Platelet-monocyte complexes in touch with the endothelium

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Molecular and functional interactions between monocytes, platelets and endothelial cells in cardiovascular diseases

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Abstract
Platelets, monocytes and endothelial cells are the key players in development and progression of cardiovascular diseases. Monocyte adhesion to and transmigration across the vascular endothelium is promoted by direct and indirect interaction with platelets, as well as by platelet stimulation of the vascular wall. The consequences of these different interactions for the development of cardiovascular diseases are discussed.

Introduction
Cardiovascular diseases are worldwide the number one cause of death and are predicted to remain so. Atherosclerosis, the primary cause of cardiovascular disease, is a systemic inflammatory disease. The inflammatory nature of atherosclerosis involves chronic stimulation of the endothelial cells (EC) that line the intima, the innermost layer of the vascular wall, and an active inflammatory response characterized by the accumulation of inflammatory cells in the intima, thus initiating the atherogenic mechanism. In this overview we focus on the most important inflammatory cell in lesion development, the monocyte. The role of platelets in atherosclerosis was first only believed to be in thrombus formation upon rupture of the more developed atherosclerotic plaques. However, together with inflammatory cells, also platelets are now regarded as important players by connecting inflammatory responses, thrombosis and atherogenesis. Activated platelets, adhered to a damaged vessel wall or to the endothelium, have been shown to further promote local recruitment of leukocytes. Since monocyte adhesion to the vascular wall, transendothelial migration and differentiation towards macrophages are critical for the formation of atherosclerotic lesions, it is important to realize that these events are subject to regulation by platelet adhesion molecules and platelet-derived chemokines and cytokines. Finally, the binding of platelets to the endothelium further promotes chronic inflammation of the vascular wall. This review will focus on the molecular mechanisms involved in cell-cell interactions between monocytes, platelets and EC, and the consequences of these interactions for the development of cardiovascular diseases.

Platelet activation and adhesion to the vascular wall
Molecular ligands
The function of blood platelets is to limit bleeding (haemostasis) by formation of stable blood clots following activation of the coagulation cascade. In addition, platelets may also contribute to the integrity of the endothelium 1 and participate in inflammatory processes 2. Healthy, non-activated endothelium normally prevents adhesion of platelets to the vessel wall by its anti-thrombotic properties, involving release of platelet activation-inhibiting substances such as nitric oxide, prostacyclin and cyclo-oxygenase 2 3. However, in an inflamed vessel wall, the endothelial phenotype can change to pro-thrombotic by release of platelet-stimulating agents such as adenosine diphosphate (ADP), high multimeric Von Willebrand factor (VWF), the expression of tissue factor (TF) and adhesion molecules 4. Especially VWF mediates direct interaction of platelets with intact, activated EC, even under high shear stress conditions 5,6. Platelet adherence is even more stimulated upon vessel wall damage, when extracellular matrix proteins are exposed. Extracellular matrix proteins, such as collagen and VWF, are strong
ligands of platelet glycoproteins. Rapid platelet adhesion to the extracellular matrix followed by their activation is the primary step in thrombus formation.

Under physiological flow, platelet adhesion at sites of vascular injury involves initial tethering and rolling over the extracellular matrix and intact endothelium. This process is mediated by adhesion to VWF via the membrane adhesion receptor GlycoProtein (GP) Ib-IX-V, also known as the VWF receptor complex and to collagen via GPVI. Rolling on intact endothelium is also mediated by binding of GPIb to activation-expressed P-selectin on EC. Additionally, P-selectin and GPIb can mediate rolling interactions between platelets that are still in the circulation and those that are already adhered to the vessel wall. Finally, already activated platelets can tether and roll on P-Selectin Glycoprotein Ligand-1 (PSGL-1) and GPIb on activated EC even under high shear (Figure 1.1).

Figure 1.1: Platelet – endothelial molecular interactions. The ligands P-selectin, PSGL-1 and GPIb are involved in the tethering and rolling of platelets on the endothelial cells. Upon further activation of integrins, platelets firmly adhere to the endothelium, mainly via the additional bridging molecules fibrinectin (Fn), fibrinogen (Fg) and VWF. Upon firm adhesion also CD40L – CD40, TNFS14 – TNFS14R, and JAM-1 – JAM-1interactions are initiated. CD40L and TNFS14 binding to the endothelial ligands induces an inflammatory response in the endothelial cells.

Stable adhesion, however, requires additional contacts between the platelets and the extracellular matrix or the endothelium. The initial contact by GPIb-IX-V and GPVI binding to VWF and collagen, respectively, results in platelet activation via a complex series of intracellular reactions. As a result, the integrins αIIbβ3 (GPIIb/IIIa, fibrinogen receptor) and α2β1 (collagen receptor) are activated. The VWF-GPIb-IX interaction has been shown to induce Syk phosphorylation and αIIbβ3 integrin activation. These activated integrins are required and essential for stable platelet adhesion to the extracellular matrix and EC. This can be through direct binding of the integrins to collagen, VWF or endothelial adhesion molecules or indirectly via additional, bridging molecules. The latter involves platelet-bound fibrinogen, fibronectin and VWF, that bind to endothelial intercellular adhesion molecule (ICAM)-1, αvβ3 integrin and GPIb, respectively (Figure 1.1). The requirement for αIIbβ3 in mediating firm
adhesion of platelets to the endothelium was shown by using platelets defective in αIIbβ3 or by adding β3-integrin antagonists or a blocking antibody 14,15. Conversely, in mice lacking ICAM-1, platelet adhesion to activated EC is strongly reduced. Furthermore, junctional adhesion molecule (JAM)-1 and platelet-associated EC superfamily 14 (TNFSF14, also known as LIGHT, identified in ADP-stimulated platelets) contribute to firm adhesion of platelets to the endothelium 16,17 (Figure 1.1). In conclusion, platelet adhesion, resulting in further platelet activation and activation of the EC, represents a positive feedback loop that amplifies recruitment of inflammatory cells.

Activation of endothelial cells by adhesion of platelets
Stable binding of platelets to the endothelium or to extracellular matrix results in strong activation of these platelets, reflected by spreading and increased surface expression of adhesion molecules, such as CD40L, TNFSF 14 and P-selectin, but also by secretion of potent inflammatory substances, such as interleukin (IL)-1β and PF4 18,19. IL-1β is synthesized and released by platelets in significant amounts and has been identified as a key mediator of platelet-induced activation of EC, inducing monocyte chemotactic protein (MCP)-1, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-6 secretion, ICAM-1 and αvβ3 integrin expression and nuclear factor-kappa B (NFκB) activation 20,21. CD40L (CD154) is stored in high amounts and released by platelets within seconds after GPIIbIIIa ligation 22,23. This results in stimulation of EC through the cognate receptor CD40, known to signal inflammatory reactions within EC, including increased secretion of IL-8 and MCP-1, expression of adhesion molecules, urokinase-type plasminogen activator and matrix metalloproteinase (MMP)-2 and -9, and production of reactive oxygen species (ROS) 22-24. Also TNFSF 14 can induce an inflammatory response in EC, reflected by up-regulation of adhesion molecules (E-selectin and vascular cell adhesion molecule (VCAM)-1) and release of chemokines (MCP-1 and IL-8) 19. E-selectin expression via activation of the NFκB pathway is also induced by platelet-released platelet factor 4 (PF4) 25. Finally, ligation of platelet P-selectin rapidly stimulates Weibel-Palade body release, resulting in, next to VWF release, P-selectin expression on the endothelium 26. In conclusion, platelet adhesion to the endothelial surface alters the properties of the endothelium in such a way that monocyte recruitment and extravasation are stimulated (Figure 1.2).

Platelets adhered to endothelial cells recruit monocytes
Atherosclerosis is characterized by monocyte and macrophage accumulation in the vascular intima. Adhered platelets efficiently mediate monocyte rolling and arrest, even at high shear. Rolling is mediated by P-selectin on activated platelets and PSGL-1 constitutively expressed on monocytes 27. Besides PSGL-1, CD15 (Lewis X) on monocytes has also been shown to bind platelet P-selectin 28. The initial association between platelet P-selectin and monocyte PSGL-1 leads to increased expression of the β2-integrin CD11b/CD18 (αMβ2, Mac-1) on the monocytes 29, which itself supports interactions with platelets. Mac-1 on leukocytes binds to GPIb 30 and to JAM-3 on platelets 31. Besides direct interaction, similar bridging mechanism between platelets and EC also mediate platelet-monocyte binding. On monocytes, fibrinogen is linked to Mac-1 and its platelet surface counterpart GPIIbIIIa 13. Also bridging by thrombospondin of the CD36 antigens (present on both monocytes and platelets) has been shown 32. Additional interactions
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between platelets and monocytes include CD40L-CD40 and monocyte TREM-1 (triggering receptor expressed on myeloid cells-1) to platelet-expressed TREM-1 ligand. Next to adhesion molecules, also chemokines deposited on the endothelium are facilitating recruitment of monocytes. For instance, RANTES (regulated upon activation, normal T cell expressed and secreted) and PF4 can be deposited on EC by activated platelets upon adhesion or even during transient, P-selectin-mediated rolling. The endothelial deposition of platelet-derived RANTES has been shown to trigger further monocyte arrest on the endothelium under high shear, but not on surface-adherent platelets. Also the chemokines platelet-activating factor and macrophage inflammatory protein are secreted by platelets adhered on the endothelium. The deposited platelet chemokines form homophilic, as well as heterophilic aggregates, which further stimulates their biological activity. For example, RANTES increases the binding of PF4 to the monocyte surface. Subsequently, PF4 drastically enhances RANTES-induced monocyte arrest on EC, predominantly mediated by CCR1. Thus, platelet adhesion to the EC or extracellular matrix and chemokines secreted by platelets, greatly contribute to subsequent monocyte adhesion to the vascular wall.

Figure 1.2: Endothelial activation upon platelet binding. Platelet interaction with endothelial cells mediates deposition of platelet-derived chemokines, such as RANTES and PF4 (1). Intracellular signalling induced upon platelet binding results in NFκB activity and ROS production (2). Furthermore, a series of several adhesion molecules is up-regulated (3) and secretion of several cytokines, VWF and MMP’s is induced by platelet binding (4).

Platelet-monocyte complexes – formation and functional consequences

Although a rare event under physiological conditions, platelets in the circulation sometimes do get activated. These activated platelets are able to bind to all types of leukocyte. Monocytes bind more activated platelets and at an initially faster rate than neutrophils. The amount of platelet-monocyte complex (PMC) formation is mostly dependent on platelet activation, and to a limited extent dependent upon monocyte activation. Different mechanisms could be responsible for the activation of circulating platelets, such as turbulent flow, activation by cytokines associated with systemic thrombo-embolic or inflammatory events, release of platelets from unstable thrombi, or by rolling interactions with activated endothelium. Whatever the cause, conditions such as systemic inflammation and acute myocardial infarction indeed increase the number of activated platelets in the circulation.
Figure 1.3: Platelet – monocyte molecular interactions. P-selectin mediates the initial binding contact with monocytes, via PSGL-1 and CD15. The ligation of PSGL-1 induces integrin activation on monocytes, resulting in further binding interactions, mediated or not by additional bridging molecules. Further interactions are through CD40L – CD40, TREM-1 ligand – TREM-1 and CD36 – CD36 via thrombospondin.

The in vivo circulation time and clearance of the complexes formed between activated platelets and monocytes is also not yet well defined. Platelets bind via P-selectin, expressed on the surface of activated platelets, to its receptor on monocytes, PSGL-1. In vivo, P-selectin is expressed upon platelet activation for several hours before it is shed from the surface. However, in a study using primates, Michelson et al. found that the life-span of PMC was not related to platelet P-selectin shedding. Huo et al. have shown that PMC, formed upon injection of activated platelets, had a short circulation time. These authors also showed that the PMC were cleared by monocyte transmigration. However, in patients with percutaneous coronary intervention, which increases the activated platelet level, PMC could be detected much longer and only returned to baseline after 24 hours. From these data we can conclude that platelet activation is crucial for PMC formation, although the kinetics and life-span of the complexes are variable, dependent on the conditions investigated.

**Monocyte activation upon platelet interaction**

Besides P-selectin – PSGL-1 interaction, additional ligand-receptor interactions have also been implicated in the binding between platelets and monocytes, as already dealt with in the section Platelets adhered to endothelial cells recruit monocytes, see also Figure 1.3. Antibody inhibition studies indicate that platelet-monocyte conjugation is abolished by blocking P-selectin, and partially inhibited by other blocking antibodies. This indicates that platelet P-selectin is the critical ligand initiating PMC formation, via binding to PSGL-1, while other ligands play only an additive role.

The binding of platelets to monocytes mediated via P-selectin – PSGL-1 interaction induces L-selectin shedding from the monocyte surface. Furthermore, this interaction between platelets and monocytes was found to increase expression and activity of the α4β1 and αMβ2 integrins.
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Similarly, engagement of CD40 with CD40L, but also TREM-1 ligation results in an increase in monocyte adhesion capacity by the synthesis of \( \beta 1 \) - and \( \beta 2 \)-integrins \(^{35,51} \). The presence of the chemokines RANTES and CXCL10, deposited by platelets onto the monocytes, augments the increase in \( \beta 2 \)-integrin avidity upon PSGL-1 cross-linking \(^{52} \).

Monocyte binding to activated platelets has also been shown to increase the production of various pro-inflammatory mediators and tissue factor expression by the monocyte. P-selectin – PSGL-1 interactions are important but not exclusively responsible for these processes. TF expression by the monocytes is reduced by a P-selectin-blocking antibody and by IL-10, but not by a CD40L antibody \(^{33,53-55} \). Monocyte expression of chemokines, induced by thrombin-activated platelets, is regulated by NF\( \kappa \)B activity \(^{56} \). Ligation of TREM-1 or the ligation of monocyte PSGL-1 together with RANTES, but not PF4, induces NF\( \kappa \)B activity and subsequently secretion of MCP-1, tumor necrosis factor (TNF)-\( \alpha \) and IL-8 \(^{35,57,58} \). Although PF4 has been shown as well to induce the secretion of TNF-\( \alpha \) by monocytes \(^{59} \). Taken together, platelet-derived chemokines together with the ligation of various adhesion molecules on the monocyte following the interaction with activated platelets induce activation of monocytes, resulting in changes in the expression of adhesion molecules and the secretion of cytokines (Figure 1.4).

**Figure 1.4: Monocyte activation upon platelet binding.** Platelet binding to monocytes leads to the deposition of platelet-derived chemokines, such as RANTES and PF4 (1). Intracellular signaling induced upon platelet binding results in L-selectin shedding (2) and increased expression and activation of \( \beta 1 \)- and \( \beta 2 \)-integrins (3). Furthermore, monocyte NF\( \kappa \)B activity (4) and the secretion of several cytokines and TF (5) are induced upon binding of activated platelets.

**PSGL-1 signalling**

PSGL-1 plays a major role in the binding of monocytes to activated platelets. PSGL-1, however, is not only an adhesion but also a signalling molecule. PSGL-1 ligation on leukocytes induces production of super-oxide anion radicals \(^{60} \), activation of GTPase Ras \(^{61} \), and tyrosine phosphorylation of various cytoplasmic proteins, such as pp125 focal adhesion kinase, ERK, Syk, Src kinase, and paxillin \(^{61-64} \). Also protein kinase C isoforms are activated, mediating integrin activation \(^{52} \).
The cytoplasmic tail of leukocyte PSGL-1 interacts with the cytoskeleton through the ezrin-radixin-moesin (ERM) proteins 65, which is crucial for leukocyte rolling 66. The ERM proteins also mediate PSGL-1 association with Syk 62, which is important for the activation of LFA-1 (αLβ2) integrins 67 and for the induction of serum response element-dependent transcriptional activity 62. Furthermore, the cytoplasmic tail of PSGL-1 interacts also with Nef-associated factor 1 (Naf1) 64. The Naf1-binding sites in the PSGL-1 cytoplasmic domain are distinct from the residues critical for the recognition of ERM proteins 68. Upon PSGL-1 engagement, Naf1 is phosphorylated via Src kinase, leading to activation of β2-integrins, which results in activation of Akt and mTOR (mammalian target of rapamycin) 64,69. The activation of mTOR is essential for the transcription and synthesis of both urokinase-type plasminogen activator and Rho kinase 1 69,70, which are both involved in adhesion and migration processes. Recently, a novel protein SLIC-1 (selectin ligand interactor cytoplasmic-1), that has no apparent signaling role upon leukocyte adhesion, was found to bind to the cytoplasmic domain of PSGL-1. SLIC-1 serves as a sorting molecule that promotes traffic of PSGL-1 to endosomes 71. From all this information it can be concluded that monocyte PSGL-1 is a signal-transducing adhesion molecule that is essential in the inflammatory response of monocytes through its role in controlling rolling, adhesion and migration.

**Platelet-monocyte complex adhesion and transendothelial migration**

The migration of monocytes across the vascular endothelium is required for immune surveillance and for recruitment at inflammatory sites. Uncontrolled monocyte transendothelial migration contributes to the development of atherosclerosis. Monocyte extravasation is tightly regulated by a multi-step process of tethering, rolling, activation, adhesion and transmigration. As described above, platelet binding induces various changes in the phenotype of the monocyte, including altered adhesion and transmigration.

*Tethering and rolling*

Like the initial interactions of platelets to EC under physiological flow, also monocyte adherence to the endothelial wall involves tethering, followed by rolling of the cells over the endothelium. Rolling is mediated by monocyte-expressed L-selectin and endothelial-expressed P- and E-selectin, interacting with PSGL-1, CD44 or E-selectin-ligand-1 (ESL-1) 72 (Figure 1.5). PSGL-1 and ESL-1 are, besides for rolling, primarily responsible for tethering of flowing leukocytes to the endothelium, CD44 is subsequently important for slowing the rolling velocity of leukocytes after they have tethered through P- or L-selectin 73. Two types of monocyte tethering can be distinguished. Primary tethering occurs directly at the endothelial surface. Secondary tethering represent monocyte adhesion, to other, already adhered monocytes 74,75. PMC show increased primary and secondary tethering, on both EC and on already adhered inflammatory cells 76,77. Platelet binding to monocytes also results in shedding of L-selectin from the monocyte surface 49, decreasing the rolling velocity of activated monocytes. The increased tethering and rolling, together with the L-selectin shedding, results in more monocyte adhesion upon platelet binding to the monocytes.
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**Monocyte activation and firm adhesion**

Slow rolling velocities increase monocyte transit time through inflamed vessels, favouring the probability of monocyte to encounter and to be activated by chemokines or lipid mediators presented on the endothelial surface \(^{78}\). During this process, chemokines present on the luminal endothelial surface, in cooperation with PSGL-1 ligation to endothelial and platelet ligands, induce a rapid increase in the binding affinity and avidity of \(\beta_2\)-integrins of the leukocytes \(^{79,80}\). Moreover, RANTES, IL-8 and MCP-1 secreted by platelets and EC trigger arrest of rolling monocytes on EC \(^{38,81}\). The high affinity binding of chemokines to specific G-protein-coupled receptors initiates the intracellular signalling cascade from these receptors to phospholipase C signalling, activation of small GTPases (Rap1) and transitional changes in integrin conformation through the association with actin-binding proteins \(^{82,83}\). On monocytes, the \(\alpha 4\beta 1\) (VLA-4) integrin is known to further slow the selectin-ligand-dependent rolling velocities, which leads to stable adhesion \(^{84}\). Leukocyte arrest is further induced by leukocyte integrins \(\alpha L\beta 2\) (LFA-1) or \(\alpha M\beta 2\) (Mac-1) and VLA-4 ligation to the endothelial immunoglobulin superfamily members ICAM-1 and VCAM-1, respectively \(^{82}\) (Figure 1.5). PMC have induced integrin expression and activity compared to platelet-free monocytes, increasing monocyte adhesion and transmigration capacity.

**Transmigration**

When the monocytes have attached, chemokines in the underlying intima stimulate them to migrate through the endothelial monolayer into the subendothelial space. The EC participate actively in the transmigration event. During transendothelial migration, the cell-cell junctions transiently and locally disengage to allow the leukocyte to cross \(^{85,86}\). Rolling and adhesion of leukocytes over activated endothelium is accompanied by a complex response from the EC, involving extensive reorganisation of the endothelial actin cytoskeleton and the activation of intracellular signalling pathways. One of the results is a pronounced

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**Figure 1.5: Monocyte – endothelial cell molecular interactions.** The initial interaction of monocytes with the endothelium is by tethering and rolling. This is mediated by the selectins and their ligands. Rolling triggers the expression and activation of monocyte integrins, resulting in firm adhesion to the endothelial cells through binding to ICAM-1, VCAM-1 or \(\alpha\nu\beta 3\) via Fn.
morphological response of the EC by forming ‘docking structures’ \(^{87}\) or ‘transmigratory cups’ \(^{88}\). In these structures, integrin ligands such as ICAM-1 and VCAM-1 are concentrated \(^{87}\). Leukocyte adhesion and ligation of ICAM-1 and VCAM-1 and the subsequent increase in endothelial actin stress fibre formation and monolayer permeability are controlled by the GTPases RhoA, Rac1 and Rap1 \(^{85}\). The junctional adhesion receptors PECAM-1, CD99 and JAMs also actively mediate leukocyte transendothelial migration through homophilic interactions \(^{89-91}\). In addition, adherent monocytes interact, via their \(\beta2\)- and \(\beta1\)-integrins, with JAM-family members at the most apical regions of the inter-endothelial junctions. Bradfield et al. \(^{92}\) discovered a novel role for endothelial JAM-3 in regulating monocyte retention in the abluminal compartment after primary transmigration in vivo. Blockade of JAM-2/-3 decreased the number of monocytes in the extra-vascular compartment by allowing multiple reverse-transmigration events.

PMC show increased transmigration compared to platelet-free monocytes \(^{49,93}\). We have observed that the platelets do not stay adhered to the monocyte upon transmigration of monocyte from the PMC \(^{93}\), but instead are shed from the monocyte as a result of monocytic PSGL-1 redistribution and mechanical stress. So far, it can be concluded that platelet binding to monocytes result in increased monocyte adhesion and transmigration and subsequent platelet deposition on the endothelium.

**Platelet-monocyte complexes in cardiovascular disease**

Atherosclerosis, causing cardiovascular diseases, is widely recognized as an inflammatory disease. The inflammatory nature of atherosclerosis involves chronic stimulation, an inflammatory response of the EC and accumulation of inflammatory cells in the intima, initiating the atherogenic program \(^{94,95}\). These conditions are likely also associated with varying degrees of platelet activation. Indeed, an increase in circulating leukocyte-platelet aggregates, particularly monocyte-platelet aggregates, was found under clinical conditions such as peripheral vascular disease, hypertension \(^{96}\), acute or stable coronary syndromes \(^{97-99}\), stroke \(^{100}\) or diabetes \(^{101}\). Increased levels of PMC are also an early marker of acute myocardial infarction \(^{99}\). Conversely, high dietary intake of omega-3 fatty acids induces a reduction in activated platelets and PMC level \(^{102}\). However, the presence of PMC is not just a sensitive marker for in vivo platelet activation and cardiovascular diseases, but is more and more also regarded as a cardiovascular risk factor \(^{100,103}\). This suggests that PMC play an active role in development and progression of cardiovascular diseases.

The importance of activated platelets and PMC in vascular disease is supported by studies demonstrating that interfering with platelet adhesion to the endothelium and monocytes is endothelial protective. Infusion of recombinant human PSGL-1 in animal models of vascular injury preserved vascular endothelial function \(^{104,105}\). Also the absence of P-selectin in mice diminishes lesion formation \(^{106,107}\). Furthermore, infusion of activated (but not P-selectin-deficient) platelets results in increased formation of atherosclerotic lesions \(^{36}\). All these data indicate a role of P-selectin – PSGL-1 interaction in atherosclerosis. Moreover, also the platelet chemokines PF4 and RANTES contribute to lesion progression by inducing monocyte survival and differentiation into macrophages \(^{59}\), which are important events in the development of an atherosclerotic plaque. PF4 also facilitates the esterification, and promotes the uptake of
oxidized low-density lipoprotein by macrophages and thereby promotes foam cell development. Additionally, RANTES contributes to smooth muscle cell proliferation, mediating progression to a fibrous plaque.

Clearly, PMC are not merely a reflection of platelet activation, but monocyte-platelet interactions lead to an activated and thus more proatherogenic monocyte phenotype. Not only by inducing expression and secretion of cytokines and active substances from both platelets and monocytes, but most importantly by amplifying monocyte adhesion and migration and by promoting monocyte differentiation towards macrophages. Increased levels of PMC in patients with cardiovascular disease have so far been regarded as a parameter reflecting disease, but in view of the above, these PMC might also play a key role in the pathogenesis of these diseases themselves.

**Intervention possibilities**

A number of therapeutic molecules have been used to investigate the inhibition of PMC, including clopidogrel (inhibition of ADP-mediated platelet activation) and abciximab (GPIIbIIIa antibody). Clopidogrel greatly reduces PMC in patients with atherosclerotic diseases and has been shown to reduce P-selectin expression and CD40L release. Although some studies suggest otherwise by reporting an increase in the expression of RANTES upon clopidogrel administration, much evidence points to an efficient inhibition of PMC formation by clopidogrel. In contrast, abciximab did not significantly reduce the formation of PMC. Although Abciximab resulted, in vitro, in less platelet binding to monocytes and a decrease in TF expression on monocytes, no effects or even an increase in PMC levels are observed. Furthermore, there are some studies with aspirin, another platelet aggregation inhibitor, that show no or very little effect on PMC formation. Because traditional platelet activation inhibitors show varying success in preventing PMC formation, P-selectin and PSGL-1 are logical potential targets for intervention with antibodies or recombinants proteins. Recombinant PSGL-1 in animal models indeed results in reduced platelet and leukocyte adhesion to the endothelium and better vascular function after injury. Also targeting CD40L or RANTES may be beneficial. RANTES receptor antagonists inhibit the infiltration of monocytes and limit atherosclerotic plaque formation in pro-atherogenic mice models. PMC represent a potential therapeutic target for limiting cardiovascular diseases. Targeting inhibition of pro-inflammatory platelet activation or interaction, in contrast to targeting platelet aggregation, are good candidates for future drugs.

**Conclusions**

Atherosclerosis and cardiovascular disease involve multifactorial mechanisms with interactions between coagulation, platelets, monocytes and EC, with multiple adhesion molecules, chemokines and receptors involved. However, the increased monocyte adhesion to and transmigration across the endothelium seems to be the most important factor in accelerating atherogenesis. Platelets and EC both can actively stimulate these processes. Platelet interaction with the monocyte – both in the circulation or at the vessel wall itself – results in activation of the monocyte, making the latter more adhesive, more migratory, more pro-coagulant (TF) and pro-inflammatory and more prone to differentiate into a macrophage. Additionally, the monocytes and platelets, each individually and also bound in a complex, contribute to an
inflammatory phenotype of the endothelium. This results in further increased adhesion of monocytes and platelets and activation of these cells. In conclusion, platelet-monocyte conjugates are now considered as pro-atherogenic particles. Diverse intervention strategies are being explored and may hold good promise, especially when platelets, monocytes and EC can be targeted simultaneously. Additionally, since it is now known from studies of our group that EC also express PSGL-1, its role, apart from binding activated platelets, might also include EC activation. This represents an important topic for further study and, perhaps, future therapy.

References
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Chapter 1


