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Platelet hyperreactivity, scavenger receptors and atherothrombosis

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Summary

Scavenger receptors (SRs) were initially identified as macrophage receptors that recognize modified lipoproteins. The lists of SRs, their ligands and cells expressing SRs have been significantly extended during the last two decades. What has become clear is that many ligands of SRs are present *in vivo* only in pathologic conditions. Several SRs have been identified on platelets with the best studied being scavenger receptors CD36 and SR-BI. Platelet SRs are multiligand receptors with properties of pattern recognition receptors. CD36 and SR-BI are exposed on resting platelets, while other SRs are rapidly expressed upon platelet activation. Thus, platelets may serve as sensors of 'pathologic ligands' in circulation. The role of platelet SRs in platelet physiology is still poorly understood. However, the data are accumulating that SR ligands, present in the circulation under pathologic conditions, interact with platelet SR and modulate platelet reactivity, thereby contributing to thrombosis and cardiovascular pathology.

Keywords

atherothrombosis; oxidized LDL; oxidized phospholipids; pattern recognition receptors; platelet hyperreactivity; scavenger receptors

Introduction

Scavenger receptors (SRs) were initially identified as macrophage receptors based on their ability to recognize modified lipoproteins. A number of different classes of SRs (from A to G) have been identified so far. Many SRs are multi-ligand receptors and some are pattern-recognition receptors. Several SRs were identified on platelets. The list of ligands for platelet SRs includes many that are present only in pathologic conditions. Platelet SRs include class B scavenger receptors, CD36 and SR-BI that are best known for their role in lipoprotein/lipid metabolism. Other platelet SRs include class E scavenger receptor LOX-1 and class D scavenger receptor CD68. There is data suggesting that scavenger receptor type A may be present on platelets as well. Expression of SR class B in platelets is mainly constitutive while other receptors are rapidly exposed upon platelet activation. Thus platelets may serve as sensors of 'pathologic ligands' for these receptors. Whether interaction of SR ligands with platelets contributes to cardiovascular pathology is yet to be determined adequately and, in general, the role of platelet SRs in platelet physiology is still poorly

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Disclosure of Conflict of Interests

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understood. We will present data suggesting that SRs on platelets may play a significant role in thrombosis only in conditions when their (pathologic) ligands are present.

Scavenger receptor CD36

CD36 is an 88-kDa heavily glycosylated transmembrane protein expressed in various cell types, including macrophages, platelets and microvascular endothelial cells [1]. It plays a significant role in many physiologic and pathologic processes including: atherogenesis, lipid metabolism, innate immune responses, angiogenesis, and diabetes [1]. Even though CD36 was recognized as a major platelet glycoprotein more than 3 decades ago, its role in platelet physiology had remained obscure. It has been suggested that CD36 may modulate platelet activation via its role in platelet uptake of arachidonic acid. Human CD36-negative platelets exhibited a mild defect in the initial stages of adhesion to fibrillar collagen I [2]. CD36 recognizes a number of distinct ligands including thrombospondin-1, oxidized LDL (oxLDL), fatty acids, microbial diacylglycerides and many others [1,3,4]. We have identified a novel family of oxidized choline glycerophospholipids (oxPC_{CD36}) that mediate CD36-dependent recognition of oxidized LDL and are formed during the oxidation of LDL by multiple distinct pathways [5]. As dyslipidemia is associated with oxidative stress, we proposed that oxPC_{CD36} may also be formed and accumulate in dyslipidemia. A number of pathophysiologic states related to dyslipidemia are associated with increased platelet reactivity and thrombogenic potential [6]. Thus, we hypothesized that the interaction of oxPC_{CD36} with platelet CD36 may alter platelet reactivity, inducing pro-thrombotic signals associated with dyslipidemia.

We found that levels of oxPC_{CD36} in plasma of hyperlipidemic *Apoe*^{-/-} and *Ldlr*^{-/-} mice was up to fortyfold higher than that in normolipidemic mice [6]. High levels of oxPC_{CD36} in the plasma of these mice were linked to increased platelet reactivity in a CD36-dependent manner [6]. Importantly, there was no significant reduction in response to agonists in platelets from *CD36*^{-/-} mice when compared with wild type on a normal chow diet, in line with our hypothesis that the role of CD36 in thrombosis may be restricted to the dyslipidemic milieu, where enhanced oxidative stress is present and specific ligands for CD36 are generated [7]. The observed platelet hyperreactivity was associated with the presence of a procoagulant factor in hyperlipidemic plasma [6]. This factor could be blocked by a CD36 peptide containing the binding site for oxPC_{CD36} (E. Podrez unpublished data). Importantly, oxPC_{CD36} induced platelet activation via CD36 and sensitized platelets to other agonists *in vitro* at concentrations found during hyperlipidemia *in vivo* [6]. oxPC_{CD36} also promoted platelet thrombus formation on collagen, a physiologic substrate. (E. Podrez, unpublished data). We observed that hyperlipidemia induced a pronounced pro-thrombotic state *in vivo* that can be rescued by genetic deletion of CD36 [6]. Platelet transfusion studies demonstrated that specifically, platelet CD36 was responsible for the observed rescue [6]. We also detected significant levels of oxPC_{CD36} in human plasma samples. The samples with the highest levels of oxPC_{CD36} reproducibly supported the highest level of platelet activation only in CD36-positive human platelets [6]. Interestingly, phosphatidylserine (PS), a well known ligand for CD36 that is present on cell-derived microparticles has been demonstrated recently to represent another endogenous CD36 ligand. It transmits an activating signal to platelets and contributes to thrombogenesis [8]. Platelet CD36 is associated with non-receptor tyrosine kinases of the src family [9], which have previously been implicated in platelet activation by oxLDL [10]. Recent studies found that hyperlipidemia *in vivo* and oxLDL *in vitro* activate JNK2 and its upstream activator MKK4 in platelets via CD36 [11]. CD36-dependent phosphorylation of platelet JNK within thrombi was demonstrated. Furthermore, pharmacologic inhibition of JNK delayed thrombosis in wild-type but not *CD36*^{-/-} mice *in vivo* [11].

The elucidation and characterization of the binding site for oxPC_{CD36} is important for understanding the contribution of the molecular mechanisms of CD36 in cardiovascular disease. We have recently identified a short stretch of amino acids in CD36 (CD36_{160–168}) as the minimal oxPC_{CD36}-binding domain [12]. This domain contains two evolutionarily conserved lysine residues at positions 164 and 166. We demonstrated that an electrostatic interaction, between the negatively charged groups in the lipids and the conserved positively charged lysines in CD36_{160–168}, is the primary mechanism of the ligand recognition. Peptides mimicking the oxPC_{CD36}-binding site were found to be potent inhibitors of oxLDL-binding to cells expressing CD36 and were proposed to be used *in vivo* to prevent pathologic effects of oxPC_{CD36} [12].

Scavenger receptor-BI

Class B scavenger receptor SR-BI (CLA-I) is close relative of CD36. It binds a variety of ligands including native and oxidized lipoproteins [13]. It is expressed on the surface of various cell types including hepatocytes and endothelial cells [13]. The major physiologic function of SR-BI is to mediate the selective transport of cholesteryl esters from HDL in the liver and steroidogenic tissues [13]. SR-BI also promotes bidirectional flux of free cholesterol between cells and lipoproteins [14]. SR-BI^{-/-}/apoE^{-/-} mice have an abnormal lipoprotein phenotype, develop severe occlusive coronary artery disease and die prematurely from myocardial infarctions [15]. SR-BI expression in extrahepatic tissues contributes to myocardial infarction [16]. It is possible that SR-BI deficiency in extrahepatic tissues contributes to acute thrombotic events, although this hypothesis has not yet been tested. Reduced expression of platelet SR-BI in patients was associated with increased platelet aggregation *in vitro* [17].

We have tested whether SR-BI, as a receptor for HDL, can be the causative agent for the reported HDL-mediated inhibition of platelet activation [18]. However, we found that even supraphysiologic concentrations of HDL do not have significant direct effects on agonist-induced platelet activation [19]. Surprisingly, HDL upon oxidative modification acquires potent antithrombotic activity [19]. OxHDL inhibits platelet aggregation via a pathway that interferes with multiple physiologic agonists [19] by inhibiting platelet α Ib β 3 activation. The inhibitory effect requires binding of oxHDL to platelet SR-BI [19]. While CD36 was reported to recognize OxHDL [20], we found that SR-BI is the major receptor on platelets for OxHDL. Even though both HDL and OxHDL bind to SR-BI, only OxHDL inhibits platelet activation, suggesting that the inhibitory activity may be attributable to the presence of oxidized moieties such as oxidized phospholipids in OxHDL. We have recently demonstrated that oxPC_{CD36} does bind specifically to SR-BI [21]. The mechanism of SR-BI-mediated inhibition of platelets-activation by oxHDL is currently under investigation. In endothelial cells, HDL binding to SR-BI stimulates eNOS via the activation of Src family kinase(s), PI3K, Akt kinase, and MAPK [22]. However, we found that this signaling pathway it is not involved in inhibition of platelet aggregation by OxHDL [19]. Interestingly, a recent study reported that platelet responses to ADP are blunted in platelets from SR-BI mice [23]. The mechanism of the effect is unknown.

There is abundant evidence for the presence of OxHDL *in vivo* [24], suggesting that the described SR-BI-dependent inhibition of platelet function is physiologically relevant. There are many other established ligands for SR-BI such as lipid-free apoE, LDL and VLDL, chemically modified lipoproteins, advanced glycation end product-modified proteins, β -amyloid, the hepatitis C virus envelope glycoprotein E2 and others. Whether these ligands have any effect on platelet function that is mediated by SR-BI remains to be established.

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1)

LOX-1 is a Class E scavenger receptor expressed on endothelial cells, macrophages, smooth-muscle cells and platelets [25,26]. It binds a number of ligands including OxLDL, apoptotic cells, activated platelets and bacteria and is implicated in the pathogenesis of atherosclerotic lesions. LOX-1 expression on the surface of platelets was found to be activation-dependent [26]. Blocking of LOX-1 inhibited ADP-induced platelet-activation *in vitro* suggesting that it may play a role in platelet physiology [27]. LOX-1 recognition of activated platelets and exposure of LOX-1 on the activated platelet surface might assist thrombosis formation. Further studies are needed to show the role of LOX-1 in thrombosis *in vivo*.

Scavenger receptor class A (SR-A)

A recent study found that p38^{MAPK} phosphorylation can be induced by oxLDL in CD36-deficient platelets but was absent in platelets of mice deficient in both CD36 and SR-A, suggesting that this receptor may be expressed on platelets in addition to other SR [28].

CD68

Class D scavenger receptor CD68 (macrosylin), a lysosomal marker that is heavily expressed in macrophages, is exposed on the surface of activated platelets in parallel with other lysosomal markers [29]. CD68 was shown to bind oxidized LDL, although it is not clear whether this plays any role in platelet recognition of oxidized LDL.

In conclusion

Published studies and our unpublished studies suggest that platelet SRs can modulate platelet activation. Specifically, in the context of dyslipidemia and enhanced oxidative stress, CD36 and SR-BI may play opposite roles. While platelet CD36 interaction with specific oxidized phospholipids present in circulation results in enhanced platelet reactivity and a prothrombotic phenotype [6], SR-BI interaction with OxHDL, a particle that is present in circulation in chronic inflammatory conditions, may inhibit platelet activation [19]. The contribution of other platelet scavenger receptors to platelet physiology is currently under investigation.

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