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Role of Platelets in the Development of Atherosclerosis

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Platelets are blood cell fragments that originate from the cytoplasm of megakaryocytes in the bone marrow and circulate in blood to play a major role in the hemostatic process and in thrombus formation after an endothelial injury. Recent studies have provided insight into platelet functions in inflammation and atherosclerosis. A range of molecules, present on the platelet surface and/or stored in platelet granules, contributes to the cross-talk of platelets with other inflammatory cells during the vascular inflammation involved in the development and progression of atherosclerosis. This review discusses the nature of these molecules and the mechanisms involved in the participation of platelets in atherosclerosis, with emphasis on P-selectin, platelet–monocyte interactions, chemokines, and inflammatory cytokines. (Trends Cardiovasc Med 2004;14:18–22) © *2004, Elsevier Inc.*

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• Platelet Activation in Atherosclerosis

Platelet activation can be seen in the different phases of atherosclerosis. Detection of activated platelets as defined by P-selectin surface expression in peripheral blood of patients with unstable atherosclerotic disease was first reported by Fitzgerald et al. (1986). These circulating activated platelets are very likely to associate with thrombotic events. Circulating activated platelets were also

detected in the blood of atherosclerotic rabbits (Tsao et al. 1994) and patients with stable coronary disease (Furman et al. 1998). Interestingly, most risk factors of atherosclerosis—including hypercholesterolemia (Broijersen et al. 1998), hypertension (Nityanand et al. 1993), cigarette smoking (Nowak et al. 1987), and diabetes (Manduteanu et al. 1992)—are able to increase the number of activated platelets in circulation. The presence of circulating activated platelets preceding massive thrombotic events such as myocardial infarction or stroke may be relevant to the development and progression of atherosclerosis.

The molecular mechanisms responsible for platelet activation in atherosclerosis are unknown. In a thrombotic event, platelet activation begins with the binding of adhesive receptors to their ligands on extracellular matrix constituents (Ruggeri 2002). This process is further strengthened by signaling through α thrombin produced on the membrane of stimulated platelets, adenosine diphosphate (ADP) generated by vascular cells and stimulated platelets, epinephrine released in response to stress, and thromboxane (TX) A_2 synthesized by stimulated platelets (Ruggeri 2002). In the early phase of atherosclerosis, platelet activation may be attributed to (a) reactive oxygen species generated by risk factors of atherosclerosis, including superoxide, hydroxyl radical, and peroxynitrite; (b) reduction in endothelial antithrombotic properties such as production of nitric oxide, prostacyclin, and CD39, an ecto-ADPase that degrades ADP; or (c) an increase in prothrombotic and proinflammatory mediators, including tissue factor and chemokines in the circulation or immobilized on the endothelium. Additionally, following formation of lesions on the vessel wall, platelet activation may be initiated by ligation of GPIb with the endothelial receptor P-selectin and endothelial von Willebrand factor (VWF) during platelet tethering on intact but dysfunctional endothelium.

Platelet activation influences the development of atherosclerosis. Inhibition of platelet TX A_2 production by aspirin (Cyrus et al. 2002) and indomethacin (Pratico et al. 2001) or TX receptors (Cayatte et al. 2000) by antagonist is able to dramatically diminish the formation of atherosclerotic lesions. Some conflicting data exist. For example, some findings did not support a beneficial effect of aspirin in atherosclerosis (Cayatte et al. 2000, Napoli et al. 2002). Also, the inhibitory effect of aspirin in atherosclerosis may be achieved by suppressing not only platelet cyclooxygenase (COX)-1, but also COX-2 in macrophages and smooth muscle cells in the vessel wall.

• Interaction of Activated Platelets with Endothelium

Activated platelets are able to interact with endothelium of postcapillary venules in mice with hypercholesterolemia (Tailor and Granger 2003) or after stimulation with the calcium ionophore A23187 (Frenette et al. 1995). Consistent with these studies, using epifluoresence microscopy, we observed that in vitro-activated, but not resting fluorescently labeled, platelets interact transiently but robustly with atherosclerotic carotid arteries of apoE$^{-/-}$ mice in vivo (Huo et al. 2003). These interactions are mainly characterized by transient tethering and rolling, but firm adhesion is rare (Huo et al. 2003). Platelet P-selectin is indispensable during the interactions of activated platelets with atherosclerotic arteries (Huo et al. 2003). The endothelial ligand for platelet P-selectin is unknown. Employing a similar in vivo model, Massberg et al. (2002) found that platelet GPIb and GPIIb/IIIa are crucial for platelet translocation and firm adhesion, respectively. In addition to P-selectin and GPIb, Theilmeier et al. (2002) classified the VWF as an important molecule in both resting and activated platelet recruitment to atherosclerosis-prone sites of arteries of rabbits with hypercholesterolemia. These authors (Theilmeier et al. 2002) also found GPIIb/IIIa-mediated platelet adhesion. Regarding platelet adhesion in these studies, the various methods of endothelial preparation and definitions of firm adhesion are likely to influence the results. Consistent with the observations by Ross (1999), under a scanning electronic microscope, we did not find platelets directly adherent to atherosclerotic endothelium, even after an injection of in vitro activated platelets. Because there is no evidence suggesting that platelets are able to transmigrate, a substantial platelet accumulation on luminal surfaces of atherosclerotic arteries should be witnessed if firm platelet adhesion on the atherosclerotic endothelium occurs. However, we did find platelets associated with monocytes adherent to the vessel wall.

Platelets interacting with the endothelium may influence the development and progression of atherosclerosis through a variety of mechanisms. The platelet monolayer formed on the injured vessel provides a platform for leukocyte recruitment to vessel walls. This pathologic process—which occurs in the thrombotic event—may not happen in the context of spontaneous atherosclerosis, because platelets adherent to the endothelium are, at most, sparse. Our study (Huo et al. 2003) showed that platelets transiently interacting with the atherosclerotic endothelium are able to deliver and deposit the chemokine RANTES. This suggests that the contribution of platelet–endothelial interactions in atherosclerosis may be to facilitate the delivery of platelet-derived proinflammatory factors to atherosclerotic arteries (Figure 1C).

The role of molecules mediating platelet–endothelial interactions in the development of atherosclerosis has been examined. Platelet P-selectin is indeed important. Repeated injections of P-selectin-expressing platelets into apoE$^{-/-}$ mice accelerated the formation of atherosclerotic lesions (Huo et al. 2003). Reconstituted mice whose platelets lack P-selectin formed smaller lesions than did control mice (Burger and Wagner 2003). The effect of VWF on the development of atherosclerosis is controversial. In pigs without VWF, inconsistent observations were reported regarding their resistance to atherosclerosis (Bowie and Fuster 1980, Nichols et al. 1998). Deficiency of VWF in a limited number of patients also did not show any protective role in development of atherosclerosis (Sramek et al. 2001). Mice deficient in VWF demonstrated decreased atherosclerosis (Methia et al. 2001). Although the effect may be accredited to the lack of VWF-mediated platelet interactions, other mechanisms may also be involved. Endothelial cells deficient in VWF have a defect in secretion of P-selectin from their Weibel-Palade bodies. Thus, endothelial P-selectin-mediated monocyte recruitment in atherosclerosis could be dramatically compromised in these VWF mutant mice (Denis et al. 2001). Chronic inhibition of GPIb with blocking antibody protected apoE$^{-/-}$ mice from atherosclerosis (Massberg

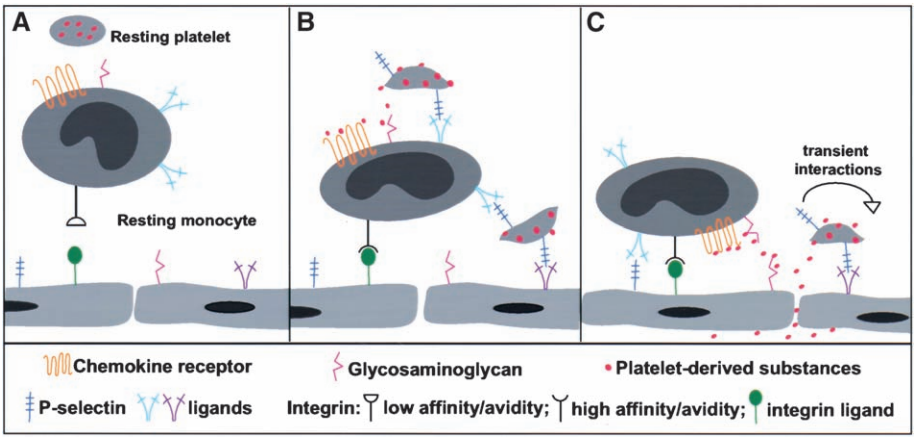

Figure 1. Mechanisms by which activated platelets participate in the development of atherosclerosis. **(A)** No interactions occur between resting platelets and monocytes. **(B)** Activated platelets promote monocyte recruitment via platelet–monocyte interactions. Activated platelets interacting with monocytes deliver their proinflammatory factors to monocytes. Consequently, affinity and/or avidity of monocyte integrins are upregulated and monocytes arrest on endothelium. Additionally, monocyte–platelet aggregates may employ platelet P-selectin to mediate aggregates to interact with endothelium. **(C)** Activated platelets promote monocyte recruitment via platelet–endothelial interactions. Activated platelets transiently interacting with endothelium may deposit their proinflammatory factors on the surface of endothelium, causing subsequent rolling monocyte arrest. Also, platelet-derived proinflammatory factors may infiltrate into the vessel wall, triggering vascular cell proliferation, migration, and inflammation.

et al. 2002). GPIIb/IIIa may be less relevant to the development of atherosclerosis. Patients with Glanzmann thrombasthenia lacking platelet glycoprotein $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) and $\alpha_v\beta_3$ receptors are not protected from atherosclerosis (Shpilberg et al. 2002). This is further demonstrated in β3-deficient mice. In atherosclerotic mice, deficiency of β3 even promoted atherosclerosis and caused significant pulmonary inflammation (Weng et al. 2003).

• Interaction of Activated Platelets with Leukocytes

P-selectin on activated platelets initiates their interactions with leukocytes. Among leukocyte subtypes interacting with activated platelets, monocytes have a competitive advantage over others in binding activated platelets (Huo et al. 2003). The property by which monocytes preferentially bind platelets, a possible basis for the role of activated platelets in the development of atherosclerosis, is unknown. Engagement of platelets with leukocytes results in activation of the leukocyte integrins Mac-1 and VLA-4. Consequently, interactions between activated platelets and leukocytes will be stabilized due to binding of leukocyte integrins to platelet intracellular adhesion molecule 2 (ICAM-2) or adhesive plasma proteins bound to activated GPIIb/IIIa complex. Recently, it was shown that GPIb is also a ligand for leukocyte Mac-1 (Simon et al. 2000). The life spans and destinies of platelet–leukocyte aggregates are not well defined. In a study using primates, Michelson et al. (2001) found that the life span of platelet–monocyte aggregates was not related to platelet P-selectin shedding. In our in vivo study (Huo et al. 2003), 2 to 3 hours after an injection of activated platelets, circulating platelet–leukocyte aggregates were no longer detectable and "normal" leukocyte subtype populations were recovered. This time course is consistent with the time that circulating activated platelets take to shed their P-selectin (Berger et al. 1998), suggesting that most platelet–leukocyte aggregates end up in disengagement. However, the possibility that aggregates sequester into peripheral tissues or monocytes phagocytose platelets bound to their surfaces cannot be excluded and needs to be investigated. Alteration in monocyte/macrophage function resulting from platelet phagocytosis may systemically regulate immune and inflammatory reactions (Mulligan et al. 1993).

Platelets binding to monocytes regulate monocyte functions. Activated platelets are able to upregulate affinity and/or avidity of monocyte/leukocyte integrins via P-selectin glycoprotein ligand-1 (PSGL-1) signaling or delivery of platelet-derived proinflammatory factors (Figure 1B). Oxidative burst occurs on monocytes in response to platelet binding. These rapid platelet-mediated regulations may play a role in atherosclerosis by promoting monocyte recruitment (Figure 1B). Activated platelets are also able to cause a variety of slow reactions. Resting monocytes do not express tissue factor, a protein involved in the initiation of blood coagulation and the formation of atherosclerotic lesions. However, upon interaction with platelet P-selectin, transcription of tissue factor is activated. Subsequently, tissue factor mRNA, protein, and activity are induced over several hours (Celi et al. 1994), although this was not confirmed in a later study (Weyrich et al. 1995). Different cytokines are induced when monocytes bind platelet P-selectin through PSGL-1 and are primed by several different synergistic activators. Exposure of monocytes to platelet P-selectin and platelet activating factor mobilizes the transcription factor nuclear factor-κB and induces expression of tumor necrosis factor α (TNFα) and monocytes chemotactic protein 1 (MCP-1) (Weyrich et al. 1995). Monocytes exposed to P-selectin and the platelet-derived chemokine RANTES secrete a different set of cytokines, including interleukin 8 (IL-8) and MCP-1 (Weyrich et al. 1996). These reactions significantly contribute to the inflammatory and procoagulant response in vascular thrombotic disease. It is not clear whether these reactions can be initiated during transient interactions between monocytes and platelets. Therefore, the relevance of these reactions in the development of atherosclerosis is unknown.

• Platelet-Derived Proinflammatory Factors in Atherosclerosis

Upon activation, platelets release a wealth of adhesive and proinflammatory substances. Main organelles involved in this release reaction are the open canalicular system, the α granules, and the dense tubular system. Among them, the α granules are the most important storage compartments within the platelets for adhesive proteins and peptides that mediate inflammation. Most of the substan-

ces that are contained within the α granules are synthesized in megakaryocytes, but it is possible that some of them are endocytosed from the blood plasma. Several groups of these substances are relevant to inflammation and atherosclerosis. Platelet factor 4 (PF-4), a member of the C-X-C subfamily of chemokines, is derived by limited proteolysis from platelet basic protein. PF-4 causes chemotaxis of monocytes and other leukocytes. Recent studies (Nassar et al. 2003) provide more evidence for the involvement of PF-4 in the development of atherosclerosis. PF-4 enhances the binding of oxidized low-density lipoprotein (oxLDL) to vascular wall cells, including endothelial cells and smooth muscle cells. PF-4, colocalized with oxLDL in atherosclerotic lesions, especially in macrophage-derived foam cells, is able to dramatically increase oxLDL esterification by macrophages. The presence of glycosaminoglycans on the cell surface is required for these PF-4-mediated reactions (Nassar et al. 2003, Sachais et al. 2002). RANTES and macrophage inflammatory protein (MIP)-1α are members of the C-C chemokine subfamily. RANTES, first purified as a product of activated T cells, is a powerful chemoattractant for memory T lymphocytes and monocytes. MIP-1α also causes chemotaxis of CD8 T lymphocytes, a lymphocyte subset found in atherosclerotic lesions of mice. Platelets are able to deposit their RANTES on the luminal surface of atherosclerotic arteries, suggesting that platelet-derived chemokines are possibly involved in the development of atherosclerosis (Huo et al. 2003, von Hundelshausen et al. 2001). Another α-granule constituent, platelet-derived growth factor (PDGF), is a cationic polypeptide composed of two chains (A and B) that are linked by interchain disulfide bridges. PDGF is the major growth factor in platelets stimulating vascular smooth muscle cell migration and proliferation associated with intimal hyperplasia. Also, PDGF is chemotactic and activates monocytes. Therefore, PDGF has long been speculated to be an important participant in the development of atherosclerosis. Endothelial cells lack PDGF receptors and are unresponsive to PDGF. Therefore, PDGF may mainly target other vascular cells in the vessel wall. Macrophages and foam cells also produce PDGF throughout the progression of atherosclerosis. To confirm that PDGF from platelets contributes to the formation of atherosclerotic lesions, direct evidence is needed to show that a portion of PDGF in atherosclerotic lesions is really "platelet derived."

In addition to the above mediators from platelet α granules, IL-1β and CD40 ligand (CD40L, CD154) from platelets have recently received intense interest. CD40L—a trimeric, transmembrane protein of the TNF family originally identified on activated T cells—has been found in platelets. CD40L is stored in the cytoplasm of resting platelets and rapidly presented on the surface after activation (Hermann et al. 2001). The surface-expressed CD40L is subsequently cleaved over a period of minutes or hours, generating a soluble but functional fragment, sCD40L. Platelet-derived CD40L is capable of initiating various inflammatory responses on endothelial cells, including production of reactive oxygen species (Urbich et al. 2002), expression of adhesion molecules (e.g., vascular cell adhesion molecule 1, ICAM-1, and E-selectin), chemokines (e.g., MCP-1 and IL-8), cytokine IL-6 (Henn et al. 1998), and tissue factor (Slupsky et al. 1998). In contrast to CD40L constitutively stored in platelet cytoplasm, platelet IL-1β is synthesized upon platelet activation (Hawrylowicz et al. 1989). A portion of IL-1β is presented in its mature form on the platelet membrane and induces endothelial cell adhesiveness (Lindemann et al. 2001). It is possible that these mediators from the platelet source are involved in the inflammatory process underlying atherosclerosis. Blockade of the CD40/CD40L pathway has been shown to dramatically diminish the development of atherosclerosis (Mach et al. 1998). The contribution of platelet-derived CD40L in this process has not been evaluated. To achieve a proinflammatory effect, membrane contact interactions are required for these factors, especially for IL-1β. Therefore, studies need to be performed to investigate whether transient interactions of platelets with endothelium are able to trigger endothelial inflammation in the context of atherosclerosis.

• Platelet Microparticles

Platelet microparticles, released from activated platelets, contain most of the platelet adhesive molecules and proinflammatory factors, and cause a variety of inflammatory reactions, as do activated platelets. The role of activated platelets in the development of atherosclerosis may be partially attributed to platelet microparticles.

• Conclusion

Thirty years after Ross et al. (1976) proposed the involvement of platelets in atherosclerosis, direct evidence now supports the conclusion that activated platelets truly play an important role in the development of atherosclerosis. Mechanisms regarding the participation of platelets in atherosclerosis, although partially suggested in primary studies, have not been fully examined yet. Investigation of these mechanisms may lead to new approaches to curb the development and progression of atherosclerosis.

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PII S1050-1738(03)00169-5 **TCM**