Platelet lipoprotein interplay: trigger of foam cell formation and driver of atherosclerosis

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In the last decade, it was recognized that platelets and lipoproteins play a pivotal role in both early and late atherogenesis. Beside cellular interactions of platelets with other blood cells and vascular cells, interactions with lipoproteins seem to be quite important. Lipoproteins are fundamental ‘players’ in atherogenesis since they change the properties of different cells involved in atherosclerosis and thrombosis. Several studies have already shown that low density lipoproteins (LDL) are involved in the initiation of platelet signalling pathways. Platelets of hypercholesterolemic patients show hyperaggregability in vitro and enhanced activity in vivo.

This review elucidates the major aspects concerning how native and modified lipoproteins influence the activation and metabolic behaviour of platelets, and shows a new way by which platelet-mediated lipoprotein transfer might contribute to foam cell formation. In hyperlipidaemia, circulating platelets are activated. This is accompanied by increased platelet aggregation, platelet-leukocyte aggregate formation, and platelet-induced superoxide anion production. Furthermore, oxidized LDL induces monocyte adhesion to the endothelium, migration and proliferation of smooth muscle cells, injures cells, interferes with nitric oxide release, and promotes procoagulant properties of vascular cells. New data about platelet-mediated lipoprotein transport and consequent foam cell formation, however, provide proof of how platelets might contribute to atheromatous lesion formation.

KEYWORDS
Platelets; Leukocytes; Receptors; Lipoproteins; Atherosclerosis

1. Introduction

The most characteristic component of the atherosclerotic plaque is the acellular lipid core, which is mainly composed of deposited lipids, such as cholesterol esters and free cholesterol derived predominantly from low-density lipoprotein (LDL) particles present in blood. The stability and vulnerability of lipid-rich plaques are mainly determined by the overlying fibromuscular cap composed of smooth muscle cells and extracellular matrix. Thinning and rupture of the cap leads to exposure of deposited lipids to blood cells and triggers inflammatory processes. There is evidence that eccentric and mainly intimal advanced atherosclerotic plaque can be reversed substantially by a reduction of blood lipid levels, whereas concentric inflammatory, lipid-rich lesions with endothelial damage are often resistant to regression. Thus, lipid accumulation inside the vessel wall determines the composition and vulnerability of the atherosclerotic plaque and the clinical consequence.

The current opinion is that atherosclerosis is an immune/inflammatory response of the intima to endothelial injury. It is also well known that the injury is mainly initiated by lipid accumulation. Native plasma lipids, in particular native LDLs, can freely enter the intima and are taken up by vascular cells via LDL receptor-mediated endocytosis. Nevertheless, they do not primarily initiate an inflammatory response, they are not phagocytosed by monocytes, and they do not initiate atherosclerotic alterations. Oxidation or other modifications of LDL, however, substantially alter its role: oxidized or modified lipids are chemotactic for monocytes, induce migration, initiate inflammatory responses, alter the endothelium, induce differentiation of monocytes into macrophages, and are avidly taken up by macrophages via scavenger receptors. Modified LDL is recognized by scavenger receptors distinct from the ‘classical’ LDL receptors present on all mammalian cells. Finally, modified forms of LDL are taken up more rapidly by monocytes/macrophages, their uptake is not feedback regulated by intracellular lipid, and they generate lipid-rich foam cells.

The central role of lipid accumulation in the intima after intimal injury is evident. Intravascular lipids are almost completely derived from plasma as a result of unphysiologically high plasma lipoprotein concentrations. It is important to note that the individual plasma lipid profile is the result...
of interactions between genetic and environmental factors.\textsuperscript{1} In summary, atherosclerosis results from multiple complex interactions among various noxa injuring the endothelium followed by healing or repair processes of the arterial wall and occurs in a hyperlipidaemic and dyslipoproteinaemia environment.

A number of different cellular components play important roles in the pathogenesis of atherosclerosis. It is well known that endothelial cells as well as intimal smooth muscle cells are involved.\textsuperscript{1,11} Furthermore, monocytes/macrophages are crucial in the early pathogenesis of atherosclerosis,\textsuperscript{6} but in the meantime it is accepted that also other blood cells, predominantly platelets and probably also progenitor cells, may play a role.\textsuperscript{12,13} In addition to the cell types mentioned here, other factors also contribute to the initiation and the development of atherosclerotic lesions. The susceptibility of pre-deposition sites within coronary or carotid arteries, such as branching sites, where blood cells adhere, shows the importance of the local haemodynamic environment in the initiation and subsequent development of atherosclerotic lesions. Furthermore, a number of inflammatory cytokines, mitogens, chemoattractants, and cell adhesion molecules also play significant roles in atherogenesis.\textsuperscript{14,15}

During the past years, evidence for an involvement of platelets in both early and late atherosclerosis has been growing rapidly.\textsuperscript{5,11,16} Many studies indicate that in addition to their classical role in thrombosis and haemostasis platelets contribute to endothelial dysfunction, modulate various inflammatory responses, and also initiate atheromatous plaque formation.\textsuperscript{17,18} About 1.5 x 10\textsuperscript{12} platelets continuously flow—without adhering or aggregating on the endothelium—in the blood stream.\textsuperscript{14,19} Functional (e.g. inflammation) or mechanical (e.g. fissure or erosion) damage to the endothelial cell surface and the resulting dysfunction induce complex interactions between circulating platelets, endothelial cells, other blood cells such as monocytes, and subendothelial structures.\textsuperscript{20} Interestingly, in vitro and in vivo studies have revealed that platelets also directly adhere to intact endothelial cells.\textsuperscript{12,21,22} This review discusses whether lipoproteins in blood influence platelet function and whether, in addition to monocytes/macrophages, platelets are also able to bind, take up, or transport lipoproteins and contribute indirectly and directly to foam cell formation in atherogenesis (Figure 1).

### 2. Role of platelets in atherosclerosis

The predominant function of platelets in atherosclerotic lesion formation is thought to be activation of the endothelium. As we and others have reported, platelets can adhere to intact endothelial monolayers, thereby inducing a proatherogenic phenotype characterized by enhanced chemotactic, adhesive, and proteolytic activity.\textsuperscript{14,16,23-30} Massberg et al.\textsuperscript{21} were the first to show in vivo that platelets adhere to the endothelium of carotid arteries in apoE-deficient mice in the absence of endothelial cell denudation before manifest atherosclerotic lesions develop. Platelet adhesion to the intact endothelium preceded leukocyte recruitment and invasion and an antibody against GPIib\textalpha attenuated atherosclerotic lesion formation.\textsuperscript{21,31} Burger and Wagner\textsuperscript{32} showed in apoE-deficient mice that platelets promote advanced atheromatous lesion development via P-selectin up-regulation. In addition, an interesting in vivo study by Huo et al.\textsuperscript{33} demonstrated that repeated infusion of circulating activated platelets and platelet-leukocyte/monocyte aggregates promote formation of atherosclerotic lesions in apoE-deficient mice. This role of activated platelets in atherosclerosis was attributed to platelet P-selectin-mediated delivery of platelet-derived proinflammatory factors to monocytes/leukocytes and the vessel wall.

Platelet interaction with the structurally intact but dysfunctional endothelium is a well-controlled process involving selectins and integrins.\textsuperscript{34} The first contact, the rolling of platelets, is dependent on endothelial cell activation that is induced by inflammatory events. Inflammation can be caused by various stimuli, like infection, mechanical alteration or ischemia and reperfusion.\textsuperscript{31,35} The rolling and adherence of platelets leads to activation both of platelet and endothelial cells.\textsuperscript{12,17} Adhesion of platelets to the endothelial surface generates signals recruiting monocytes to the site of inflammation.\textsuperscript{19} Subsequently, extravasation of monocytes and their differentiation to macrophages initiate fatty streak and atheromatous plaque formation.\textsuperscript{11}

### 3. Effects of lipoproteins on platelets

#### 3.1 Influence of native lipoproteins on platelet function

Recent reports have established platelets as a major player in the initiation of the atherosclerotic process in addition to their role in thrombus formation after plaque rupture in late atherosclerosis.\textsuperscript{36,37} In patients with hypercholesterolaemia, the abnormalities in platelet composition and function indicate that circulating lipoproteins in blood influence platelet properties.\textsuperscript{38,39} In fact, there is increasing evidence that lipoproteins affect platelet functions (Table 1).\textsuperscript{40} LDL, very low-density lipoproteins (VLDL), and especially oxidized LDL (OxLDL), which all contain apoprotein B-100, are atherogenic lipoproteins and increase platelet activation, whereas high-density lipoprotein (HDL) shows antiatherogenic effects on platelet function. In patients, hypercholesterolaemia is associated with increased platelet activity, such as hyperaggregability, and the application of a lipid-lowering drug resulted in a reduction of platelet reactivity.\textsuperscript{41,42} These changes in reactivity are probably the result of cholesterol-phospholipid uptake by platelet membranes during platelet formation or of their direct interaction with plasma lipoproteins.

Interestingly, in vitro data revealed that LDL is able to activate platelets whereas HDL desensitizes platelets underlining the anti-atherosclerotic properties of HDL.\textsuperscript{40} Radioiodination experiments in the early 80s already demonstrated the existence of a uniform class of saturable specific binding sites for each of the lipoprotein ligands.\textsuperscript{43} Several reports showed that native LDL and HDL do bind to platelets in a time-dependent and temperature-dependent manner,\textsuperscript{44,45} but that the so-called native LDL site on platelets is not the same as the ‘classical’ LDL receptor of most extrahepatic cells. It was estimated that unstimulated human platelets contain 1500 binding sites for LDL and 3200 for HDL, respectively.\textsuperscript{44} The interactions with unmodified native lipoproteins probably lead to an exchange of lipids between platelets and lipoproteins, to the loss of platelet lipids and to the transfer of lipids to other cells where they are further
processed. Although it is not fully clear which receptor is responsible for the binding of native lipoproteins, some data indicate that LDL receptor-related protein-8, a member of the LDL receptor family, might be capable of binding apoB-100 and also be responsible for LDL-induced platelet sensitization.46 The binding and activation of this LDL receptor on platelets alter signal transduction cascades inside the platelet increasing sensitivity to platelet-activating agents.47

3.2 Binding and internalization of modified LDL by platelets

In blood, circulating platelets are continuously exposed to LDL and HDL. Furthermore, upon endothelial damage or plaque rupture of lipid-rich plaques, adhering platelets are also in close contact to OxLDL inside the vulnerable plaque. The emerging concept that platelets may contribute to both early and advanced atherosclerosis by binding, uptake, and transport of modified lipoproteins is the subject of considerable interest.

It is well known that lipoproteins affect platelets via specific binding receptors on the platelet. However, there are different opinions concerning these binding receptors. In addition to the classical receptors for LDL known for most cell types, platelets possess a number of other receptors.

Furthermore, it is still not sufficiently elucidated whether native LDL particles are simply bound to the platelet surface or really internalized into vesicles inside platelets via endocytosis. Native LDL alters platelet function also by inducing resynthesis or remodelling of phospholipids in the cell membrane and by insertion of phospholipids from circulating lipoproteins thereby changing the composition of membrane phospholipids.48 Thus, platelets are sensitized by native LDL through the activation of a signal transduction pathway as well as by the exchange of lipids. In contrast to native LDL, binding of OxLDL induces platelet activation followed by quick changes in shape and aggregation contributing to

Figure 1 Hypothetical role of platelet/CD34+ interaction and the resulting foam cell formation for atherogenesis. Platelets bind LDL in blood, are activated and adhere to the endothelium, predominantly at bifurcation sites. Adhering platelets alter endothelial function and recruit progenitor cells/monocytes. These cells are able to phagocytose LDL-laden platelets and migrate into the intima of the vessel wall where they develop into macrophages. Native LDL (orange) is modified to oxidized LDL (yellow) by endothelial cells and smooth muscle cells in the intima but also by platelets and monocytes outside the vessel wall. Both macrophages and platelets express scavenger receptors that bind OxLDL. At lesion prone sites, OxLDL diffuses passively through the functionally altered endothelium, but OxLDL is also transported into the intima by platelets after scavenger receptor-mediated uptake. All illustrated OxLDL transport pathways result in transformation of macrophages into foam cells, the main component of fatty streaks and atheromatous plaques. Specific inhibitors, such as the immunoadhesin CD68-Fc, are able to block several steps in foam cell formation.

Figure 2 Co-cultivation of CD34+ progenitor cells with platelets for 5-10 days induces foam cell generation in vitro. (A) Representative phase contrast image of CD34+-progenitor cells and macrophage foam cells: foam cells show an increased diameter of 25 μm and a high granularity and are surrounded by a platelet-free area. (B) Lipid staining with Sudan red III marks large granular and lipid-rich cells. (C) Macrophage foam cells internalize OxLDL which was labelled with a red fluorochrome (Dil-OxLDL). (D) Intracellular and surface-bound OxLDL of macrophage foam cells was detected by anti-OxLDL antibodies. These original photomicrographs belong to the series reported by Daub et al.91
thrombus formation after plaque rupture. Most recent evidence indicates that modified LDL bound by platelets via scavenger receptors might exert an additional, ‘pro-atherothrombotic’ effect.

Foam cell formation, the key step in atherosclerotic plaque formation, occurs as a result of unregulated uptake of modified lipoproteins by scavenger receptors with deposition of cholesterol esters in the cytoplasm. The ‘classical’ and best studied pathway of lipid transfer into the vessel wall leading to foam cell formation, and subsequent acceleration of atherosclerotic lesion formation, is the scavenger receptor-mediated uptake of modified LDL into monocyte-derived macrophages. Monocytes, which transmigrate into the vessel wall and differentiate into macrophages secrete and are activated by cytokines, such as interleukin-10 and transforming growth factor-beta. However, only activated macrophages express scavenger receptors. In contrast to LDL receptors which are widely expressed in both liver and peripheral tissue, scavenger receptor expression on macrophages in the arterial vessel wall is not down-regulated by increasing LDL levels, and foam cell formation proceeds by persistent uptake of modified LDL. On macrophages, a variety of different scavenger receptors have been identified, belonging to SR-classes A, B, and D, such as SR-A1, SR-B, CD36, and CD68. Scavenger receptors on macrophages predominantly bind acetylated LDL (AcLDL) as well as different forms of OxLDL.

### 3.3 Scavenger receptor types on platelets

**3.3.1 Class B scavenger receptor CD36**

The class B scavenger receptor CD36, also called platelet glycoprotein IV, is an 88 kDa glycoprotein expressed on the surface of smooth muscle cells, endothelial cells, monocytes, certain microvascular endothelium and serves as a receptor for thrombospondin, collagen, *Plasmodium falciparum*-infected erythrocytes, apoptotic cells, and oxidized LDL. The presence of CD36 on platelets was confirmed by inhibition studies with antibodies directed against the OxLDL binding domain of CD36. Antibody blockade of CD36 resulted in a reduction of Ox-LDL binding to platelets. In vitro, the affinity of Ox-LDL binding to human platelets was found to be within the range of OxLDL binding to macrophages with a $k_d$ of 4.17 $\mu$g/mL. Interestingly, no specific binding of AcLDL to platelets could be observed indicating that platelets do not express the classic scavenger receptors of the SR-A class. Thus, platelets exclusively bind OxLDL via the class B scavenger receptor CD36.

Antibody blockade experiments demonstrated that CD36 triggers the activation of monocytes and platelets. CD36 serves primarily as a receptor for both the adhesive glycoprotein thrombospondin and for collagen. As a thrombospondin 1 receptor on platelets, CD36 strengthens the binding between fibrinogen and the $\alpha_{IIb}\beta_3$ integrin during platelet aggregation. Finally, CD36 has been shown to be responsible for a part of the OxLDL binding activity on platelets.

On macrophages, CD36 plays an important role in foam cell formation. Incubation of CD36-deficient monocytes/macrophages with OxLDL resulted in a 40–60% reduction in OxLDL binding and uptake compared to wild-type CD36-expressing cells. Macrophages isolated from CD36-null mice have a profound defect in OxLDL uptake and foam cell formation. Crossing the CD36 deficiency into a proatherogenic apoE-null background yields animals that are significantly protected from lesion development. Animals fed a Western diet showed a >70% reduction in aortic lesion size and distribution.

Although CD36 was already recognized as a major platelet glycoprotein several decades ago, its role in platelet physiology, OxLDL binding and uptake and its contribution to foam cell formation is not fully understood until now. However, very recent data published by Podrez et al. suggest that CD36 might serve as a kind of primer or sensitizer for platelet activation leading to platelet hyperreactivity in response to various forms of OxLDL or specific oxidized choline glycerophospholipids (oxPC$_{CD36}$) that are formed during LDL oxidation. In hyperlipidaemic CD36/−/− mice, the absence of CD36 significantly protected these mice from the hyperlipidaemia-related prothrombotic phenotype when compared to hyperlipidaemic apoE/−/− mice without CD36 deficiency.

In vitro, various forms of OxLDL and oxPC$_{CD36}$ bind to human and mouse platelets in a CD36-dependent manner. Binding of these ligands to CD36 results in platelet activation characterized by activation of integrin $\alpha_{IIb}\beta_3$ and an increase in P-selectin surface expression.

In addition, another study recently published by Korporaal et al. provided interesting data about a new mechanism for OxLDL-induced platelet activation that is initiated by the combined action of CD36 and SR-A. In a previous study, this group described that platelets are activated by native LDL through apoE Receptor 2 (apoER2)-mediated signalling to p38MAPK and by oxidized LDL through lysophosphatidic acid signalling to RhoA and Ca$^{2+}$ and that these two pathways are independent. To elucidate OxLDL-induced activation mechanisms of platelets, activation of the p38MAPK signalling induced by OxLDL was then compared with that induced by native LDL. They found that the oxidation of LDL led to a strong increase in p38MAPK signalling caused by the loss of apoER2 activation and that both the OxLDL binding domain of CD36.

### Table 1 Effects of native or oxidized low-density lipoprotein (LDL) and high density lipoprotein (HDL) on platelets

<table>
<thead>
<tr>
<th>LDL</th>
<th>HDL</th>
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<tbody>
<tr>
<td>Binding and activation of platelets</td>
<td>Desensitization of platelets</td>
</tr>
<tr>
<td>Increase of platelet sensitivity to platelet-activating agents</td>
<td>Anti-atherosclerotic effects</td>
</tr>
<tr>
<td>Change of the composition of membrane phospholipids</td>
<td></td>
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<tr>
<td>Exchange of lipids, transfer of lipids to other cells</td>
<td></td>
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<tr>
<td>Conversion to OxLDL by platelet ROS → lipid peroxidation</td>
<td></td>
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<tr>
<td>Oxidized LDL</td>
<td></td>
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<tr>
<td>Binding to platelets via SR-B, CD36, LOX-1</td>
<td></td>
</tr>
<tr>
<td>Induction of platelet hyperreactivity</td>
<td></td>
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<tr>
<td>Fast platelet shape change and aggregation → thrombus formation</td>
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<tr>
<td>Cholesterol release by activated platelets to macrophages</td>
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<tr>
<td>Foam cell formation after platelet phagocytosis by macrophages</td>
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<tr>
<td>Induction of foam cell formation in platelet/progenitor cell co-culture</td>
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*Table 1 Effects of native or oxidized low-density lipoprotein (LDL) and high density lipoprotein (HDL) on platelets*
activity of CD36 and SR-A was involved. The adhesion of platelets to fibrinogen under flow was found to be enhanced by Ox-LDL signalling in which both CD36 and SR-A were involved.

### 3.3.2 Class B scavenger receptor B1 (SR-B1)
In addition to CD36, human scavenger receptor B1 (SR-B1), which is known to be a receptor for 'protective' HDL (Table 2), was described on the surface and inside human platelets. The levels of SR-B1/CLA-1 expression inversely correlated with cholesterol ester content and aggregation of platelets obtained from patients with atherosclerotic disease as opposed to those in control subjects.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Class</th>
<th>Expressing cell type</th>
<th>References</th>
</tr>
</thead>
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<tr>
<td>CD36</td>
<td>SR-A</td>
<td>Macrophages, monocytes, adipocytes, specific endothelial, and epithelial cell populations</td>
<td>Tandon et al.</td>
</tr>
<tr>
<td>SR-Bi, CLA-1, SR-Bii</td>
<td>SR-B</td>
<td>Platelets, monocytes/macrophages, dendritic cells, hepatocytes</td>
<td>Imachi et al.</td>
</tr>
<tr>
<td>SR-Ci</td>
<td>SR-C</td>
<td>Macrophages, hemocytes (Drosophila)</td>
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<tr>
<td>LOX-1</td>
<td>SR-E</td>
<td>Platelets, vascular endothelial cells and monocytes/macrophages</td>
<td>Endemann et al.</td>
</tr>
<tr>
<td>SREC</td>
<td>SR-F</td>
<td>Endothelial cells</td>
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<tr>
<td>SR-PSOX/CXCL16</td>
<td>SR-I</td>
<td>Macrophages</td>
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<tr>
<td>FEEL1/2, CD163</td>
<td>SR-H, I, J</td>
<td>Placenta, thymus, lymphatic vessels</td>
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### 4. Phagocytosis of platelets by monocytes/macrophages

#### 4.1 Lipophages and thrombus organization
It is well established that erythrocytes can infiltrate the atherosclerotic plaque via leakage and/or rupture of newly formed microvessels and there is evidence that platelets can also use this route. Inside the plaque they are then in close contact with macrophages and phagocytosis of platelets by macrophages was proposed as an alternative mechanism of foam cell formation several decades ago. The first evidence for this hypothesis was provided 36 years ago by Chandler et al. In *vitro* human thrombus formation experiments performed by this group and observations in rabbits indicated that phagocytosed platelets might be a source of lipids leading to the development of so-called 'lipophages'. Furthermore, human *ex vivo* thrombi in the early stages of organization were examined and found to contain lipophages similar to those in atherosclerotic plaques. Thus, the hypothesis was born that mural thrombi can develop into atherosclerotic plaques. Poole et al. also proposed that phagocytosis of platelets by monocytes might occur in general during the organization of thrombi in *vivo* and studied the deeper layers of mural thrombi in baboons using electron microscopy looking for evidence of phagocytosis. The authors concluded that platelets are phagocytosed by monocytes and illustrated the early stages of the morphological changes that the engulfed platelets undergo. Three decades later, De Meyer *et al.* confirmed *in vitro* that platelet phagocytosis by murine macrophages results in the formation of lipid-laden macrophages and that platelet-derived amyloid precursor protein is proteolytically processed to beta-amyloid peptide, resulting in iNOS induction. The authors concluded that macrophages become activated after phagocytosis of platelets and that this may trigger matrix degradation in atherosclerosis.

#### 4.2 Phagocytosis mediated by scavenger receptors
Phagocytosis is mostly mediated by scavenger receptors. This also seems to be the case for platelets. Brown *et al.* described that constitutive death of platelets by a caspase-independent cell clearance program (similar to apoptosis) leads to phagocytosis mediated through class A scavenger receptors. They suggest that platelets can undergo a form of programmed cell death that can be regulated by exogenous influences, in particular plasma-derived survival factors. Inhibitor studies in this report indicated that phagocyte class A scavenger receptors mediate recognition and ingestion of aged platelets. Scavenger receptor A has already been shown to play a role in the clearance of other apoptotic cells, e.g. thymocytes by mouse
macrophages. Studies by Miao et al. indicate that CD36 associates with CD9, a member of the tetraspanin family of transmembrane receptors, as well as integrins on human blood platelets. Their data implicate that these associated proteins may mediate or participate in some of the diverse biological functions of CD36 and suggest that CD36 might also be involved in platelet recognition by macrophages. Based on these reports it can be concluded that the organization of mural thrombi can explain at least to some extent that phagocytosis of platelets by macrophages also play a role in the pathogenesis of atherosclerosis. In vivo experiments in rabbits have already shown in 1965 that rabbits can incorporate dietary cholesterol into organizing mural thrombi of the aorta. However, it is not clear if and how large amounts of lipids are transported into the atheromatous plaque and accumulate inside the lipid core. Examination of platelet/monocyte ultrastructure by electron microscopy indicate that platelets, after being phagocytosed by monocytes, undergo further changes and disintegration, resulting in the appearance of fat deposits in the cytoplasm of the monocytes and development of typical foam cells. However, at present it seems likely that more than one mechanism is involved in foam cell formation and the development of lipid-rich atherosclerotic plaques.

5. Platelet-mediated conversion of monocytes into foam cells

At sites of endothelial damage, a close association between platelets and monocytes which could enhance macrophage accumulation of cholesteryl esters has been documented. In vitro-studies provided evidence that activated platelets may release cholesterol, which can be accumulated by smooth muscle cells and macrophages and stored as lipid droplets. Furthermore, Curtiss et al. showed that platelets enhance both the rate of cholesteryl esters formation and the total cholesteryl esters accumulation in cultured peripheral blood mononuclear cell-derived macrophages. They also demonstrated that this process can be induced by products released from activated platelets. It therefore appears likely that platelets accumulate and are activated at sites of vessel wall injury, enhance the conversion of monocytes into foam cells, and contribute to fatty streak and atherosclerotic plaque formation.

6. Platelet-induced modification of LDL by oxidation

It is well known that vascular cells, such as endothelial cells and smooth muscle cells, as well as blood derived cells, predominantly macrophages are able to convert native LDL to oxidized LDL resulting in scavenger receptor-mediated uptake of OxLDL by macrophages and its intracellular degradation. However, OxLDL can also be generated by platelets by lipid peroxidation. Fogelman et al. showed in vitro that after exposure of native LDL to platelets that aggregate and release mediators, a significant amount of malondialdehyde was bound to platelet-modified LDL and that after incubation with platelet-modified LDL for 3 days, human monocyte-derived macrophages showed a dramatic increase in cholesteryl ester content. There is further evidence that both resting and activated platelets produce reactive oxygen species (ROS), such as superoxide anion. An enhanced ROS-production of human platelets compared to progenitor cells could be also confirmed by our group (unpublished data). Furthermore, Krotz et al. found that platelets express four subunits of NADPH oxidase leading to superoxide anion release which stimulates platelet recruitment. Evidence that platelets modify LDL leading to an enhanced LDL uptake by macrophages was recently published by Carnevale et al. Their data demonstrated that upon stimulation with collagen, platelets are able to oxidize LDL via an NADPH-oxidase dependent mechanism, and suggested that the NADPH oxidase subunit gp91phox plays a crucial role in LDL modification. Furthermore, the role of phospholipid during generation of modified LDL was shown by inhibiting phospholipase A2 in collagen-stimulated platelets which resulted in a reduction of LDL modification. It was suggested that this may represent a novel pathway through which platelets trigger the progression of atherosclerosis. Finally, the in vitro findings of this interesting study were supported by ex vivo experiments. Platelets from hypercholesterolemic patients were found to have a higher potential to oxidize LDL and to promote LDL uptake by macrophages compared to platelets from healthy donors. The authors concluded that platelets in patients at risk of atherosclerotic disease could contribute to the progression of atherosclerotic plaque formation by an oxidative stress mechanism.

7. Platelet-induced foam cell generation from progenitor cells

Circulating progenitor cells play an important role in repair mechanism at sites of vascular lesions. Our group was able to show that human platelets recruit CD34+ progenitor cells via the specific adhesion receptors P-selectin/PSGL-1, β1- and β2-integrins. However, platelets are also able to induce the differentiation of CD34+ progenitor cells into mature foam cells. In our in vitro-atheroscreen model, foam cell formation could be induced reproducibly in vitro after co-incubation of human CD34+ progenitor cells with activated platelets. These foam cells are characterized by their typical morphology, the expression of the scavenger receptor CD68 and intracellular lipids (Figure 2). They take up red-labelled LDL (Dil-OxLDL) and accumulate lipids inside their cytoplasm. We also found a dose- and time-dependent uptake of Dil-OxLDL into macrophage foam cells at least partially mediated by CD68. However, Dil-OxLDL was also taken up by platelets themselves. Moreover, when platelets were pre-incubated with OxLDL and added to macrophages, foam cell formation occurred also by platelet-mediated ‘transport’ of OxLDL into macrophage foam cells via phagocytosis (Figures 2 and 3). Thus, platelets are able to take up modified LDL which is then stored in dense granula and rapidly internalized into macrophage foam cells. So far, platelets were not known to play such a direct role in macrophage-foam cell formation. In addition, we also found an enhanced release of ROS by platelets contributing to the modification of native LDL. Thus, uptake of modified LDL by platelets and phagocytosis of LDL-laden platelets by macrophages may be critical steps for the development of lipid-rich plaques (Figure 1).
8. Inhibition of platelet-mediated foam cell formation

The ability to understand and modulate these mechanisms may offer new treatment strategies for patients at high risk for atherosclerotic diseases. Recently, we could demonstrate that platelet-induced foam cell generation from progenitor cells could be partially prevented by HMG-CoA reductase inhibitors and agonists of peroxisome proliferator-activated receptor-α and -γ.91 Furthermore, we cloned and characterized an immunoadhesin which resembles the scavenger receptor CD68 and binds its ligand OxLDL with high affinity. CD68-Fc was found to bind to lipid rich human atherosclerotic plaque specimens. In our in vitro-atheroscreen model, CD68-Fc was able to inhibit platelet-mediated macrophage foam cell formation and specific functions, such as matrix metalloproteinase-9 activity in vitro. Thus, the inhibition of platelet-mediated foam cell formation may be a promising option to influence atherosclerotic plaque formation (Figure 1).

9. Atherogenic role of platelets

Beside their classical role in thrombosis, platelets contribute to endothelial dysfunction, initiate atheromatous plaque formation, and modulate various inflammatory responses. Continuously elevated blood levels of LDL exert ‘pro-atherogenic’ activating effects on platelet function resulting in hyperaggregability. In contrast, HDL desensitizes platelets and has ‘anti-atherogenic’ effects. When platelets adhere to the inflamed or functionally disturbed, but otherwise uninjured endothelium, they are able to initiate and accelerate atherosclerosis (Figure 1). Adhering platelets recruit monocytes that transmigrate into the subendothelial space and develop into macrophages and foam cells (Figure 3).
1. Platelets also recruit endothelial progenitor cells when they adhere to the endothelium. These progenitor cells differentiate into endothelial cells for vascular regeneration but also into foam cells depending on the micro-environmental conditions.

We propose a new way of LDL transport and deposition into the arterial vessel wall and subsequent foam cell formation by the following mechanisms: first adhering platelets bind and take up modified LDL via scavenger receptors, such as CD36 or LOX-1. Secondly, they contribute to the further oxidation of lipoproteins by a substantial release of ROS. Thirdly, platelets transfer bound modified LDL into macrophages by which they are phagocytosed and further processed (Figure 4).

10. Conclusion

Currently, it is not clear, whether OxLDL uptake by platelets and phagocytosis by monocytes/macrophages takes place in circulating blood or after adherence of platelets to the endothelium. However, it is tempting to speculate that chronically elevated LDL levels in blood alter platelet function leading to platelet adhesion at lesion-prone site, such as CD36 or LOX-1. Secondly, they contribute to the further oxidation of lipoproteins by a substantial release of ROS. Thirdly, platelets transfer bound modified LDL into macrophages by which they are phagocytosed and further processed.

Although our group has published some in vitro data about phagocytosis of a platelet lawn by progenitor cells and subsequent foam cell formation, no adequate model exists until now to study this process in vivo. We are just beginning to learn more about the relationship between platelets and lipoproteins and about the structural changes in the vascular wall. Further research elucidating the role of platelets as inducer or trigger of foam cell formation will be of great interest and an antiatherosclerotic therapy blocking the platelet-mediated LDL uptake and foam cell generation may be a promising new therapeutic strategy.

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