lipidol* 2007; 18: 534–539.
48. Xu Q. The impact of progenitor cells in atherosclerosis. *Nat Clin Pract Cardiovasc Med* 2006; 3: 94–101.
49. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997; 275: 964–967.
50. Asahara T, Masuda H, Takahashi T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 1999; 85: 221–228.
51. Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. *Circ Res* 2004; 95: 343–353.
52. Ruggeri ZM. Platelets in atherothrombosis. *Nat Med* 2002; 8: 1227–1234.
53. George JN. Platelets. *Lancet* 2000; 355: 1531–1539.
54. Langer H, May AE, Daub K, et al. Adherent platelets recruit and induce differentiation of murine embryonic endothelial progenitor cells to mature endothelial cells in vitro. *Circ Res* 2006; 98: e2–10.
55. Langer HF, May AE, Vestweber D, et al. Platelet-induced differentiation of endothelial progenitor cells. *Semin Thromb Hemost* 2007; 33: 136–13.
56. de Boer HC, Verseyden C, Ulfman LH, et al. Fibrin and activated platelets cooperatively guide stem cells to a vascular injury and promote differentiation towards an endothelial cell phenotype. *Arterioscler Thromb Vasc Biol* 2006; 6: 1653–1659.
57. Lev EI, Estrov Z, Aboulfatova K, et al. Potential role of activated platelets in homing of human endothelial progenitor cells to subendothelial matrix. *Thromb Haemost* 2006; 96: 498–504.
58. Chavakis E, Aicher A, Heeschen C, et al. Role of β 2-integrins for homing and neovascularization capacity of endothelial progenitor cells. *J Exp Med* 2005; 201: 63–72.
59. Berg C, Hammarstrom S, Herbertsson H, et al. Platelet-induced growth of human fibroblasts is associated with an increased expression of 5-lipoxygenase. *Thromb Haemost* 2006; 96: 652–659.
60. Daub K, Langer H, Seizer P, et al. Platelets induce differentiation of human CD34+ progenitor cells into foam cells and endothelial cells. *FASEB J* 2006; 20: 2559–2561.
61. Lindemann S, Kramer B, Seizer P, et al. Platelets, inflammation and atherosclerosis. *J Thromb Haemost* 2007; 5 (Suppl 1): 203–211.
62. Lindemann S, Kramer B, Daub K, et al. Molecular pathways used by platelets to initiate and accelerate atherogenesis. *Curr Opin Lipidol* 2007; 18: 566–573.
63. Daub K, Lindemann S, Langer H, et al. The evil in atherosclerosis: adherent platelets induce foam cell formation. *Semin Thromb Hemost* 2007; 33: 173–178.
64. Stellos K, Langer H, Daub K, et al. Platelet-derived SDF-1 regulates adhesion and favours differentiation of human CD34+ progenitor cells to endothelial cells. *Circulation* 2008; 117: 206–215.
65. Zernecke A, Schober A, Bot I, et al. SDF-1alpha/CXCR4 axis is instrumental in neointimal hyperplasia and recruitment of smooth muscle progenitor cells. *Circ Res* 2005; 96: 784–791.
66. Wartiovaara U, Salven P, Mikkola H, et al. Peripheral blood platelets express VEGF-C and VEGF which are released during platelet activation. *Thromb Haemost* 1998; 80: 171–175.
67. Boehlen F, Clemetson KJ. Platelet chemokines and their receptors: what is their relevance to platelet storage and transfusion practice? *Transfus Med* 2001; 11: 403–417.
68. Brandt E, Ludwig A, Petersen F, et al. Platelet-derived CXC chemokines: old players in new games. *Immunol Rev* 2000; 177: 204–216.
69. Struyf S, Burdick MD, Proost P, et al. Platelets release CXCL4L1, a nonallelic variant of the chemokine platelet factor-4/CXCL4 and potent inhibitor of angiogenesis. *Circ Res* 2004; 95: 855–857.
70. Klinger MH. Platelets and inflammation. *Anat Embryol (Berl)* 1997; 196: 1–11.
71. Gear AR, Camerini D. Platelet chemokines and chemokine receptors: linking hemostasis, inflammation, and host defense. *Microcirculation* 2003; 10: 335–350.
72. Power CA, Clemetson JM, Clemetson KJ, et al. Chemokine and chemokine receptor mRNA expression in human platelets. *Cytokine* 1995; 7: 479–482.
73. Schober A, Zernecke A, Liehn EA, et al. Crucial role of the CCL2/CCR2 axis in neointimal hyperplasia after arterial injury in hyperlipidemic mice involves early monocyte recruitment and CCL2 presentation on platelets. *Circ Res* 2004; 95: 1125–1133.
74. Klinger MH, Wilhelm D, Bubel S, et al. Immunocytochemical localization of the chemokines RANTES and MIP-1 alpha within human platelets and their release during storage. *Int Arch Allergy Immunol* 1995; 107: 541–546.
75. Kameyoshi Y, Dorschner A, Mallet AI, et al. Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. *J Exp Med* 1992; 176: 587–592.
76. Fujisawa T, Fujisawa R, Kato Y, et al. Presence of high contents of thymus and activation-regulated chemokine in platelets and elevated plasma levels of thymus and activation-regulated chemokine and macrophage-derived chemokine in patients with atopic dermatitis. *J Allergy Clin Immunol* 2002; 110: 139–146.