# Platelets in Atherosclerosis

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**Abstract**

Platelets play a critical role in haemostasis and thrombosis. Recent discoveries provide evidence that platelets promote atherogenesis and augment vascular inflammation and remodelling of the arterial wall, resulting in formation of atherosclerotic plaques. Upon activation, platelets adhere to the endothelial monolayer and release a variety of inflammatory mediators. Platelet-derived inflammatory mediators promote activation of the endothelial monolayer and recruitment of circulating blood cells, including monocytes and endothelial progenitor cells (EPCs). Further, platelets stimulate differentiation of monocyte and endothelial progenitor cells into macrophages and foam cells. Thus, at the site of platelet accumulation on the arterial wall, platelets form an inflammatory 'hot spot' that constitutes an early trigger for atherosclerotic lesions. On the other hand, platelets also control healing of vascular lesions by recruitment and differentiation of circulating endothelial progenitor cells to promote repair of the endothelial monolayer. Thus, platelets ensure both repair of vascular lesions ('healing') and foster lesion formation and progression, depending on atherosclerotic cofactors such as oxidized LDL (oxLDL). The diverse mechanisms by which platelets accomplish a balance between vascular injury and repair encompass a variety of inflammatory mediators and receptors. Recent clinical studies provide evidence that an intensified and prolonged antiplatelet therapy improves clinical prognosis of patients with atherosclerotic disease. This chapter highlights the recent developments concerning platelets in the context of atherosclerosis and highlights the novel therapeutic strategies.

## Introduction

The pathogenesis of atherosclerosis encompasses a strong inflammatory component that involves various cell types including platelets and inflammatory mediators (Lusis 2000; Libby et al. 2011). Not long ago the pathophysiological contribution of platelets to atherosclerosis was contemplated mainly in the context of the coagulation cascade, participating in the final step of atherosclerosis, and eventual thrombus build up following plaque rupture causing thrombotic narrowing or occlusion of a vessel. However, abundant experimental evidences have changed this point of view and established platelets as early responders seeding atheroprogression. Platelets are the prime responders to vascular injury and ensure haemostasis and vascular integrity to prevent extravasal blood loss (Ruggeri 2002, 2009). Platelets rapidly adhere to vascular lesions via adhesion receptors (e.g. GPIb, GPVI), aggregate via the fibrinogen receptors $\alpha_{IIb}\beta_3$ and become activated during the adhesion process. Usually, the intact endothelial monolayer prevents platelet attachment and accumulation at the vessel wall. However, inflamed endothelial cells (ECs) develop properties that render them adhesive for platelets. In accordance with the 'response-to-

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P. Gresele et al. (eds.), *Platelets in Thrombotic and Non-Thrombotic Disorders*, DOI 10.1007/978-3-319-47462-5_66

injury' hypothesis of atherogenesis, atherosclerotic lesions result in an orchestrated response to a localized injury to the vascular endothelium, which subsequently triggers platelet adhesion, aggregation and release of pro-inflammatory mediators like regulated upon activation normal T-cell expressed and secreted (RANTES), epithelial neutrophil-activating peptide (ENA-78), IL-1β, chemotactic (e.g. stromal-derived factor-1, SDF-1) and growth factors (e.g. platelet-derived growth factor, PDGF) from activated platelets. Upon activation platelets also release substantial amounts of alarmin or danger signals which function as inflammatory mediators and *damage-associated molecular pattern* proteins (DAMPs) (Gleissner et al. 2008; Gawaz et al. 2005; Karshovska et al. 2013; Gawaz and Vogel 2013). Activated platelets recruit circulating blood cells (e.g. monocytes and endothelial progenitor cells) at the site of vascular lesions and interact with resident vascular cells such as endothelial and smooth muscle cells (Langer and Gawaz 2008). Under physiological conditions all these mechanisms contribute to vascular repair and preservation of vessel integrity.

Platelets adhere under pathophysiological conditions to endothelial cells via multiple adhesion receptors including selectins, integrins and immunoglobulin-type receptors (Gawaz 2004). During the adhesion process, platelets become activated and release an arsenal of potent inflammatory and mitogenic substances into the local microenvironment, thereby alter the characteristics of the endothelial monolayer into a proatherogenic substrate (Gleissner et al. 2008; Karshovska et al. 2013). Platelet-derived chemokines and DAMPs attract circulating monocytes and endothelial progenitor cells towards the inflamed endothelium, which is a critical step in atherosclerotic lesion formation (Karshovska et al. 2013; von Hundelshausen and Schmitt 2014; Chatterjee and Gawaz 2013). Once circulating blood cells are recruited towards the atherogenic *hot spot*, platelets induce differentiation of recruited bone-marrow-derived cells into macrophages/foam cells (Massberg et al. 2006; Stellos and Gawaz 2007a, b; Langer et al. 2007; Stellos et al. 2008, 2010; Daub et al. 2006). A key mechanism in this differentiation process is the generation and uptake of oxidized low-density lipoprotein (oxLDL). OxLDL is bound to the surface and phagocytosed by platelets by means of scavenger receptors like CD36, LOX-1 and CXCL16/PS-OX, making platelets a major vehicle for oxLDL (Fig. 1)

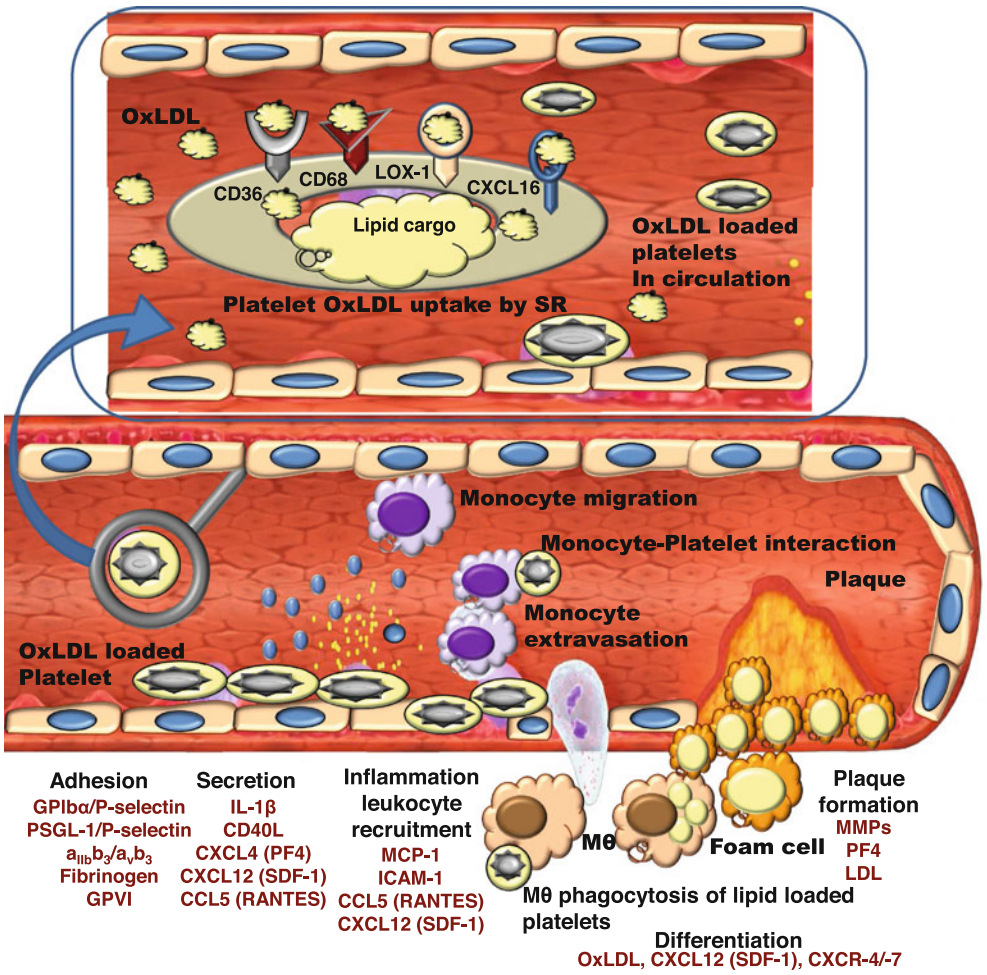


**Fig. 1** Platelets orchestrate atherogenesis. Above: Platelets interact with circulatory lipoproteins. Platelets can take up oxLDL by scavenger receptors like CD68, CD36, LOX-1 and CXCL16/PS-OX. Therefore platelets serve as lipid vehicles in circulation. Below: Activated lipid-laden platelets in circulation interact with the vascular endothelium, release pro-inflammatory factors and also induce a proatherogenic phenotype of ECs. Subsequently, adherent platelets recruit circulating monocytes, bind them and inflame them by receptor interactions and paracrine pathways, thereby initiate monocyte transmigration, their phagoctytic uptake of lipid-loaded apoptotic platelets and differentiation into macrophages and foam cells. Thus, platelets provide the inflammatory basis for plaque formation before physically occluding the vessel by thrombosis upon plaque rupture

(Siegel-Axel et al. 2008; Magwenzi et al. 2015; Badrnya et al. 2014). OxLDL-rich platelets are rapidly taken up by monocytes and stimulate transformation into macrophages and foam cells (Stellos et al. 2010; Daub et al. 2006, 2007; Chatterjee et al. 2015a, b; Nurden 2011). Thus, platelet adhesion, release of inflammatory mediators, recruitment of monocytes, uptake of lipid-laden platelets by monocytes and their differentiation into foam cells represent a critical step in early lesion formation of atherosclerotic plaques (Fig. 1). On the other hand, platelet interaction and phagocytosis by endothelial progenitor cells can induce differentiation into an endothelial phenotype that favours vascular repair and regeneration and may limit atherosclerotic lesion progression (Massberg et al. 2006; Stellos and Gawaz 2007a, b; Langer et al. 2007; Stellos et al. 2008, 2010; Daub et al. 2006) (Fig. 2). The key mechanisms that flip the switch between regeneration versus disease progression remain unknown. Thus, understanding the mechanistic basis that modulates platelet-dependent vascular inflammation and formation of atherosclerotic lesions is an attractive strategy to prevent or to control the consequences of atherosclerosis such as myocardial infarction or stroke.

## Platelet Adhesion to the Endothelium

Under pathophysiological circumstances platelets adhere even to the intact endothelial monolayer especially at the lesion-prone sites of vessel bifurcation governed by altered shear stress. Normal 'resting' endothelium represents a nonadhesive and non-thrombogenic surface that prevents extravasation of circulating blood cells. In contrast, activated endothelial cells are pro-adhesive and promote the adhesion of circulating blood platelets (May et al. 2008; Gawaz et al. 1991, 1996, 1998). Adhesion of platelets to the intact but activated endothelium in the absence of previous endothelial denudation involves a surface receptor-dependent process that allows *capturing* or tethering of circulating platelets towards the vessel wall even under high shear stress, followed by rolling and subsequent firm adhesion. Platelets are well equipped to adhere to endothelial cells in vitro and in vivo. Platelet interaction with intact endothelium is a well-orchestrated procedure (Fig. 3). 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 interacting cell partner involved in adhesion, for instance, endothelial cells. Initially, platelets roll loosely on the endothelial layer. Rolling is often dependent on endothelial activation induced by inflammatory assaults inflicted by infection, mechanic erosion or ischaemia and reperfusion. High levels of C-reactive proteins are associated with high rate of vascular events and promote platelet adhesion to endothelial cells. Platelets get activated during rolling interaction with the endothelium and subsequently adhere more and more tightly. Endothelial cells in turn get activated, too, and both cells express or secrete chemokines to perpetuate vascular inflammation. Activated endothelial cells surface-express ICAM-1, vascular cell adhesion molecule-1, E-selectin and P-selectin and release the chemokines MCP-1, SDF-1 and interleukin-8. Both activated platelets and endothelial cells can actively release pro-inflammatory interleukin-1β and CD40L. Platelet-specific release is chiefly characterized as RANTES and ENA-78. This vast array of secretory products, besides mediating interaction with leukocytes and endothelium, acts as inflammatory cues for the recruitment of immune or inflammatory cells, e.g. monocytes, also prompt their differentiation into macrophages to seed the process of plaque formation and atherosclerosis. Rolling is followed by firm adhesion that is mediated by integrin binding. Firm platelet adhesion triggers maximal platelet activation, instigating shape change, cytoskeletal rearrangement and release of potent inflammatory and mitogenic mediators into the local microenvironment. GPIIb-IIIa ($\alpha_{IIb}\beta_3$) is the major integrin on platelets and plays a key role in platelet accumulation on the activated endothelium. Among the integrins expressed on the luminal side of endothelial cells, the vitronectin receptor ($\alpha_v\beta_3$) plays a crucial role in promoting platelet adhesion. Another adhesion molecule for the contact of platelets with the vascular wall is GPIb-IX. Taken together platelet–endothelial cell interactions involving selectins, integrins and immunoglobulin-like adhesion receptors perpetuate transcellular communications leading to a pro-inflammatory status of both endothelial cells and inflammatory cells recruited to the site of action, thus contribute to vascular inflammation and atherosclerosis.

*The Role of Selectins* The initial loose contact between circulating platelets and the vascular endothelium ('platelet rolling') is mediated by selectins, present on both endothelial cells and platelets (Frenette et al. 1995, 1998a, b; Massberg et al. 1998; Subramaniam et al. 1996). P-selectin (CD62P) is rapidly expressed on the endothelial surface in response to inflammatory stimuli. In addition, P-selectin is stored in platelet α-granules and can rapidly translocate to the platelet surface upon activation. Endothelial P-selectin has been demonstrated to mediate platelet rolling in both arterioles and venules in acute inflammatory processes, such as ischaemia/reperfusion. Further, P-selectin glycoprotein ligand 1 (PSGL-1) is expressed on platelets and can mediate platelet–endothelial interaction in vivo (Lam et al. 2011; Frenette et al. 2000). E-selectin, which is also expressed on inflamed endothelial cells, allows a loose contact between platelets and the endothelium in vivo (Frenette et al. 1998a, b). In line with the concept of endothelial inflammation as a trigger for platelet accumulation, the process of platelet rolling does not require previous platelet activation, since platelets from mice lacking P- and/or E-selectin roll as efficiently as wild-type platelets (Frenette et al. 1995).

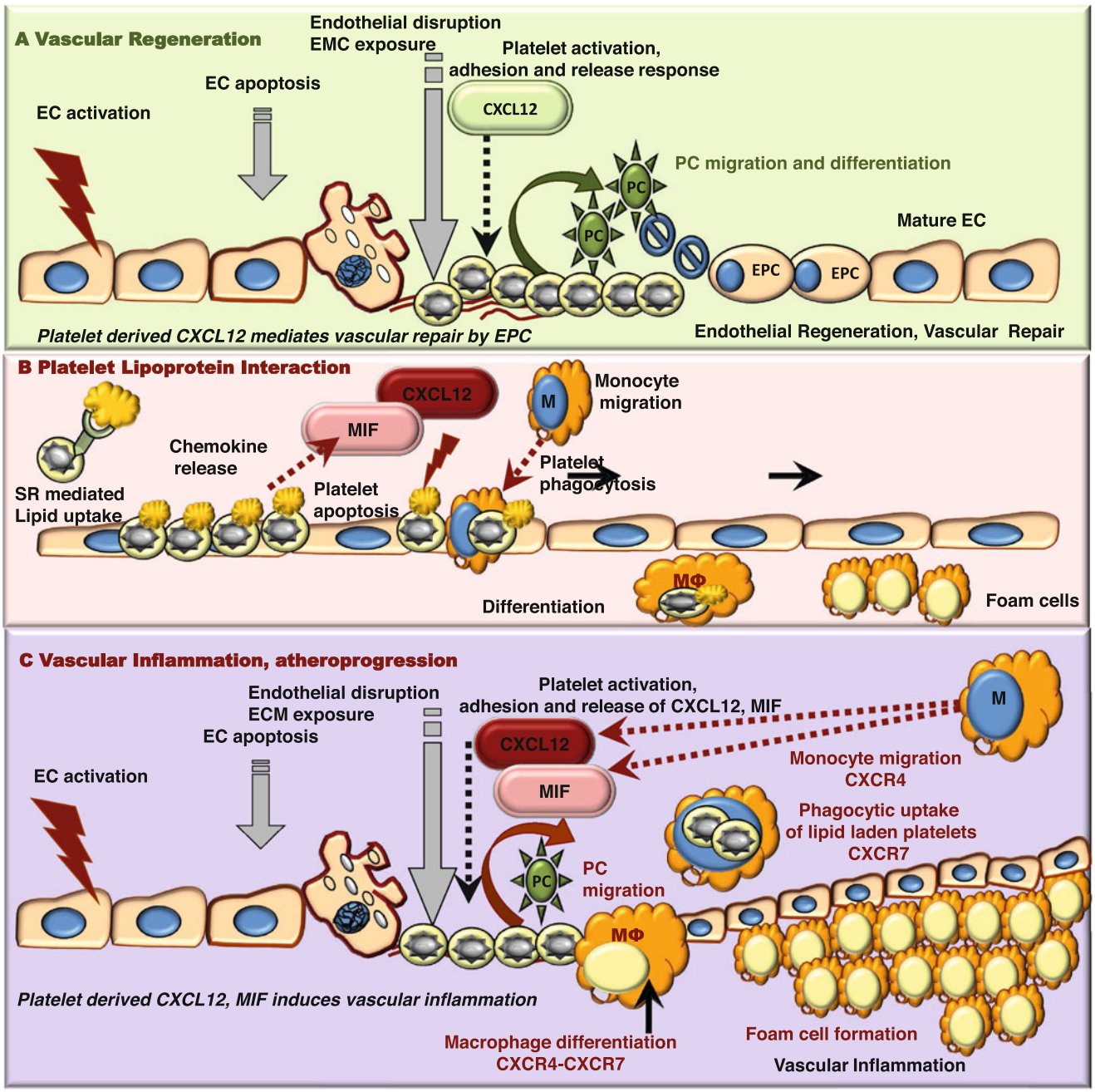


**Fig. 2** Platelet chemokines in balancing atheroprogression and vascular regeneration. Platelets adhere to and interact with the injured or inflamed endothelium (EC, endothelial cell) or exposed sub-endothelial matrix components (EMC) and secrete CXCL12 and MIF upon activation. Platelet-derived CXCL12 mediates the migration and differentiation of progenitor cells (PC) into an endothelial phenotype (endothelial progenitor cell, EPC) to promote vascular repair or regeneration (**a**) and also supports the migration of inflammatory monocytic cells (M). Monocytes phagocytose activated platelets and CXCL12 present in the immediate microenvironment supports their differentiation into foam cells. This contributes to vascular inflammation and injury (**c**). Platelets bind lipids like LDL and OxLDL in circulation through scavenger receptors. Lipid-loaded activated platelets adhere to the endothelium and release chemotactic factors like CXCL12 and MIF. Monocytes migrate towards CXCL12 and MIF. These infiltrated monocytes phagocytose lipid-laden apoptotic platelets and subsequently migrate into the intima of the vessel wall where they differentiate into macrophages and foam cells (**b**). Thus platelet–lipoprotein interaction (**b**) and platelet-derived factors can decide the balance of vascular regeneration (**a**) versus vascular inflammation and atheroprogression (**b–c**)

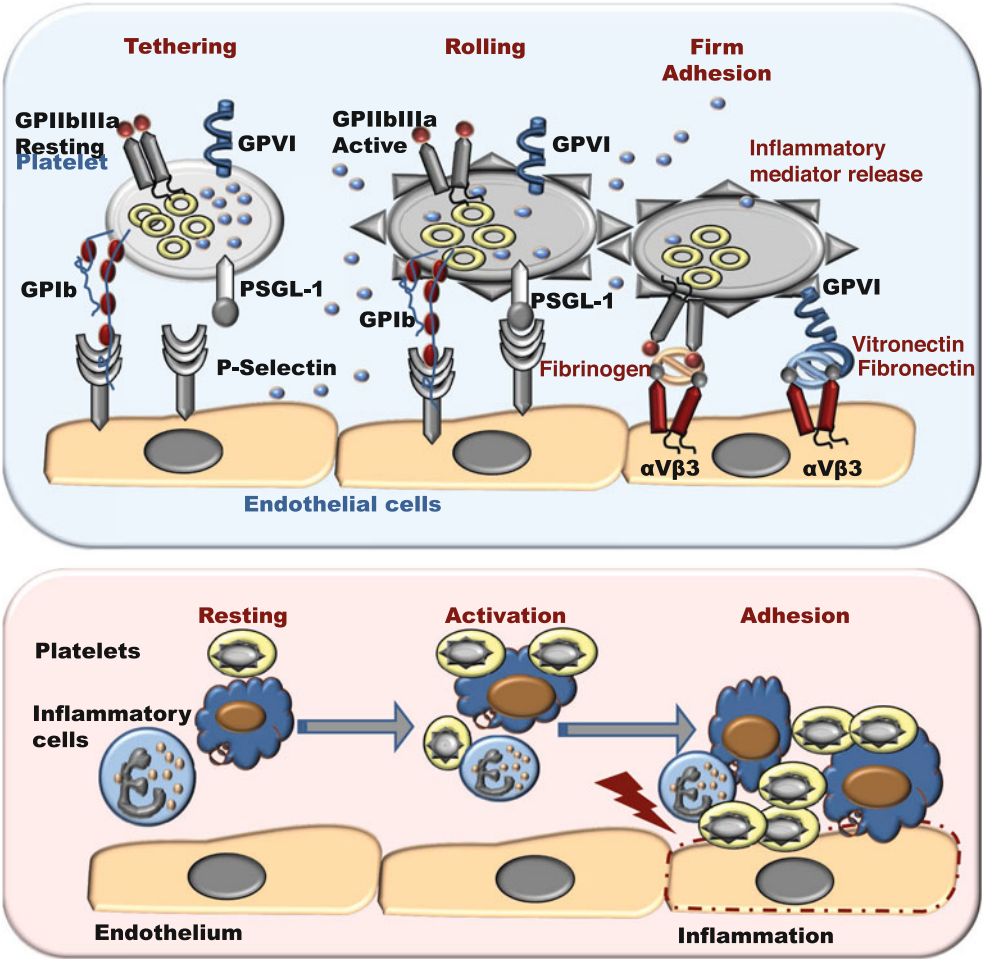


**Fig. 3** Platelet interaction with the vascular endothelium. Above: Platelet–endothelium adhesion is a multistep process involving several adhesion receptors. Activated endothelium surface expresses P-selectin. Platelet surface receptors GPIbα and PSGL-1 interact with endothelial P-selectin and mediate platelet rolling. Subsequent firm adhesion is mediated through β3 integrins. Below: Platelets recruit inflammatory cells like monocytes and neutrophils. Activated platelets in circulation form co-aggregates with inflammatory cells and foster their subsequent interaction with intact or inflamed endothelial cells and inflame monocytes. Thus, platelet–monocyte interaction provides an atherogenic milieu at the vascular wall that supports plaque formation

*The Role of GPIb-IX-V* Glycoprotein Ib (GPb) mediates platelet–endothelium adhesion (von Hundelshausen and Weber 2007). Glycoprotein Ib has been identified as counter-receptor for P-selectin (Etingin et al. 1993; Theilmeier et al. 2002). Platelet rolling on the activated endothelium can be inhibited by antibodies against both P-selectin and GPIbα (Frenette et al. 1995, 1998a, b; Massberg et al. 1998). Interactions of selectins with their counter-receptors are characterized by high on- and off-rates, enabling platelets to rapidly attach to the endothelial monolayer with high resistance to shear stress. However, due to their biophysical characteristics, selectin–ligand interactions are not sufficient to promote firm adhesion of platelets in the bloodstream. This implicates that these tighter interactions between platelets and the vascular wall involve the interplay of platelets and endothelial integrins as well as immunoglobulin-like adhesion molecules.

*The Role of Integrins* In the presence of soluble fibrinogen, $\alpha_{IIb}\beta_3$ mediates heterotypic cell adhesion to $\alpha_v\beta_3$-expressing cells including endothelial cells (Gawaz et al. 1991, 1996, 1997; Bombeli et al. 1998). Moreover, platelets firmly adhere to activated endothelial cells via $\alpha_{IIb}\beta_3$, a mechanism that can be blocked by antagonists of β3-integrins (Gawaz et al. 1997). In vivo, firm platelet adhesion to the endothelium can be inhibited by anti-$\alpha_{IIb}\beta_3$ mAb, and platelets defective in $\alpha_{IIb}\beta_3$ do not firmly adhere to activated endothelial cells (Gawaz et al. 1997).

*The Role of Immunoglobulin-Type Receptors* The intercellular adhesion molecule-1 (ICAM-1) is surface expressed on the inflamed endothelium and acts as an endothelial fibrinogen receptor, promoting fibrinogen deposition at the inflamed endothelium (Springer 1994). ICAM-1-fibrinogen interactions have been demonstrated to promote cell adhesion to activated endothelial cells (Bombeli et al. 1998). Another immunoglobulin-type platelet receptor GPVI mediates platelet adhesion to immobilized collagen, fibronectin and vitronectin, the matrix proteins which are exposed upon erosion of the inflamed endothelium (Bültmann et al. 2010; Schönberger et al. 2012).

*The Role of Platelet Adhesion Receptors in Atheroprogression* With the help of appropriate atherosclerotic animal models, it has become evident that platelets

adhere to the arterial wall early in the process of atherosclerosis before atherosclerotic plaques are morphologically detectable (Massberg et al. 2002, 2005; Huo et al. 2003). Bone marrow transplantation experiments demonstrated that mice receiving P-selectin-deficient platelets develop smaller lesions (Burger and Wagner 2003). Even more drastic findings were obtained in a model of wire-induced arterial injury in *P-selectin*$^{-/-}$*Apoe*$^{-/-}$ double knockout mice (Manka et al. 2004). The most striking effects in inhibiting atherosclerosis could be achieved with a combined deficiency of E-selectins and P-selectins, showing 80 % and 40 % protection in the early and advanced stages of the disease (Dong et al. 1998). Another critical adhesion molecule mediating platelet contact with the vascular wall is GPIb-IX, the significance of which is exemplified by the fact that prolonged antibody blockade of platelet GPIbα profoundly reduced leukocyte accumulation in the arterial intima and attenuated atherosclerotic lesion formation (Massberg et al. 2002). The contribution of platelet GPIIb ($\alpha_{IIb}$) was also validated in atherosclerotic lesion formation in the carotid artery and aortic arch among *GPIIb*$^{+/+}$*Apoe*$^{-/-}$ and *GPIIb*$^{-/-}$*Apoe*$^{-/-}$ mice where the absence of GPIIb attenuated lesion formation at both vascular locations (Massberg et al. 2005). Further, administration of activated platelets and platelet–leukocyte/monocyte aggregates in *Apoe*$^{-/-}$ mice promote formation of atherosclerotic lesions (Huo et al. 2003). In *Apoe*$^{-/-}$ mice, platelet adhesion to atherosclerotic arteries was significantly inhibited by i.v. injection of soluble GPVI-Fc (Schulz et al. 2008; Schonberger et al. 2008; Ungerer et al. 2013). Long-term administration of an anti-GPVI antibody attenuates atherosclerosis in *Apoe*$^{-/-}$ mice (Schulz et al. 2008). Further, gene transfer of GPVI-Fc to the carotid vascular wall significantly attenuates atheroprogression and endothelial dysfunction in atherosclerotic rabbits in vivo (Bültmann et al. 2010). Inhibition of GPVI both via GPVI-Fc and anti-GPVI antibodies results in protection against atherosclerosis in both cholesterol-fed rabbits and *Apoe*$^{-/-}$ mice (Bültmann et al. 2010).

## Platelet Interaction with Leukocytes

Activated platelets promote leukocyte arrest on the vascular endothelium, which is a key process of vascular inflammation during the progression of atherosclerosis (Lusis 2000; Libby et al. 2011). The interaction of platelets with leukocytes has been extensively described (Zarbock et al. 2007). Briefly, platelets physically interact with leukocytes (Rinder et al. 1991; Gawaz et al. 1994; Ott et al. 1996; May et al. 1997) and EPCs (Stellos et al. 2013). Platelet–leukocyte coaggregation can foster adhesion to the intact arterial wall. This interaction can occur in variable sequences: first, platelets can coaggregate with leukocytes in circulation and thereby support leukocyte recruitment to the endothelium by activating leukocyte adhesion receptors or by directly serving as bridging cells. For example, platelet–monocyte co-aggregates can attach to the vascular endothelium by both platelet and endothelium or by monocyte–endothelium contacts. Second, when adhered to the endothelium, platelets can chemoattract leukocytes and then provide a surface for their adhesion to the vascular wall. During these interactions involving platelets, leukocytes and the endothelium, all cell types involved become activated in a cascade-like manner (Fig. 3). Upon adhesion, platelets rapidly translocate P-selectin from α-granules to the plasma membrane. This allows leukocytes to tether to platelets via PSGL-1/P-selectin interaction. Subsequently, monocytes or polymorphonuclear cells firmly adhere to platelets in a Mac-1-dependent (CD11b/CD18, αMβ2) manner (Chavakis et al. 2003). On platelets, various counter-receptors of Mac-1 have been identified: GPIbα (Simon et al. 2000), junctional adhesion molecule-C (JAM-C, JAM-3) (Santoso et al. 2002), CD40L (Zirlik et al. 2007), ICAM-2 (Diacovo et al. 1994) as well as bridging proteins, such as fibrinogen (bound to $\alpha_{IIb}\beta_3$) (Altieri et al. 1988) or high-molecular-weight kininogen (Chavakis et al. 2003). However, the exact contribution of each receptor system awaits clarification. During this adhesive process, receptor engagement of PSGL-1 and Mac-1, together with platelet-derived inflammatory mediators, induces complex activation cascades in monocytes (Neumann et al. 1997; McEver and Cummings 1997; Weyrich et al. 1996) including NFkB activation and thereby promotes monocyte or neutrophil adhesion (by upregulation and activation of Mac-1 and VLA-4), thrombosis (mediated through monocyte secretion of tissue factor), monocytic chemokine and cytokine release (IL-1β, IL-8, MCP-1, TNF-α) (Neumann et al. 1997; McEver and Cummings 1997; Weyrich et al. 1996; Celi et al. 1994) or the oxidative burst of neutrophils (Zarbock et al. 2007). In addition, engagement of PSGL-1 by P-selectin also drives translationally regulated expression of proteins, such as the urokinase receptor (uPAR), a critical surface protease receptor and regulator of integrin-mediated leukocyte adhesion (May et al. 1998) in vivo. Additional adhesion receptor pairs also appear to be involved to induce vascular inflammation. For example, we have recently identified the extracellular matrix metalloproteinase (MMP) inducer (EMMPRIN, CD147) as a monocyte receptor that induces MMP-9 synthesis and secretion on cellular interactions (Schmidt et al. 2006; Lindemann et al. 2007). Thus multifaceted heterotypic platelet–endothelial cell, platelet inflammatory cell or progenitor cell interactions allow transcellular communication via soluble mediators inflicting pro-inflammatory damage to the vascular endothelium and subsequently recruit inflammatory cells to the site of atheroprogression (Fig. 4).

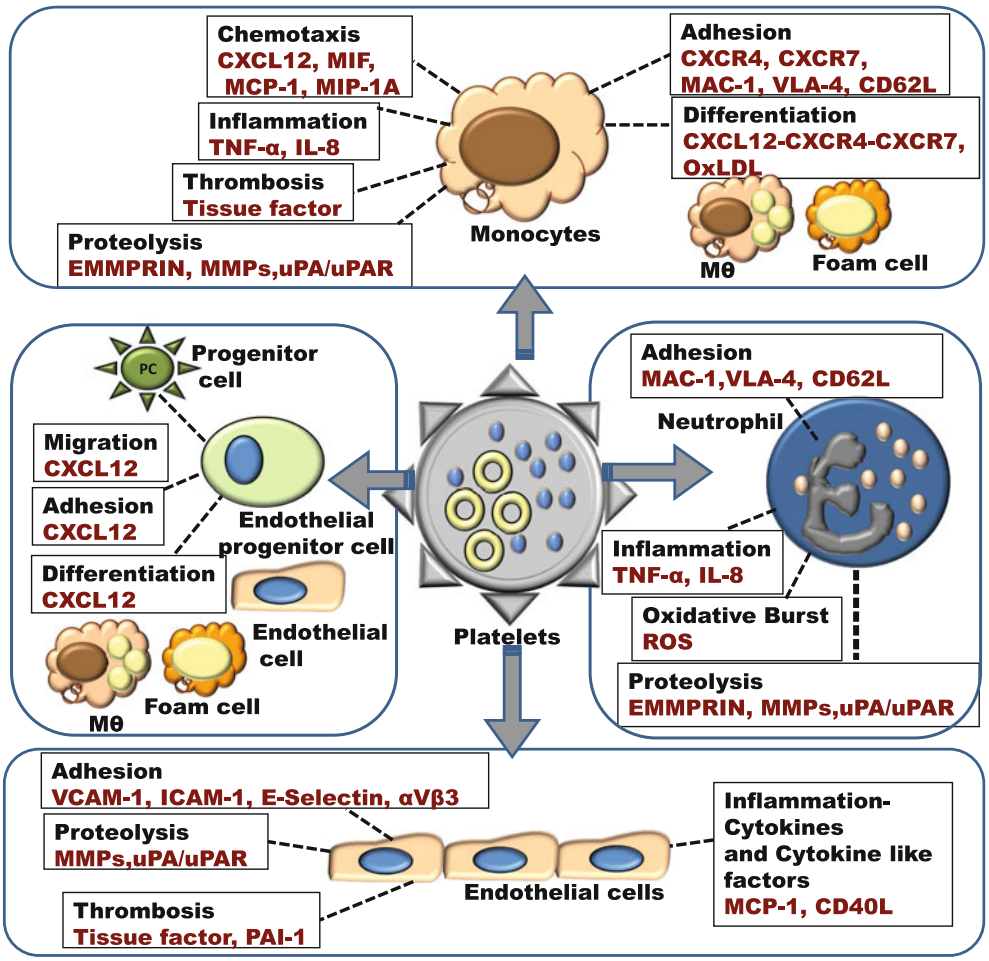


**Fig. 4** Platelets have the ability to modulate atherothrombosis via interaction with other vascular cells. Adherent platelets inflame ECs. By adhesion to endothelial cells, platelets induce release of chemoattractants, upregulation of endothelial adhesion molecules and secretion of metalloproteinases. This is mediated by interference with the IκB and NF-κB pathway in ECs. Distinct receptor–ligand pairs mediate the interaction of platelets with endothelial cells involving $\alpha_{IIb}\beta_3$ induces platelet surface exposure of P-selectin (CD62P) and release of CD40L and IL-1β, which stimulate ECs to provide an inflammatory milieu that supports proatherogenic alterations of the endothelium. Platelet adhesion thereby contributes to atheroprogression, a process that involves complex and interacting steps, diverse cell types and mediators. Adherent platelets recruit and inflame monocytes. Adherent and/or activated platelets mainly interact with monocytic PSGL-1 via P-selectin and with monocytic Mac-1 (αMβ2) via $\alpha_{IIb}\beta_3$ (and fibrinogen bridging) or GPIbα. Thereby, platelets initiate monocyte secretion of chemokines, cytokines and procoagulatory tissue factor, upregulate and activate adhesion receptors and proteases and induce monocyte differentiation into macrophages. Thus, platelet–monocyte interaction provides an atherogenic milieu at the vascular wall that supports plaque formation. On the other hand, platelets serve as a bridging mechanism for circulating endothelial progenitor cells and can contribute to atheroprogression and vascular regeneration. Platelet derived inflammatory substances like CXCL12 along with potent 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 Inflammatory Mediators

Platelet–endothelium interaction occurs in the macro- and microcirculation of inflamed tissue and during reperfusion of ischaemic organs. Platelets adhere to the intact or inflamed endothelium or exposed sub-endothelial matrix components and release pro-inflammatory and mitogenic mediators including cytokines, chemokines and DAMPs that alter physiological characteristics of the vascular endothelium and either prompt tissue (Stellos and Gawaz 2007a, b) restoration or inflict vascular injury (Gawaz and Vogel 2013; Lindemann et al. 2007; Langer et al. 2007) (Fig. 2). Proteomic analysis of platelets has identified more than 300 inflammatory mediators including growth factors, chemokines, cytokines and angiogenic compounds that are released into the microenvironment of accumulating platelets (Maynard et al. 2007). Most of these inflammatory

mediators have been evaluated for paracrine cell function and are potent chemotactic compounds for monocytes. Some of the platelet-derived inflammatory mediators have been also shown to promote and modulate platelet function (*autocrine loop*) (Fig. 5). Atherosclerotic plaques are rich in chemokines including RANTES (Gear et al. 2001). Platelet-derived PF4 and RANTES immobilized on endothelial cells induce monocyte arrest on the activated microvascular or aortic endothelium (von Hundelshausen et al. 2005), promote survival of emigrated monocytes and their differentiation into macrophages (Scheuerer et al. 2000). Atherosclerotic plaque is enriched with CXCL12 deposited from adherent platelets, and a counter-regulation between CXCL12-driven domiciliation of endothelial progenitors (Massberg et al. 2006; Stellos et al. 2008) and PF4–RANTES-triggered recruitment of monocytes (Scheuerer et al. 2000) might influence the outcome of vascular regeneration as opposed to atheroprogression. Other CXC chemokines like fractalkine (Schäfer et al. 2004) and CXCL16 (Borst et al. 2012; Seizer et al. 2011) can heighten atherothrombosis by prompting platelet activation, degranulation and adhesion at sites of atherosclerotic developments. Evidently, fractalkine and CXCL12 are upregulated in neointimal SMCs, which become luminally exposed after arterial denudation (Scheuerer et al. 2000), whereby platelet activation under the pro-inflammatory influence of SMCs might inflict further vascular damage. This part of the chapter summarizes the celebrated platelet-derived accomplices in atheroprogression and particularly highlights the recently unravelled contribution of MIF, CXCL12, CXCL16, HMGB1 and CyPA which have emerged as new molecular targets.

**Cytokines** Platelet activation results in the release of interleukin-1 (IL-1β) and CD40 ligand (CD40L) (Gawaz et al. 2000; Henn et al. 1998). Activated platelets rapidly synthesize IL-1β via an extranuclear polysomal translation mechanism (Lindemann et al. 2001). Platelet–IL1β induces activation of human endothelial cells and smooth muscle cells and augments neutrophil adhesion (Gawaz et al. 2002; Massberg et al. 2003). CD40L, which belongs to the TNF family is cleaved and released from activated platelets (Henn et al. 1998). Platelet–CD40L induces synthesis and secretion of chemokines, adhesion molecules and metalloproteinases in endothelial cells and promotes neutrophil adhesion. Engagement of $\alpha_{IIb}\beta_3$ on platelets upregulates CD40L and triggers CD40L-dependent matrix degradation by endothelial cells (May et al. 2002). Administration of activated wild-type platelets but not CD40L-deficient platelets stimulates atherosclerotic lesion formation (Lievens et al. 2010).

**Macrophage Migration Inhibitory Factor (MIF)** MIF is an inflammatory cytokine with chemokine-like functions that plays a role in atherosclerosis (Morand et al. 2006; Bernhagen et al. 2007; Strüßmann et al. 2013). Expression of the pleiotropic inflammatory mediator is enhanced and intricately

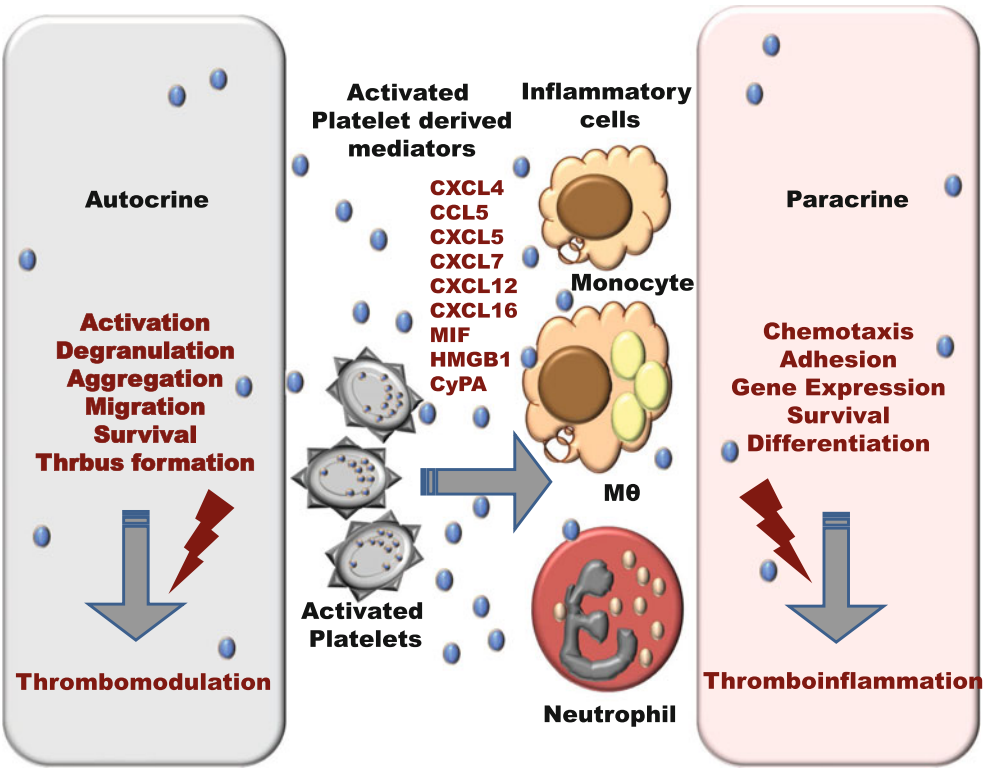


**Fig. 5** Autocrine and paracrine effects of platelet-derived factors influence thrombomodulation and thromboinflammation. Platelet-derived inflammatory mediators play a critical role for thromboinflammation (paracrine) and thrombomodulation (autocrine), important mechanisms involved in vascular inflammation and atherosclerosis. Autocrine effects of platelet-derived CCL5, CXCL4, CXCL7, CXCL12, CXCL16, MIF, HMGB1 and extracellular CyPA modulate platelet activation, aggregation and thrombotic events, whereas in a paracrine mode of action, they influence chemotactic migration, adhesion, survival, differentiation and other pro-inflammatory attributes of inflammatory leukocytes by regulating expression of pro-inflammatory genes

associated with course of atherosclerotic progression. The therapeutic impact of MIF in atherosclerosis is exemplified by the fact that peripheral MIF depletion in *Apoe*$^{-/-}$ mice reduces atheroprogression (Bernhagen et al. 2007). MIF is a major platelet-derived chemotactic factor with a retarded release kinetics secreted by the nonclassical secretory pathway independent of ER–Golgi network, however results from degranulation of 60 % of total MIF reserve (Strüßmann et al. 2013; Wirtz et al. 2015) following thrombin and GPVI stimulation. MIF shows a diffused granular pattern of intracellular distribution but does not share co-localization with other α-granule constituents like PF4 and VEGF (Strüßmann et al. 2013). The chemotactic capacity of stimulated platelet supernatants is substantiated by MIF, suggesting a role for atherogenic cell recruitment (Strüßmann et al. 2013; Wirtz et al. 2015). The presence of neutralizing anti-MIF antibody significantly reduces the chemotactic potential of thrombin-activated platelet supernatant, whereas chemotactic potential of activated platelet supernatant derived from *mif*$^{-/-}$ mice is drastically reduced (Wirtz et al. 2015). Moreover, endothelial monolayers incubated with supernatants from MIF-enriched thrombin-stimulated platelets show significantly enhanced monocyte adhesion, supporting its potential as a platelet-derived pro-inflammatory mediator (Wirtz et al. 2015). MIF binds to several chemokine receptors including CXCR-2, CXCR-4 and CXCR-7 (Strüßmann et al. 2013; Wirtz et al. 2015; Chatterjee et al. 2014a, b; Alampour-Rajabi et al. 2015). Plasma levels of MIF are enhanced in acute coronary syndrome and associated with the inflammatory response (Müller et al. 2012, 2013, 2014). MIF binds to the DAN–protein gremlin-1, and a complex formation of MIF with gremlin-1 inhibits its chemotactic activity and reduces atheroprogression in *Apoe*$^{-/-}$ mice (Müller et al. 2013). Plasma levels of MIF and gremlin-1 are associated with acuity of coronary artery disease, and the MIF/gremlin-1 ratio might determine the grade of plaque stability in humans (Müller et al. 2014).

**Chemokines** The CC and CXC chemokines such as CCL5 (RANTES), CXCL4 (PF4), CXCL5 (ENA-78), CXCL7 (β-TG), CXCL12 (SDF-1) and CXCL16 are the most intensively studied platelet-derived chemokines in the context of atherosclerosis. *CCL5 (RANTES)* is highly expressed and stored in platelets (Karshovska et al. 2013). Platelet–CCL5 is deposited on the endothelial monolayer and favours monocyte activation and adhesion (von Hundelshausen et al. 2001). CCL5 forms heteromers with *CXCL4 (PF4)*, and this complex synergistically supports monocyte/endothelium adhesion (Koenen et al. 2009). Administration of activated platelets in *Apoe*$^{-/-}$ mice enhances CCL5/CXCL4 deposition on the arterial wall and promotes atheroprogression via P-selectin (Huo et al. 2003). Plasma levels of CXCL5 and CXCL7 are increased in *Apoe*$^{-/-}$ mice (Rousselle et al. 2013). *CXCL5* enhances cholesterol efflux in macrophages and seems to be atheroprotective (Rousselle et al. 2013). *CXCL7* cleavage results in formation of platelet basic protein (PBP), beta-thromboglobulin (β-TG) and CTAPIII; all of these cleaved proteins are not chemotactic. After release those proteins are further activated by N-terminal shortening and promote migration of neutrophils and endothelial progenitor cells. *CXCL12 (SDF-1)* is stored in α-granules (Chatterjee et al. 2011) and gets surface expressed and released upon activation (Massberg et al. 2006; Stellos et al. 2008). Platelet–CXCL12 expression is increased in patients with myocardial infarction and correlates with circulating progenitor cells (Stellos et al. 2009). Platelet–CXCL12 and platelet surface expression of CXCR4–CXCR7 is associated with prognosis and recovery of myocardial function in patients with acute coronary syndromes (Geisler et al. 2012; Wurster et al. 2013; Rath et al. 2014, 2015). CXCL12 is a key mediator of regenerative mechanisms and regulates homing and trafficking of EPCs towards vascular and tissue lesions. Platelet–CXCL12 enhances neovascularization by mobilization of CXCR4$^{+}$ cells in the mice hind limp ischaemia model (Jin et al. 2006) indicating recruitment of bone marrow-derived progenitors to support vascular repair. Platelet–CXCL12 interacts with CXCR4-positive EPCs and stimulates adhesion and endothelial differentiation. Systemic application of CXCL12 promotes mobilization of smooth muscle progenitor cells and accumulation in vascular lesions resulting in a stable plaque phenotype in *Apoe*$^{-/-}$ mice (Akhtar et al. 2013). Further, recombinant SDF1–GPVI triggers chemotaxis of CXCR4+ cells, preserves cell survival, enhances endothelial differentiation of BMCs in vitro and reveals proangiogenic effects. In a mouse model of myocardial infarction, administration of the bifunctional recombinant protein SDF1–GPVI leads to enhanced recruitment of BMCs, increases capillary density, reduces infarct size and preserves cardiac function (Ziegler et al. 2012). Thus, platelet–CXCL12 may be an atheroprotective chemokine which needs to be further explored. **CXCL16** is a multifaceted chemokine. Membrane-associated CXCL16/PS-OX is an OxLDL scavenger receptor which facilitates OxLDL binding to activated platelets, in its solubilized form exerts a stimulatory effect on their haemostatic and thrombotic attributes and also mediates inflammatory associations with the endothelium and eryptotic erythrocytes to corroborate atheroprogressive complications. Platelet–CXCL16 surface expression is further enhanced upon activation, and in ACS patients as compared to SAP, shows positive correlation with plasma C-reactive protein and markers of myocardial necrosis (Borst et al. 2012; Seizer et al. 2011). Recent results of a population-based HUNT2 cohort in Norway indicate that high levels of soluble CXCL16 is associated with risk of MI in healthy subjects (Laugsand et al. 2015). CXCL16 is detected in the human carotid atherosclerotic lesions from complex carotid endarterectomy specimens, sequesters circulating platelets through CXCR6 engagement and supports vWF-mediated platelet associations (Meyer Dos Santos et al. 2015). Moreover, high CXCL16 expression is

observed in the endothelium in close proximity to mural thrombus enriched in vWF and platelet GPIbα (Meyer Dos Santos et al. 2015). CXCL16 supplementation also supports platelet adhesion to the injured carotid artery in vivo (Borst et al. 2012). Platelets also release CXCL16 following PAR-1 activation by TRAP and therefore could contribute to CXCL16 plasma levels (Seizer et al. 2011). Moreover, since activated platelets release CXCL16 (Seizer et al. 2011), they might be an enriched source of circulatory CXCL16 levels among ACS patients, which serves as a peripheral biomarker and is associated with long-term motility. *CXCL16*$^{-/-}$ mice show decreased cholesterol efflux and attenuated atheroprogression. The role of platelet–CXCL16 however remains unclear and megakaryocyte–platelet-lineage-specific *CXCL16*$^{-/-}$ needs to be developed.

**DAMPs** Damage-associated molecular pattern molecules (DAMPs) are a heterogeneous group of nuclear or cytosolic host proteins that can initiate and perpetuate a noninfectious inflammatory response. DAMPs are released or exposed on the cell surface following tissue injury. Recently, two DAMPs have been recognized in platelets which contribute to regulation of thromboinflammatory events, namely, cyclophilin A (CypA) (Coppinger et al. 2004; Seizer et al. 2014, 2015; Elvers et al. 2012) and high-mobility group box 1 (HMGB1) (Rouhiainen et al. 2000; Ahrens et al. 2015; Vogel et al. 2014, 2015a, b). Platelet-bound CypA is enhanced in stable CAD patients, and its surface expression is associated with hypertension and hypercholesterolemia (Seizer et al. 2015). In patients with acute myocardial infarction (AMI), platelet-bound CyPA is significantly decreased (Seizer et al. 2015). CyPA stimulates vascular smooth muscle cell migration and proliferation, endothelial cell adhesion molecule expression and inflammatory cell chemotaxis (Seizer et al. 2015). *Apoe*$^{-/-}$ mice develop more severe atherosclerosis compared with *Apoe*$^{-/-}$/*CypA*$^{-/-}$ mice (Nigro et al. 2011). Platelets are a recently recognized source of high-mobility group box 1 (HMGB1) (Rouhiainen et al. 2000; Ahrens et al. 2015; Vogel et al. 2014, 2015a, b), the circulatory levels of which are elevated in multiple inflammatory diseases (de Souza et al. 2012). Platelets express HMGB1, which is translocated towards surface following activation and subsequently released (Rouhiainen et al. 2000; Ahrens et al. 2015; Vogel et al. 2014, 2015a, b). HMGB1 expression is increased in macrophages and SMCs at atherosclerotic lesions and is implicated in the progression of plaque (de Souza et al. 2012). The contribution of platelet-derived HMGB1 to influence infiltration of inflammatory cells during atheroprogression remains to be seen. Activated platelets interfere with recruitment of mesenchymal stem cells to apoptotic cardiac cells via HMGB1/TLR-4-mediated downregulation of hepatocyte growth factor receptor MET (Vogel et al. 2014). Recently, using a platelet-specific *Pf4-cre-HMGB1*$^{-/-}$ transgenic mice, we have identified platelet-derived HMGB1 as a critical mediator of thrombosis and inflammation in vivo (Vogel et al. 2015a, b). HMGB1 regulates microvascular endothelial inflammation (Fiuza et al. 2003) and leukocyte recruitment (Venereau et al. 2013); platelet-derived HMGB1 instigates neutrophil extracellular trap (NET) formation (Maugeri et al. 2014) and thrombosis (Vogel et al. 2015a, b) suggesting probable inflammatory potential of platelet-derived HMGB1 in linking atheroprogression and atherothrombosis. These evidences encourage further investigations to validate the potential of platelet-derived HMGB1 in influencing the cellular composition of atherosclerotic plaque and thereby its immunogenicity and vulnerability. HMGB1 is highly expressed in platelet-rich human coronary artery thrombi (Ahrens et al. 2015) pointing towards a central role for HMGB1 in atherothrombosis, also suggesting the therapeutic potential of platelet-targeted anti-inflammatory therapeutic strategies for CAD patients.

Platelet-derived HMGB1 might confer heterotypic plaque cellularity rendering them vulnerable, which combined with a pro-thrombotic disposition is an active accomplice in atherothrombosis and subsequent ischaemic events.

## Regulation of Platelet Function and Thrombosis by Platelet-Derived Inflammatory Mediators

Upon release platelet-derived compounds can mediate both paracrine and autocrine effects (Fig. 5). Whereas the role of platelet-derived inflammatory mediators for paracrine actions and tissue inflammation has been extensively investigated, the autocrine effects of these molecules on platelet function are far less understood. Platelets express several chemokine receptors like CXCR-4, CXCR-6, CXCR-7 which render them susceptible to autocrine modulation imposed by factors such as CXCL12, CXCL16 and MIF (Chatterjee et al. 2015a, b). Further, platelets express receptors for CypA and HMGB1 (e.g. EMMPRIN, RAGE, TLR4) that significantly modulate platelet functions (Fig. 6). **CXCL12** substantiates platelet aggregation and thrombosis (Abi-Younes et al. 2000; Walsh et al. 2014; Kowalska et al. 2000; Gear et al. 2001; Shenkman et al. 2004). CXCL12 enhances platelet activation through Gαi-coupled CXCR4 (Abi-Younes et al. 2000; Walsh et al. 2014; Kowalska et al. 2000; Gear et al. 2001; Shenkman et al. 2004), antagonizes adenylate cyclase activity and counteracts $PGI_2$ analogue-induced cAMP levels (Walsh et al. 2014) and further substantiates granular release, PLC activation triggering aggregation. CXCL12–CXCR4-induced primary phase of aggregation occurs through PI3K, whereas the secondary wave involves engagement of downstream tyrosine kinases

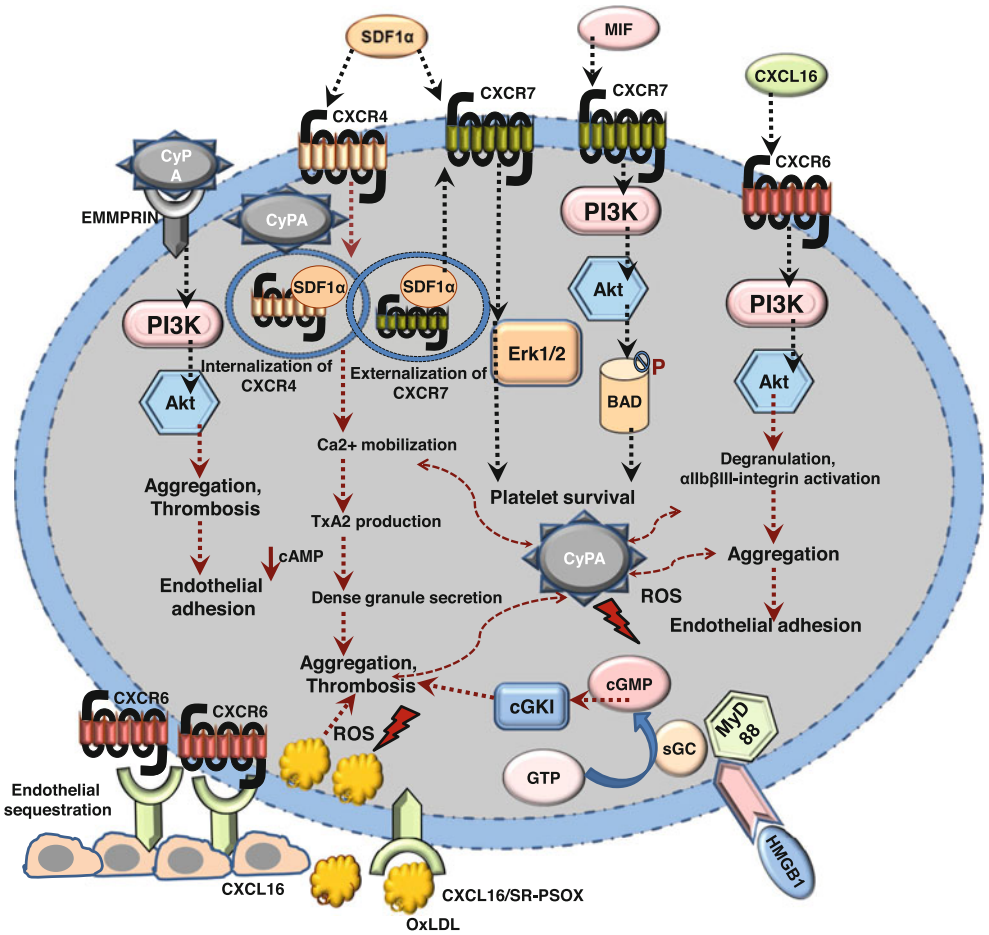


**Fig. 6** New platelet-derived mediators in the context of atherothrombosis and atherosclerosis. CXCL12 through CXCR4 ligation substantiates platelet activation, aggregation and thrombotic potential. MIF exerts a prosurvival effect through CXCR7 and downstream activation of the PI3K–Akt pathway culminating in phosphorylation-mediated inactivation of pro-apoptotic protein BAD. The membrane-associated form of CXCL16/SR-PS-OX functions as a scavenger receptor facilitates OxLDL binding and sequesters CXCR6+ platelets from circulation to CXCL16-expressing endothelial cells at atherosclerotic sites. Soluble CXCL16 acts through CXCR6–PI3K–Akt pathway to promote degranulation, cytoskeletal reorganization and shape change, $\alpha_{IIb}\beta_{III}$-integrin activation and adhesion to endothelial layer. Intracellular cyclophilin A (CyPA) is involved in bidirectional trafficking of CXCR4–CXCR7 and extracellular CyPA acting through EMMPRIN engagement regulates aggregation, thrombotic potential of platelets. HMGB1 enhances platelet activation, dense and α-granule secretion, aggregatory response to GPVI stimulation and thrombus formation. HMGB1 exerts its effects through TLR4, myeloid differentiation factor 88-dependent (MyD88), recruitment of guanylyl cyclase (GC) towards platelet membrane and subsequent activation of cGMP-dependent protein kinase I (cGKI) in platelets

and prostanoids to achieve maximal irreversible aggregation and degranulation. CXCL12 co-stimulates aggregation induced by sub-threshold concentrations of agonists under arterial and lower shear conditions (Abi-Younes et al. 2000; Walsh et al. 2014; Kowalska et al. 2000; Gear et al. 2001; Shenkman et al. 2004) and exhibits selective synergistic aggregatory response with serotonin (5HT) but not epinephrine (Abi-Younes et al. 2000; Walsh et al. 2014; Kowalska et al. 2000; Gear et al. 2001; Shenkman et al. 2004). Chemokines are capable of heterophilic interactions exerting synergistic or antagonizing effects. RANTES noncompetitively reduces the stimulatory effect of CXCL12 on aggregation and adhesion to endothelial monolayers under venous flow conditions (Shenkman et al. 2004). Mature platelets also exhibit transmigration through endothelial layer towards CXCL12, mediated through CXCR4–Gαi–PI3K pathway which triggers platelet activation (Kraemer et al. 2010, 2011). Platelets preferentially accumulate at areas with high CXCL12 under flow conditions and exhibit flow-directed migration (Kraemer et al. 2010, 2011).

The receptors of CXCL12, CXCR4 and CXCR7 are constitutively expressed in platelets at transcript and protein levels. The relative surface expression of CXCR4 appears to be much higher than that of CXCR7 at resting state (Chatterjee et al. 2014b). Surface expression of CXCR4–CXCR7 exhibits a unique dynamism in the presence of their ligands, CXCL12 (Chatterjee et al. 2014b) and MIF (Chatterjee et al. 2014a) which influences their relative abundance on platelet surface,

thereby their frequency of participation in effector functions. CXCR4 is internalized by CXCL12 and MIF, while CXCR7 is preferentially externalized in response to CXCL12 but not MIF (Chatterjee et al. 2014b). CXCL12 induced bidirectional trafficking of CXCR4, and CXCR7 is a coupled process executed through the downstream signalling intermediates like Erk1/2 and the PPIase activity of intracellular molecular chaperone CyPA culminating in ubiquitination-driven externalization of CXCR7 (Chatterjee et al. 2014b). However, chemokine-induced receptor trafficking in platelets is a comparatively new idea and warrants further investigations in targeted pathophysiologies where platelet responsiveness and their inflammatory potential are altered. Moreover, CXCL12-executed effects on receptor trafficking can be influenced by the presence of other factors in the immediate microenvironment having differential binding affinities towards their cognate receptors (Chatterjee et al. 2014b). CXCL12 binds to CXCR4 on platelet surface with approximately 2000 sites/platelet and an affinity of 24 nmol/L but shares CXCR4 with MIF. Once released MIF can engage CXCR4 and CXCR7, while CXCR2 surface expression is relatively low and CD74 is absent on platelets. MIF ligates but does not influence CXCR7 availability on platelets. This discrepancy with CXCL12 is attributed to the absence of CD74 (which acts as a co-receptor for MIF–CXCR4 axis) and, therefore, lack of Erk1/2 activation downstream of CXCR4–MIF ligation, which is essential for CXCR7 externalization (Chatterjee et al. 2014a). At CXCL12/MIF-enriched atherosclerotic plaques, this dynamic receptor trafficking could influence relative CXCR4–CXCR7 availability with major functional implications. Both CXCL12 and MIF are ligands for the chemokine receptors CXCR4 and CXCR7 (Chatterjee et al. 2014a, b; Alampour-Rajabi et al. 2015). Unlike CXCL12, MIF does not seem to modulate platelet activation, degranulation or prompt release of chemokines (CXCL4, CXCL12) either alone or in combination with other agonists (Strüßmann et al. 2013; Wirtz et al. 2015). MIF does not amend aggregation by itself or that elicited by ADP and TxA2 analogue or influence integrin activation and spreading over fibrinogen (Strüßmann et al. 2013; Wirtz et al. 2015). MIF, unlike CXCL12, is unable to mobilize intracellular calcium pools in TxA2 receptor presensitized platelets; however, both CXCL12 and MIF significantly block/desensitize increases in calcium transient in response to ADP (Strüßmann et al. 2013; Wirtz et al. 2015). Thus, CXCL12 and MIF although sharing receptors show distinct effects on platelet functionality, possibly executed through distinct intracellular signalling cascades. However, MIF, like CXCL12, as a survival factor, rescues platelets from activation and BH3-mimetic induced apoptosis through CXCR7 engagement (Chatterjee et al. 2014a). The MIF–CXCR7-triggered anti-apoptotic effect is mediated through the PI3K–Akt pathway which culminates in phosphorylation-induced inactivation of pro-apoptotic effector BAD. CXCL12-induced surface availability of CXCR7 can mediate subsequent prosurvival benefits of both CXCR12 and MIF (Chatterjee et al. 2014a). Unlike CXCL12, as a prosurvival agent, MIF attenuates pro-thrombotic phosphatidylserine exposure on platelet surface, whereby MIF–CXCR7 also exerts an antithrombotic effect (Chatterjee et al. 2014a). Thus, current experimental evidence points towards a functional preference in executing haemostatic and thrombotic functions through CXCR4 (by CXCL12), while supports platelet survival through CXCR7 (by CXCL12 and MIF).

CXCL16/SR-PS-OX is a transmembrane chemokine which is cleaved and shedded from the plasma membrane via ADAM10 (Seizer et al. 2011). Soluble CXCL16 chiefly functions as a chemokine for inflammatory cells through CXCR6/BONZO (Borst et al. 2012). CXCL16 can induce platelet degranulation, $\alpha_{IIb}\beta_{III}$-integrin activation, cytoskeletal reorganization and shape change and adhesion to the endothelium under arterial flow conditions in vitro. CXCL16 enhances aggregatory response to sub-threshold ADP concentrations and fibrinogen (Borst et al. 2012). CXCL16 effects mediated through CXCR6 lead to downstream activation of PI3K–Akt pathway and are therefore significantly abrogated in $CXCR6^{-/-}$ and $Akt^{-/-}$ mice (Borst et al. 2012) and following pharmacological inhibition of the PI3K–Akt pathway. Moreover, CXCL16-triggered platelet activation is diminished in the presence of apyrase and purinergic $P_2Y_1$ and $P_2Y_{12}$ receptor antagonists suggesting a feedback loop mediated through ADP (Borst et al. 2012). Immobilized or microsphere-bound CXCL16, resembling membrane-tethered version of the chemokine, enhances intracellular calcium mobilization and integrin activation, degranulation and aggregation responses to ADP (Borst et al. 2012; Meyer Dos Santos et al. 2015). Therefore, CXCL16 both as a soluble mediator and membrane-associated form can modulate activation and haemostatic functions of platelets.

The DAMP protein CyPA is an abundantly expressed intracellular protein which executes a variety of functions due to its PPIase activity. Intracellular CyPA participates in the bidirectional CXCR4–CXCR7 trafficking (Chatterjee et al. 2014b), whereas extracellular CyPA functions as a redox–stress-sensitive pro-inflammatory cytokine that contributes to atherosclerosis, cardiac hypertrophy myocardial infarction and myocarditis (Seizer et al. 2014). Intracellular CyPA also influences thrombotic and haemostatic functions (Elvers et al. 2012). $Cypa^{-/-}$ mice have prolonged bleeding time; exhibit impaired platelet degranulation, spreading, cytoskeleton reorganization, shape change and aggregation; and show attenuated thrombus formation despite comparable platelet counts and lack of severe haematologic abnormalities (Elvers et al. 2012). Therefore CyPA exerts dual effects in regulating platelet

function: it regulates $Ca^{2+}$ signalling, and when released into the extracellular space, CyPA binds to its extracellular receptor CD147 (EMMPRIN) and thereby initiates a cascade of inflammatory processes. CyPA has been identified in the proteomic analysis of activated platelet releasate as one of >300 proteins, also detected at atherosclerotic plaques where platelets accumulate (Coppinger et al. 2004). Extracellular CyPA activates platelets via CD147/EMMPRIN-mediated PI3K–Akt, leading to enhanced platelet adhesion and thrombus formation in vitro even among *Cypa*$^{-/-}$ mice, independent of intracellular CyPA (Seizer et al. 2015). Thus platelet-derived CyPA is a potential therapeutic target in inflammatory cardiovascular ailments.

Another platelet–DAMP protein is HMGB1. Extracellular HMGB1 enhances platelet activation, dense and α-granule secretion, aggregatory response to GPVI stimulation, adhesion and spreading (Vogel et al. 2015a, b). Collectively, these functional modulations drive a pro-thrombotic disposition. HMGB1 exerts its effects through TLR4, myeloid differentiation factor 88-dependent (MyD88) recruitment of guanylyl cyclase (GC) towards platelet membrane and subsequent activation of cGMP-dependent protein kinase I (cGKI) in platelets. With platelets and pro-thrombotic aspects of HMGB1 come to light, the pathophysiological implication of platelet-derived HMGB1 needs to be evaluated in atherosclerosis (Vogel et al. 2015a, b).

In summary, platelet-derived inflammatory mediators play a critical role for thromboinflammation (paracrine) and thrombomodulation (autocrine), important mechanisms involved in vascular inflammation and atherosclerosis.

## Effect of Low-Density Lipoproteins on Platelet Function

Lipoproteins are fundamental 'players' in atherogenesis since they change the properties of different cells involved in atherosclerosis and thrombosis. Low-density lipoproteins (LDL) and its oxidized form bind to the platelet surface via scavenger receptors SR-B, CD36, LOX-1 or CXCL16 (Siegel-Axel et al. 2008). Both oxLDL and LDL activate platelets and induce thrombus formation (Siegel-Axel et al. 2008). Platelets uptake and store both oxLDL and LDL in significant amounts in dense granules (Daub et al. 2007). Lipid-laden platelets are phagocytosed by macrophages (Daub et al. 2006) and induce foam cell development from monocytes and $CD34^+$ progenitor cells (Daub et al. 2006). The soluble scavenger receptor CD68 inhibits platelet-dependent foam cell generation in vitro (Daub et al. 2010) and atheroprogression in *Apoe*$^{-/-}$ mice (Zeibig et al. 2011). Further, oxLDL-laden platelets activate the endothelium, and the number of $CD34^+$ progenitor cells (colony-forming units), which would otherwise transform into endothelial cells, is significantly reduced in the presence of oxLDL loaded–platelets (Daub et al. 2007; Lindemann et al. 2007). Patients with ACS show significantly enhanced oxLDL binding on platelets as compared to patients with stable coronary CAD. Platelet-bound oxLDL positively correlates with the degree of platelet activation and plasma oxLDL levels. Preincubation of isolated platelets with OxLDL, but not with native LDL, results in enhanced platelet adhesion to collagen and activated endothelial cells under high shear stress in vitro, as well as after carotid ligation in *Apoe*$^{-/-}$ and wild-type mice (Stellos et al. 2012). Recently we found that oxLDL uptake by platelets induces platelet apoptosis, like other platelet agonists like thrombin and collagen-related peptide (CRP). CXCL12 facilitates phagocytosis of lipid-laden platelets by monocytes and M1–M2 macrophages and also promotes their differentiation into foam cells via CXCR4 and CXCR7 (Chatterjee et al. 2015a, b). Stimulation of platelets with oxLDL results in the formation of platelet–monocyte aggregates (PMA) and phagocytosis of platelets thereby increases oxLDL uptake by monocytes, dependent on platelet CD36 and CXCL4 release (Badrnya et al. 2014). Thus, platelets have the capacity to store and to transfer significant amounts of oxLDL to sites of atherosclerotic lesions as vehicles, which strengthens the significance of platelets in atherogenesis.

## Platelet-Based Theranostics in Atherosclerosis

Apart from inflammatory mediators as highlighted in this chapter and summarized in Table 1, glycoprotein receptors like GPVI offer a platform to regulate platelet responsiveness, thrombotic propensity, inflammatory disposition and immune reactivity particularly in atheroprogression (Fig. 7). Platelet GPVI surface expression is enhanced following acute ischaemic events like myocardial infarction and cerebral stroke, thereby it serves as a biomarker and is associated with poor prognosis (Gawaz et al. 2014). Platelet adhesion to atherosclerotic lesions or ruptured plaques is primarily mediated through GPVI which favours atherothrombosis (Gawaz et al. 2014). Several diagnostic biomarkers have been developed in recent years for evaluation of thromboischaemic coronary and cerebrovascular diseases. These have significantly improved diagnosis and patient care and facilitated individualized risk assessment. A non-invasive platelet-based diagnostic and therapeutic strategy has emerged, which utilizes GPVI for lesion-directed antithrombotic therapy or to counteract atherosclerotic disposition. The objective of this approach is to ameliorate care of patients particularly in the context of cardiocerebrovascular medicine (Gawaz et al. 2014). Conventional imaging modalities to define atherosclerotic vessel disease and luminal stenosis have poor prognostic impact in

**Table 1** Platelet-derived mediators contributing to atherosclerosis and atherothrombosis

| Platelet-derived inflammatory mediator | Mediator | Receptor | Mechanisms effecting platelet biology | Refs. |
|---|---|---|---|---|
| Cytokines | Interleukin-1β | IL-1R | Induces activation of human endothelial cells and smooth muscle cells and augments neutrophil adhesion | Gawaz et al. (2000, 2002, 2005), Henn et al. (1998), Koenen et al. (2009), Lievens et al. (2010), Lusis (2000), Magwenzi et al. (2015), Maugeri et al. (2014) |
| | CD40L[a] | | Induces synthesis and secretion of chemokines, adhesion molecules and MMPs in endothelial cells and promotes neutrophil adhesion and matrix degradation by endothelial cells | Gawaz et al. (2005), Henn et al. (1998), Huo et al. (2003), Koenen et al. (2009), Lievens et al. (2010), Lindemann et al. (2007), Magwenzi et al. (2015), May et al. (1998) |
| | MIF[a] | CXCR-4 and CXCR-7 | Anti-apoptotic, antithrombotic and atherosclerotic progression, plasma levels elevated in CAD and correlate with disease severity | Alampour-Rajabi et al. (2015), Chatterjee et al. (2014a), Müller et al. (2012, 2014), Naghavi et al. (2003), Theilmeier et al. (2002), Zarbock et al. (2007) |
| Chemokines | | | | |
| CCL type | CCL-1 (I-309) | CCR8 | Proatherogenic | Gawaz et al. (2005), Henn et al. (1998), Koenen et al. (2009), Lievens et al. (2010), Magwenzi et al. (2015) |
| | CCL-2 (MCP-1) | CCR2 | Proatherogenic, chemotactic. CCL2 presentation by platelets supports monocyte adhesion in vitro and neointima formation in vivo | Gawaz et al. (2005), Henn et al. (1998), Koenen et al. (2009), Lievens et al. (2010), Magwenzi et al. (2015), May et al. (1997) |
| | CCL-3 (MIP-1a) | CCR-1, CCR-2 and CCR-3 | Proatherogenic, facilitates neointimal formation | Gawaz et al. (2005), Henn et al. (1998), Koenen et al. (2009), Lievens et al. (2010), Magwenzi et al. (2015) |
| | CCL5 (RANTES)[a] | CCR-1, CCR-3 and CCR-5 | Proatherogenic, facilitates neointimal formation, platelets deliver CCL5 (and CXCL4) to monocyte surface and the endothelium, resulting in increased leukocyte adhesion | Gawaz et al. (2005), Henn et al. (1998), Koenen et al. (2009), Lievens et al. (2010), Magwenzi et al. (2015), Massberg et al. (1998), Nurden (2011) |
| | CCL-7 (MCP-3) | CCR-1, CCR-2 and CCR-3 | Proatherogenic | Gawaz et al. (2005), Henn et al. (1998), Koenen et al. (2009), Lievens et al. (2010), Magwenzi et al. (2015) |
| | CCL-17 (TARC)[a] | CCR-4 and CCR-8 | Synergistic platelet agonist, proatherogenic | Gawaz et al. (2005), Gear et al. (2001), Henn et al. (1998), Koenen et al. (2009), Lievens et al. (2010), Magwenzi et al. (2015) |
| CXCL type | CXCL-1 (Gro-a) | CXCR-1 and CXCR-2 | Support arrest of human monocytic cell lines and primary monocytes under flow conditions. Oxidative stress, eNOS downregulation in endothelial cells | Gawaz et al. (2005), Henn et al. (1998), Koenen et al. (2009), Lievens et al. (2010), Magwenzi et al. (2015) |
| | CXCL-4 (PF4) | CXCR-3B | Synergistic platelet agonist, proatherogenic. EC activation (E-selectin expression). Induces monocyte activation (oxidative burst, induction of CCL3, CCL4 and CXCL8), macrophage differentiation, foam cell formation | Gawaz et al. (2005), Henn et al. (1998), Koenen et al. (2009), Lievens et al. (2010), Magwenzi et al. (2015), Scheuerer et al. (2000), von Hundelshausen and Schmitt (2014) |
| | CXCL-5 (ENA-78) | CXCR-2 | Endothelial progenitor cell domiciliation, neutrophil chemoattractant | Gawaz et al. (2005), Henn et al. (1998), Koenen et al. (2009), Lievens et al. (2010), Magwenzi et al. (2015), Rousselle et al. (2013) |
| | CXCL-7 (β-TG, NAP-2) | CXCR-2 | Endothelial progenitor cell domiciliation | Gawaz et al. (2005), Henn et al. (1998), Koenen et al. (2009), Lievens et al. (2010), Magwenzi et al. (2015) |

(continued)

**Table 1** (continued)

| Platelet-derived inflammatory mediator | Mediator | Receptor | Mechanisms effecting platelet biology | Refs. |
|---|---|---|---|---|
| | CXCL-11 (ITAC)[a] | CXCR-7 | CXCR7 internalization, survival | Chatterjee et al. (2014a) |
| | CXCL-12 (SDF-1)[a] | CXCR-4 and CXCR-7 | Receptor internalization, activation, aggregation, pro-thrombotic, adhesion to immobilized collagen–fibrinogen, migration and survival. Atheroprogression, vascular regeneration and/or remodelling, PC mobilization, differentiation | Abi-Younes et al. (2000), Akhtar et al. (2013), Chatterjee and Gawaz (2013), Chatterjee et al. (2015b), Daub et al. (2006), Gear et al. (2001), Geisler et al. (2012), Jin et al. (2006), Langer et al. (2006), Massberg et al. (2006), Rath et al. (2014, 2015), Stellos et al. (2008, 2009, 2010, 2012), Stellos and Gawaz (2007a, b), Walsh et al. (2014), Wurster et al. (2013) |
| | CXCL-16[a] | CXCR-6 | Platelet surface expression elevated in ACS, plasma levels enhanced, OxLDL binding, pro-thrombotic, expression increased in macrophages and SMCs at atherosclerotic lesions, contributes to progression of plaque | Borst et al. (2012), Laugsand et al. (2015), Meyer Dos Santos et al. (2015), Seizer et al. (2011) |
| DAMPs | HMGB1[a] | TLR-4 RAGE | Pro-inflammatory, pro-thrombotic, proatherogenic, present in intracoronary thrombi and atherosclerotic plaques. Anti-HMGB1 strategies prevent atheroprogression | Ahrens et al. (2015), Rouhiainen et al. (2000), Vogel et al. (2014, 2015b), von Hundelshausen et al. (2005) |
| | CypA[a] | EMMPRIN GPVI | Platelet activation, intracellular calcium mobilization, pro-thrombotic, CXCR4-CXCR7 trafficking, atherogenic | Elvers et al. (2012), Seizer et al. (2014, 2015, 2016) |

[a]Autocrine effects on platelet functions are defined

predicting subsequent thromboischaemic complications since they are caused by rupture-prone vulnerable plaques (Naghavi et al. 2003) and luminal collagen exposure towards the direction of circulation that are hardly detectable by conventional imaging tools (Langer et al. 2008). The soluble dimeric form of GPVI (GPVI-Fc) exhibits high affinity to collagen (Massberg et al. 2004), competes with platelet GPVI for binding collagen and thereby prevents platelet adhesion onto collagen in vitro and in vivo (Massberg et al. 2004; Schulz et al. 2008; Schonberger et al. 2008). GPVI-Fc binds to collagenous components at the core of human atheromatous plaque (Gawaz et al. 2014; Naghavi et al. 2003; Langer et al. 2008; Massberg et al. 2004; Schulz et al. 2008; Schonberger et al. 2008) and to vascular lesions in mice. Therefore, thrombogenecity of atherosclerotic plaques may be detected by using labelled GPVI. Utilizing $^{124}$I-GPVI-Fc and in vivo scintigraphy, a sensitive, non-invasive imaging method to detect thrombogenicity of vascular lesions has been developed (Schonberger et al. 2008; Bigalke et al. 2013). PET imaging reveals an increased uptake of $^{124}$I-GPVI-Fc at atherosclerotic lesions in the aortic arch of high-fat diet-fed *Apoe*$^{-/-}$ mice (Bigalke et al. 2013). To allow for clinical translation, $^{64}$Cu may be preferred to $^{124}$I due to its improved spatial resolution and adequate half-life time for delayed PET studies (Gawaz et al. 2014). Similarly, after systemic administration of GPVI-Fc-FITC, increased signals were recovered from the sites of the injured carotid artery in *Apoe*$^{-/-}$ mice (Wadas et al. 2010; Bigalke et al. 2011). Targeted contrast-enhanced ultrasound (CEU) using target molecules that are conjugated to the surface of microbubble (MB) agents has evolved as a non-invasive imaging technique to evaluate atheroprogression, a method which provides high prospective for early risk stratification. Recently an ultrasound-guided molecular imaging with GPVI-targeted MB ($MB_{GPVI}$) has been utilized to detect atherosclerotic lesions in mice at the aortic arch and truncus brachiocephalicus (Metzger et al. 2015) following systemic administration of $MB_{GPVI}$.

GPVI critically influences atherothrombosis. It ensues thrombus growth and stability in a coordinated action with thrombin. Targeting the GPVI axis does not compromise physiological haemostasis, which is mainly ensured by the GPIb–vWF axis (Gawaz et al. 2014). GPVI-Fc owing to its preferential deposition at sites of injured vessels therapeutically blocks GPVI-binding sites on exposed collagen (Fig. 7) at atherosclerotic lesions (Gawaz et al. 2014; 143–145, 149)

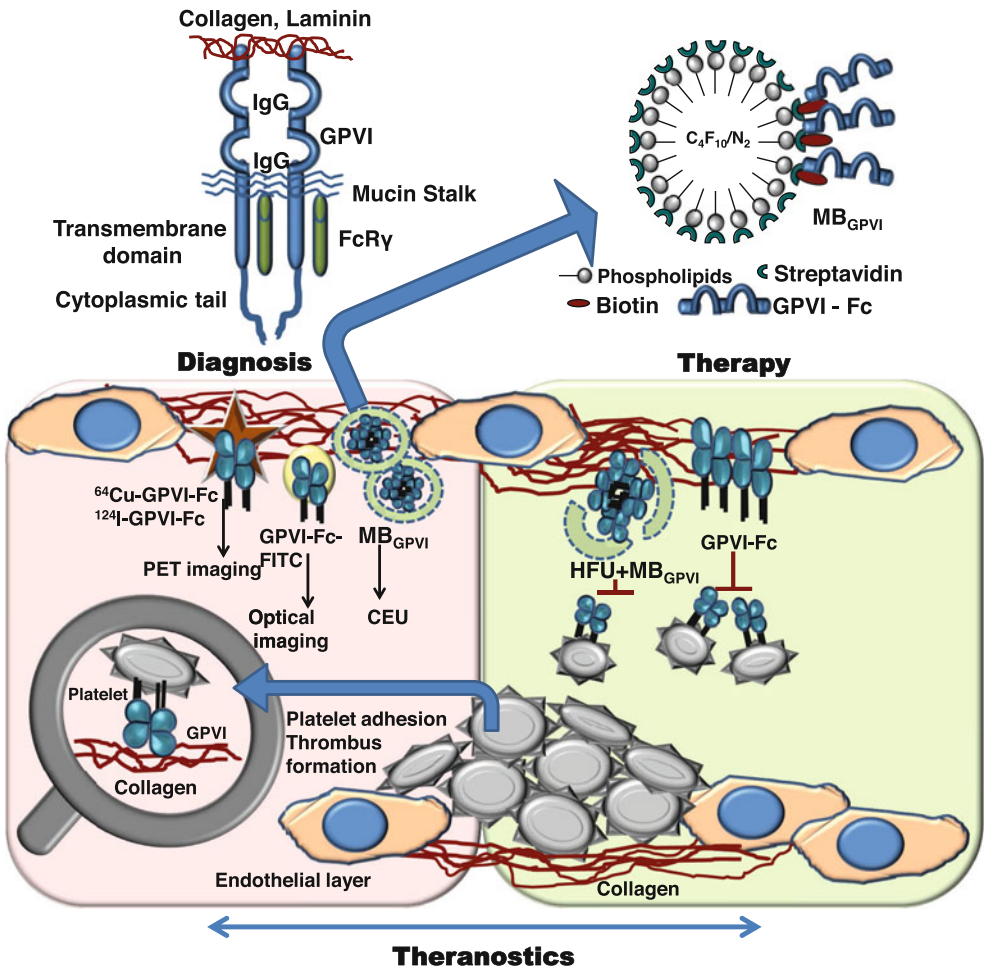


**Fig. 7** Platelet theranostics to diagnose and combat atherosclerosis. GPVI is an ~62 kDa protein belonging to the immunoglobulin superfamily. The diagnostic and therapeutic potential of GPVI-based molecules combines to generate GPVI theranostics. Soluble dimeric GPVI-Fc can bind to extracellular matrix collagen in atheromatous plaque. Thrombogenecity of atherosclerotic plaques or non-invasive detection of vascular lesions at risk might be executed by using variously labelled GPVI derivatives like $^{124}$I-GPVI-Fc, $^{64}$Cu-GPVI-Fc, GPVI-Fc-FITC, GPVI-targeted microbubble agent ($MB_{GPVI}$) and their subsequent monitoring by PET imaging, optical imaging and contrast-enhanced ultrasound (CEU). Schematic representation of $MB_{GPVI}$ is elaborated in the figure. $MB_{GPVI}$ competes with platelet surface GPVI for binding collagen and attenuates platelet adhesion to immobilized collagen. High-frequency ultrasound (HFU)-guided disruption of $MB_{GPVI}$ enables localized drug delivery at target site, significantly checks atherosclerotic development

and thereby counteracts stable arrest and aggregation of platelets without affecting bleeding times. Further, prolonged administration of GPVI-Fc attenuates atheroprogression (Gawaz et al. 2014) and arterial remodelling after mechanical injury in *Apoe*$^{-/-}$ mice. High-frequency ultrasound (HFU)-guided disruption of $MB_{GPVI}$ injected in *Apoe*$^{-/-}$ mice by rapid ultrasonic 'burst' enhances GPVI accumulation at atherosclerotic lesions, covers the collagen-enriched surface therein, interferes with accumulation of GPIbα-positive platelets, checks lipid-rich plaque formation and exhibits noticeable reduction in plaque area limiting atherosclerotic development (Metzger et al. 2015). Therefore, GPVI-Fc, which ensures targeted delivery appears to be a promising diagnostic and therapeutic agent to combat atherothrombotic and inflammation involving platelet vascular interactions mediated through collagen and GPVI.

## Does Uncontrolled Platelet Activation Promote Atherosclerosis in Humans?

There is convincing experimental evidence that platelets are a major driver of atherosclerosis. There are several supportive arguments that platelets initiate atherosclerotic lesions, foster atheroprogression and are critical in development of atherothrombosis leading to acute coronary or cerebrovascular events. Much clinical data has been published that link increased systemic platelet activation and hyperreactivity with poor clinical prognosis and clinical progression of coronary artery disease. Platelet reactivity is influenced by various clinical risk factors including diabetes mellitus, increased body mass index, left ventricular ejection fraction, renal failure, acute coronary syndrome, advanced age and

congestive heart failure (Geisler et al. 2008). Previously, a simple clinical risk score, *R*esidual *P*latelet Aggr*e*gation after *D*eployment of *I*ntra*c*oronary Sten*t* (PREDICT), was developed to identify patients with coronary artery disease at risk for increased platelet reactivity (Geisler et al. 2008; Droppa et al. 2015). The score encompasses different variables including acute coronary syndrome, older age, diabetes mellitus and renal and left ventricular function impairment. After weighing these variables according to their effects size in multivariate analysis, the score ranged from 0 to 9 with higher score levels being significantly associated with both platelet reactivity and cardiovascular outcome. Thus, comorbidity has a major impact on individual responsiveness to antiplatelet drugs. Clinical studies have shown that an increase in activation of circulating platelets is associated with the severity of coronary artery disease and progression of atherosclerosis (Gawaz et al. 2005). Platelet reactivity correlates with coronary plaque burden and calcification as assessed by cardiac computed tomography (Burgstahler et al. 2009). Further, systemic platelet activation is associated with progression of carotid artery disease in patients with diabetes (Fateh-Moghadam et al. 2005) and cardiac transplant vasculopathy (Fateh-Moghadam et al. 2000) within 1 year. Recently, the ADAPT-DES study showed that platelet reactivity is associated with atherosclerotic plaque burden and unstable plaque morphology in patients with coronary artery disease (Wang et al. 2016). In a clinical study, Trip and coworkers showed that spontaneous platelet aggregation in vitro is a useful biologic marker for the prediction of coronary events and mortality in survivors of a myocardial infarction (Trip et al. 1990). A recent systematic review and meta-analysis of individual patient data on major adverse cardiac events (MACE) outcomes (ACS, ischaemic strokes and vascular deaths) in relation to platelet reactivity and its interaction with cardiovascular risk levels were reported (Reny et al. 2015). 6478 patients, out of which 421 who experienced MACE (6.5 %) during a median follow-up of 12 months, were studied. The strength of the association between the risk of MACE and platelet reactivity increased significantly with the number of risk factors present (age $\geq$ 75 years, ACS at inclusion, diabetes and hypertension). Thus, it seems that the level of cardiovascular risk factors determines platelet reactivity and occurrence of MACE.

Currently, targeting cholesterol plasma levels in cardiovascular patients is the cornerstone in secondary prevention of atherosclerosis. To date statins and newly introduced PCSK9 inhibitors are established therapies for clinical progression of atherosclerosis. Besides lipid-lowering strategies, an intensified antiplatelet therapy is critical in treatment of patients with advanced atherosclerosis. Several oral antiplatelet drugs such as aspirin, $P2Y_{12}$ inhibitors (clopidogrel, prasugrel, ticagrelor) or PAR-1 antagonists (vorapaxar) as mono- or combination therapy are established treatment options for secondary prevention in patients with CAD. Since antiplatelet therapy not only reduces the thromboischaemic risk but also decreases ongoing platelet-driven systemic inflammation, possibly it also influences atheroprogression and occurrence of vulnerable plaques. Recent large clinical studies (PEGASUS, TRA2P, DAPT, OPTIDUAL) suggest that a prolonged and intensified antiplatelet therapy reduces progression of CAD as evidenced by reduction of ischaemic events, however, at the cost of higher bleeding event rates. Thus, targeting molecular mechanisms of thromboinflammation may turn out as innovative strategy for disease control among cardiovascular patients in the future.

**Take-Home Message**

Platelets accumulated at the site of vascular lesion or lesion-prone areas form a regulatory 'hot spot' that determines trigger for atherosclerotic lesions, plaque instability and subsequent atherothrombotic vessel occlusion.

Platelet–endothelial and inflammatory cell interactions mediated through ever-expanding array of adhesion molecules, pro-inflammatory, mitogenic factors and DAMPs promote atherogenesis and vascular remodelling.

Platelets recruit progenitor cells to the injured endothelium and platelet-derived mediators determine the balance between vascular inflammation and regeneration.

Intensified and prolonged antiplatelet therapy improves clinical prognosis of patients with atherosclerotic disease.

Advanced diagnostic and therapeutic strategies target platelet inflammatory biomarkers and mediators to diagnose atherosclerotic predisposition and prevent subsequent thromboischaemic complications.

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