REVIEW ARTICLE

MECHANISMS OF DISEASE

Platelet Activation and Atherothrombosis

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Platelets are essential for primary hemostasis and repair of the endothelium, but they also play a key role in the development of acute coronary syndromes and contribute to cerebrovascular events. In addition, they participate in the process of forming and extending atherosclerotic plaques. Atherosclerosis is a chronic inflammatory process,[1] and inflammation is an important component of acute coronary syndromes.[2] The relation between chronic and acute vascular inflammation is unclear, but platelets are a source of inflammatory mediators,[3] and the activation of platelets by inflammatory triggers may be a critical component of atherothrombosis.[4] This review article describes the role of platelets in atherothrombosis by integrating our knowledge of basic mechanisms with the results of mechanistic studies in humans and clinical trials of inhibitors of platelet function.

PLATELETS IN PRIMARY HEMOSTASIS

Platelets are produced by megakaryocytes as anucleate cells that lack genomic DNA[5] but contain megakaryocyte-derived messenger RNA (mRNA) and the translational machinery needed for protein synthesis.[6] Pre-mRNA splicing, a typical nuclear function, has been detected in the cytoplasm of platelets,[7] and the platelet transcriptome contains approximately 3000 to 6000 transcripts. Analysis of the platelet proteome is more complex.[8-11] After leaving the bone marrow, platelets circulate for about 10 days. Their primary function is to stop hemorrhage after tissue trauma and vascular injury.

PLATELET ACTIVATION

The initial tethering of platelets at sites of vascular injury is mediated by glycoprotein Ib/V/IX, a structurally unique receptor complex expressed in megakaryocytes and platelets. Von Willebrand factor is the major ligand for one component of this complex, glycoprotein Ib, and the absence of the factor causes defects in primary hemostasis and coagulation.[12] Besides glycoprotein Ib, several collagen receptors with a tethering function are found on the platelet surface, notably glycoprotein VI and glycoprotein Ia, members of the immunoglobulin superfamily.[4]

After the initial adhesion of platelets to the extracellular matrix, the repair process requires a rapid response to autocrine and paracrine mediators, including adenosine diphosphate (ADP), thrombin, epinephrine, and thromboxane A_2. These mediators amplify and sustain the initial platelet response (Fig. 1), and they recruit circulating platelets from the flowing blood to form a growing hemostatic plug. Most agonists that activate platelets operate through G-protein–coupled receptors.[14] The final pathway for all agonists is the activation of the platelet integrin glycoprotein IIb/IIIa (αIIbβ3), the main receptor for adhesion and aggregation.[15] The

phenotype of mice lacking the β3 integrin resembles that of patients with Glanzmann's thrombasthenia, in which platelets cannot aggregate and have a greatly reduced uptake of fibrinogen.[16]

Several adhesive substrates bind to glycoprotein IIb/IIIa.[13,16] Fibrinogen plays an important role in maintaining the stability of a thrombus, by bridging glycoprotein IIb/IIIa integrins between platelets; von Willebrand factor is necessary to facilitate interplatelet bridges at low shear rates in vitro (Fig. 1). Quiescent platelets contain the pre-mRNA of the molecule termed tissue factor, the primary initiator of the coagulation cascade that leads to the conversion of prothrombin to thrombin and fibrinogen to fibrin.[17] Signal-dependent splicing of tissue-factor pre-mRNA allows for the synthesis of bioactive tissue-factor protein and therefore provides platelet-derived tissue factor for propagating and stabilizing the thrombus.[17]

The vascular endothelium controls platelet reactivity by means of three pathways: the arachidonic acid–prostacyclin pathway, the L-arginine–nitric oxide pathway, and the endothelial ecto-adenosine diphosphatase (ecto-ADPase) pathway.[18] Endothelial cells convert arachidonic acid into prostacyclin with the help of cyclooxygenase-1 or cyclooxygenase-2 (COX-1 or COX-2) and prostacyclin synthase. COX-2 appears to be important in prostacyclin synthesis, on the basis of the effects of selective COX-2 inhibitors on the excretion of prostacyclin metabolites.[19-21] Prostacyclin inhibits platelet function by elevating intracellular cyclic AMP levels.[22]

Nitric oxide diffuses into platelets, stimulates the production of cyclic guanosine monophosphate (GMP), and regulates cyclic GMP–dependent protein kinases, causing a secondary decrease in intracellular Ca^{2+} flux. This reduction in intracellular Ca^{2+} levels suppresses the conformational change in glycoprotein IIb/IIIa that is required for binding of the integrin to fibrinogen, thereby decreasing the number and affinity of fibrinogen binding sites on the platelet's surface.[22]

Ecto-ADPase, an integral component of the endothelial-cell surface, limits the plasma level of nucleotides (ADP and ATP) and is substrate-activated. The activity of this enzyme abrogates the critical recruitment phase of platelet reactivity, because it removes nucleotides from the fluid environment.[23]

CLINICAL IMPLICATIONS

The importance of the role of thromboxane A_2 and ADP in amplifying platelet activation during hemostasis is supported by the twofold increase in the incidence of major bleeding complications (mostly in the upper gastrointestinal tract) associated with the use of low-dose aspirin or the thienopyridines ticlopidine and clopidogrel.[24] The clinical relevance of adhesive interactions with platelet glycoprotein IIb/IIIa in primary hemostasis is known largely from the study of Glanzmann's thrombasthenia[25] and the association of bleeding complications with the use of pharmacologic blockers of glycoprotein IIb/IIIa.[24]

The impairment of primary hemostasis by antiplatelet drugs cannot be dissociated from their effects in the prevention of arterial thrombosis, which suggests that similar molecular mechanisms contribute to both processes. However, the transient incomplete blockade of platelet COX-1 and of glycoprotein IIb/IIIa by some traditional nonsteroidal antiinflammatory drugs and oral inhibitors of glycoprotein IIb/IIIa, respectively, has been associated with an increased risk of bleeding and a lack of antithrombotic efficacy.[24] This suggests that the extent and duration of platelet inhibition required to impair hemostasis may differ from that required to prevent atherothrombosis.

The ex vivo measurement of platelet responses to various agonists provides an index of the functional capacity of platelets,[26-29] but such measurements by no means reflect the extent of platelet activation in vivo.[30-32] The maximum capacity of platelets to synthesize thromboxane A_2 in vitro is approximately 5000 times the basal rate of thromboxane biosynthesis in vivo,[32] and only a fraction of this biosynthetic capacity appears to contribute to platelet activation, as reflected by excretion of thromboxane metabolites.[30,32]

PLATELETS AND DEVELOPMENT OF ATHEROSCLEROTIC LESIONS

Platelets that adhere to the vessel wall at sites of endothelial-cell activation contribute to the development of chronic atherosclerotic lesions, and when these lesions rupture, they trigger the acute onset of arterial thrombosis. Platelets adhere to the endothelium of carotid arteries in apolipoprotein E $(apoE)^{-/-}$ mice before atherosclerotic lesions are visible.[33] Von Willebrand factor, when

Figure 1 (facing page). Agonists, Receptors, and Effector Systems in Platelet Activation.
The activation of platelets is induced by the interaction of several agonists with receptors expressed on the platelet membrane. Panels A, B, and C depict outside-in signaling mediated by thromboxane A_2 (TXA_2), adenosine diphosphate (ADP), and thrombin, respectively. TXA_2 is synthesized by activated platelets from arachidonic acid (AA) through the cyclooxygenase (COX) pathway (Panel A). Once formed, TXA_2 can diffuse across the membrane and activate other platelets. In platelets, there are two splice variants of the TXA_2 receptor: TPα and TPβ, which differ in their cytoplasmic tail. TPα and TPβ couple to the proteins G_q and G_{12} or G_{13}, all of which activate phospholipase C (PLC). This enzyme degrades the membrane phosphoinositides (such as phosphatidylinositol 4,5-bisphosphate [PIP_2]), releasing the second messengers inositol triphosphate (IP_3) and diacylglycerol (DAG). DAG activates intracellular protein kinase C (PKC), which causes protein phosphorylation. The release of IP_3 increases cytosolic levels of Ca^{2+}, which is released from the endoplasmic reticulum. ADP is released from platelets and red cells. Platelets express at least two ADP receptors, $P2Y_1$ and $P2Y_{12}$, which couple to G_q and G_i, respectively (Panel B). The activation of $P2Y_{12}$ inhibits adenylate cyclase, causing a decrease in the cyclic AMP (cAMP) level, and the activation of $P2Y_1$ causes an increase in the intracellular Ca^{2+} level. The $P2Y_{12}$ receptor is the major receptor able to amplify and sustain platelet activation in response to ADP. Thrombin is rapidly generated at sites of vascular injury from circulating prothrombin and, besides mediating fibrin generation, represents the most potent platelet activator (Panel C). Platelet responses to thrombin are largely mediated through G-protein–linked protease-activated receptors (PARs), which are activated after thrombin-mediated cleavage of their N-terminal exodomain. Human platelets express PAR1 and PAR4. PAR1 couples to members of the $G_{12/13}$, G_q, and G_i protein families. The α-subunits of G_{12} and G_{13} bind Rho guanine-nucleotide exchange factors (Rho GEFs), providing for Rho-mediated cytoskeletal responses that are probably involved in the change in platelet shape. The G_q and G_i signaling pathways lead to increased intracellular Ca^{2+} and decreased cAMP, respectively. Panel D depicts inside-out signaling. The effects of agonists mediated by the decrease in cAMP levels and increase in intracellular Ca^{2+} levels lead to platelet aggregation through the change in the ligand-binding properties of the glycoprotein IIb/IIIa (αIIbβ3), which acquires the ability to bind soluble adhesive proteins such as fibrinogen and von Willebrand factor. The release of ADP and TXA_2 induces further platelet activation and aggregation. The small-peptide sequence arginine–glycine–aspartic acid (RGD) of the adhesive proteins binds to the glycoprotein IIb/IIIa receptor. Fibrinogen contains two RGD sequences on its α-chain, one in the N-terminal region and the other in the C-terminal region. The study of fibrinogen$^{-/-}$ mice has shown that von Willebrand factor alone is not sufficient to achieve stable platelet aggregation,[13] supporting the hypothesis that the concurrent binding of von Willebrand factor to glycoprotein IIb/IIIa and glycoprotein Ibα allows for initial contact between platelets, whereas fibrinogen is necessary for a permanent linkage between activated glycoprotein IIb/IIIa on adjacent platelets to ensure stable aggregate formation. TXAS denotes thromboxane synthase, PGH_2 prostaglandin H_2, and PLA_2 phospholipase A_2.

secreted in large amounts by endothelial cells in response to inflammatory stimuli, can recruit platelets to the site; the interaction between glycoprotein Ib and von Willebrand factor allows platelets to roll on endothelial cells. The atherosclerotic lesions in von Willebrand factor$^{-/-}$ mice are smaller than the lesions in wild-type mice.[34]

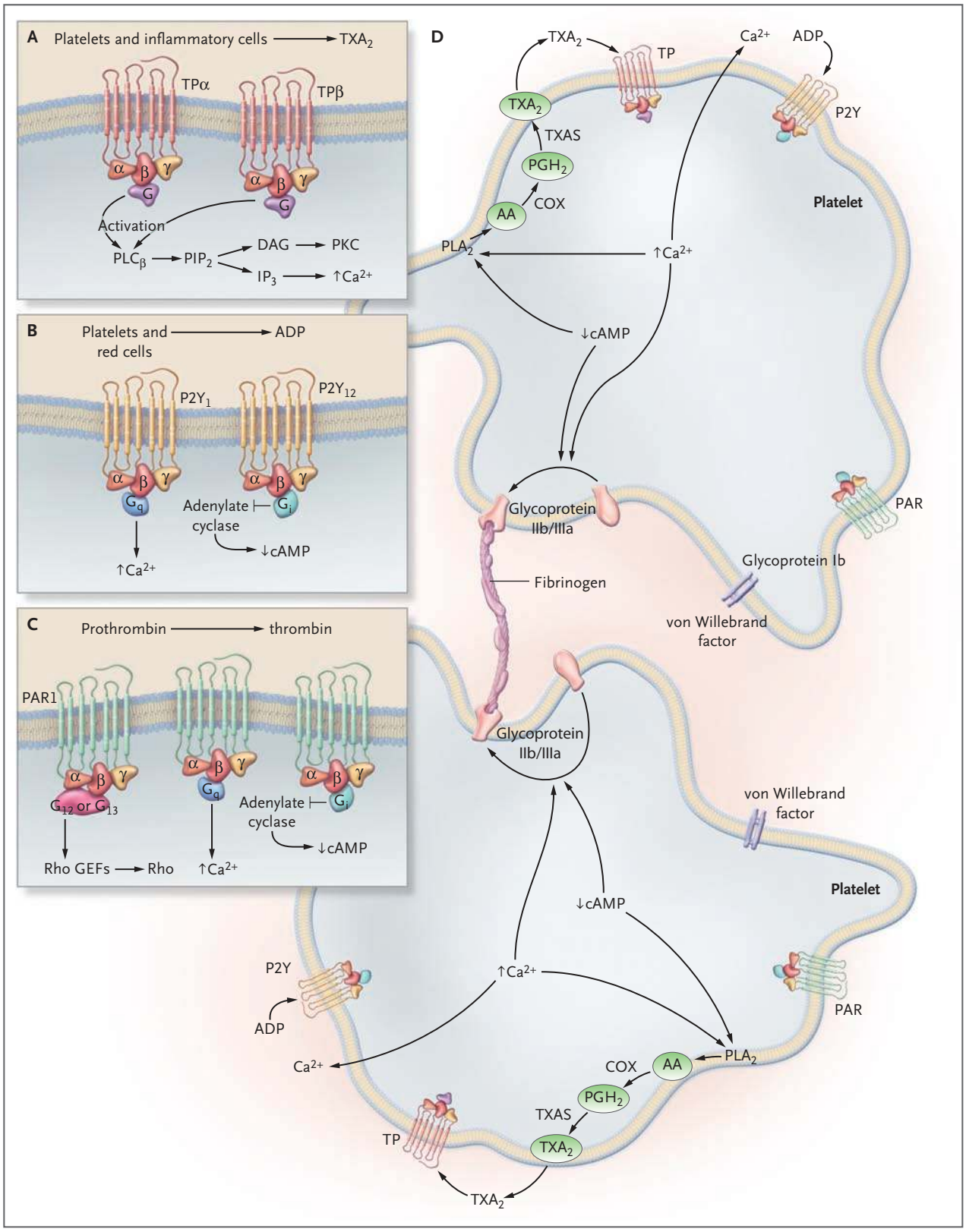
The acceleration of atherogenesis by COX-1–dependent thromboxane in low-density lipoprotein (LDL)–receptor$^{-/-}$ mice suggests that platelet activation increases the rate of plaque formation.[35] The inhibition of the synthesis of platelet thromboxane,[36] as well as the antagonism[37] or deletion[38] of the thromboxane receptor, delays atherogenesis in murine models.

Activated platelets can also influence the progression of plaque formation by releasing adhesive ligands, such as P-selectin, that become expressed on the platelet membrane and mediate platelet–endothelium interactions.[4] Signaling by P-selectin stimulates monocytes and macrophages to produce chemoattractants or growth factors. Moreover, engagement by P-selectin of the P-selectin glycoprotein ligand 1 on the monocyte surface initiates the formation of platelet–monocyte aggregates and outside-in signaling that induces the transcription of *COX-2*.[39] Prolonged adhesion-dependent signaling promotes the expression of interleukin-1β. This cytokine enhances the stability of COX-2 mRNA, thereby promoting synthesis of the enzyme.[39] Activated platelets exacerbate atherosclerosis in apoE$^{-/-}$ mice in a P-selectin–dependent manner.[40]

PLATELET-DERIVED MEDIATORS OF INFLAMMATION

Activated platelets release inflammatory and mitogenic mediators into the local microenvironment, thereby altering the chemotactic and adhesive properties of endothelial cells.[41] These platelet-induced alterations of endothelial-cell function support the chemotaxis, adhesion, and transmigration of monocytes to the site of inflammation (Fig. 2).

CD40 ligand released from platelets induces inflammatory responses in the endothelium.[42] This ligand, originally identified on activated T cells,



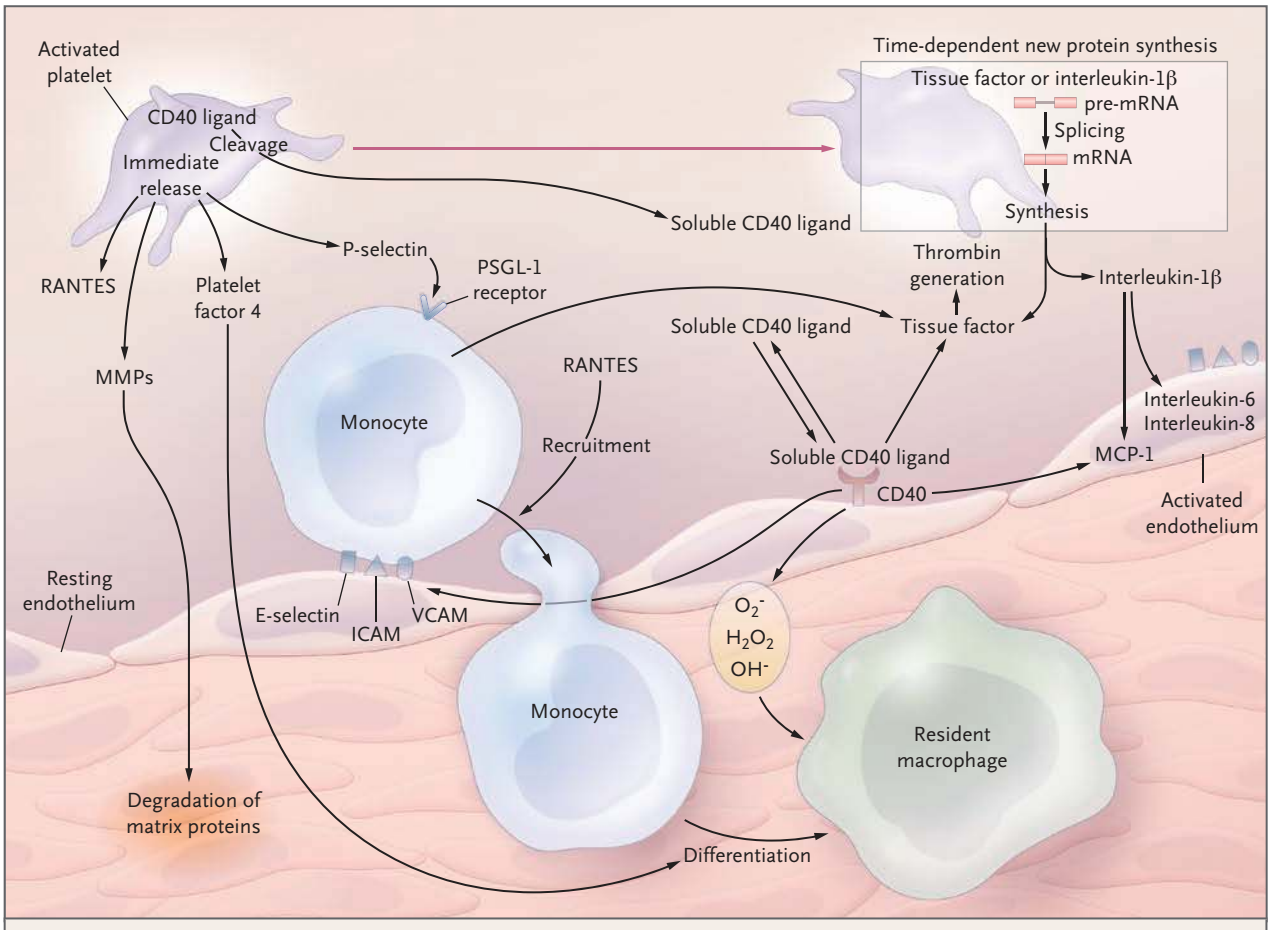


Figure 2. Platelet-Derived Mediators of the Inflammatory Response.

Activated platelets release inflammatory and mitogenic substances into the microenvironment, primarily altering the chemotactic, adhesive, and proteolytic properties of the endothelium. Preformed platelet mediators, stored in α-granules, can be released immediately after platelet activation through a process of exocytosis triggered by increased intracellular calcium levels. Activated platelets are also capable of time-dependent synthesis of protein mediators, such as tissue factor and interleukin-1β. CD40 ligand is stored in the cytoplasm of resting platelets and rapidly presents on the surface after platelet activation. After cleavage, to generate a soluble, functional fragment (soluble CD40 ligand), the mediator is released into the extracellular environment, inducing inflammatory responses in the endothelium by binding CD40 on endothelial cells. P-selectin is released from platelet granules and binds to the P-selectin glycoprotein ligand 1 (PSGL-1) receptor on monocytes, enhancing the adhesion of the monocytes to vascular-cell adhesion molecule (VCAM) 1 and the other adhesins expressed on activated endothelial cells and inducing the production of tissue factor by monocytes. Activated platelets also release chemokines that trigger the recruitment of monocytes (e.g., regulated on activation normal T-cell expressed and secreted [RANTES]) or promote the differentiation of monocytes into macrophages (e.g., platelet factor 4), as well as matrix-degrading enzymes such as matrix metalloproteinase (MMP) 2 or 9. Interleukin-1β is a major mediator of platelet-induced activation of endothelial cells, causing enhanced chemokine release and up-regulation of endothelial adhesion molecules to promote the adhesion of neutrophils and monocytes to the endothelium. ICAM denotes intracellular adhesion molecule, mRNA messenger RNA, MCP-1 monocyte chemoattractant protein 1, and OH^- hydroxyl radical.

is a trimeric transmembrane protein in the tumor necrosis factor family. CD40 ligand is stored in the cytoplasm of resting platelets and rapidly appears on the surface after platelet activation.[43] On the platelet membrane, the CD40 ligand undergoes cleavage over a period of minutes or hours, generating a functional soluble fragment. Platelet-derived CD40 ligand can induce endothelial cells to produce reactive oxygen species,[44] adhesion molecules, chemokines,[42] and tissue factor,[45] all of which are components of an inflammatory response (Fig. 2). Blockade of the CD40–CD40 ligand signaling pathway markedly inhibits the formation of atherosclerotic plaque and arterial lipid deposition in LDL-receptor$^{-/-}$ mice.[46] A prospective study of healthy women found that high plasma levels of soluble CD40 ligand are associated with an increased risk of vascular events.[47]

Moreover, several cardiovascular risk factors, including cigarette smoking[48] and type 2 diabetes mellitus,[49] are associated with platelet activation and increased release of the CD40 ligand. The combination of hyperinsulinemia and hyperglycemia up-regulates the release of platelet CD40 ligand and monocyte-derived tissue factor.[50]

In contrast to the CD40 ligand, which is stored in the platelet cytoplasm, interleukin-1β is synthesized on platelet activation.[7,51] The amount of interleukin-1β that activated platelets synthesize, by way of the signal-dependent translation of interleukin-1β mRNA, is sufficient to induce endothelial cells to express genes that mediate the adhesion of leukocytes.[7] Interleukin-1β is an important mediator of the platelet-induced activation of endothelial cells, causing them to increase the release of chemokines and up-regulate molecules that promote adhesion of neutrophils and monocytes to the endothelium (Fig. 2).[41]

The stimulation of platelets by strong agonists can cause the shedding of small membrane vesicles from the platelet surface.[52] At sites of vascular injury, the expression of P-selectin by activated endothelial cells or platelets can trigger the recruitment of microparticles bearing the P-selectin glycoprotein ligand 1 and tissue factor.[52] Microparticles have also been implicated in the up-regulation of COX-2–dependent prostanoid formation in monocytes and endothelial cells, involving the transcellular metabolism of arachidonic acid.[53,54]

Activated platelets or platelet microparticles also release chemokines that can trigger the recruitment of monocytes[55] or promote their differentiation into macrophages.[56,57] Platelet factor 4, a platelet-specific chemokine released upon platelet activation, induces the expression of E-selectin by endothelial cells.[58] Activated platelets also release the matrix-degrading enzymes matrix metalloproteinases 2 and 9 (Fig. 2).[41] Platelets are a rich source of stimulators and inhibitors of angiogenesis and play a central role in that process.[59]

REACTIVE OXYGEN SPECIES AND PLATELET ACTIVATION

The enhanced release of reactive oxygen species (e.g., O_2^-) from the vessel wall (where endothelial and smooth-muscle cells express a variety of enzymes that generate these species) indirectly affects the activation of platelets because the species scavenge nitric oxide.[60] Activated platelets can also generate reactive oxygen species (Fig. 3). The metabolism of arachidonic acid by means of the COX-1 pathway contributes to the production

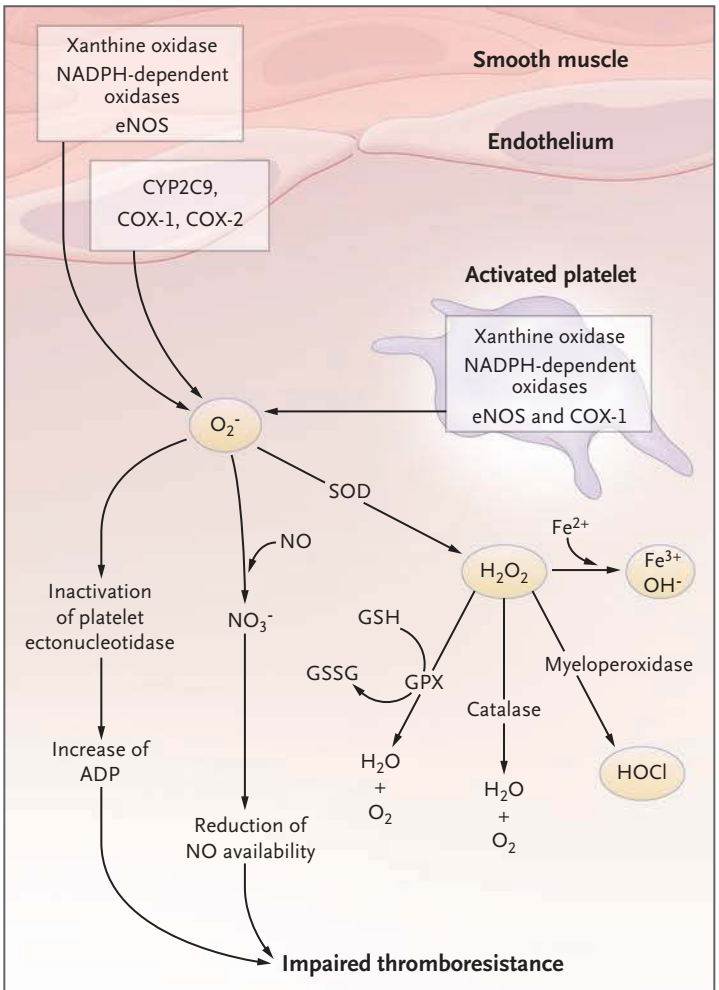


Figure 3. Role of Reactive Oxygen Species in Platelet Activation.

The production of reactive oxygen species is promoted in vascular endothelial cells and smooth-muscle cells in response to injury through several enzymatic pathways and by the expression of enzymes by activated platelets. The production of O_2^- by platelets that is dependent on NADPH oxidases enhances the recruitment of platelets to a growing thrombus, most likely by inactivating a platelet ectonucleotidase, thereby increasing the bioavailability of adenosine diphosphate (ADP). Furthermore, O_2^- can scavenge nitric oxide (NO) to form peroxynitrate (NO_3^-), thereby impairing the antiplatelet activity of NO. O_2^- can be converted to H_2O_2 by superoxide dismutase (SOD); SOD and NO are competitors in O_2^- scavenging. H_2O_2 also serves as a substrate for the production of other detrimental reactive oxygen species, such as hypochlorous acid (HOCl), generated by enzymatic conversion by neutrophil myeloperoxidase. In addition, H_2O_2 reacts with ferrous iron (Fe^{2+}) to generate ferric iron (Fe^{3+}) and the hydroxyl radical (OH^-). In the presence of catalase, H_2O_2 is degraded to water and oxygen, and glutathione peroxidase (GPX) also exerts an antioxidant enzymatic function, because it catalyzes a reaction that degrades H_2O_2 by oxidizing reduced glutathione (GSH) to its disulfide form (GSSG). COX denotes cyclooxygenase and eNOS endothelial nitric oxide synthase.

of reactive oxygen species by activated platelets. Agonists that induce platelet activation also activate the platelet isoform of NADPH oxidase.[61] The production of O_2^- by platelets through the pathway dependent on these oxidases enhances the recruitment of platelets to a growing thrombus.[62]

O_2^- is a functionally relevant scavenger of nitric oxide and regulates the redox-sensitive ectonucleotidases on platelet and endothelial-cell membranes.[60,63] The scavenging of nitric oxide by reactive oxygen species prevents its participation in the late disaggregation of a thrombus. Moreover, the accelerated removal of nitric oxide can occur through its reaction with COX-1–derived products.[64]

The increased generation of reactive oxygen species can induce enhanced lipid peroxidation of cell-membrane phospholipids or circulating LDL, leading to the increased generation of F_2-isoprostanes, a family of prostaglandin isomers produced from arachidonic acid by a mechanism catalyzed by free radicals (Fig. 4).[65,66] F_2-isoprostanes can modulate the adhesive reactions and activation of platelets induced by low levels of other agonists.[67]

The consistent relationship between the rates of formation of F_2-isoprostanes and thromboxane in obese women[68] and in patients with hypercholesterolemia,[69] type 2 diabetes mellitus,[70] or homozygous homocystinuria[71] suggests that a low-grade inflammatory state associated with these metabolic disorders may be the primary trigger of thromboxane-dependent platelet activation that is mediated, at least in part, by enhanced lipid peroxidation (Fig. 4).

PLATELETS IN ATHEROGENESIS IN HUMANS

The evidence for a role of platelets in atherogenesis in humans is limited and largely indirect. Several platelet-derived chemokines and growth factors are detectable in atherosclerotic plaques.[9,72] Moreover, platelet activation is associated with increased wall thickness of the carotid artery[73] and with progressive thickening of the artery in patients with type 2 diabetes mellitus.[74] Persistent platelet activation, as reflected by enhanced excretion of thromboxane metabolites, has been reported in association with major cardiovascular risk factors that accelerate atherogenesis.[75-79] These studies suggest that platelet activation links diverse metabolic and hemodynamic abnormalities to accelerated atherogenesis.[80] Given the advanced age of the participating subjects and the nature of the vascular end points, trials of aspirin were not designed to address this hypothesis.[81] The same considerations apply to clinical trials of selective COX-2 inhibitors.[82]

PLATELETS AND ARTERIAL THROMBOSIS

Atherosclerosis without flow-limiting thrombosis is a slowly progressive disease. The usual mechanism responsible for the sudden transition from a stable, often clinically silent, disease to a symptomatic life-threatening condition is the denudation and erosion of the endothelial surface or plaque disruption followed by thrombosis.[83] Platelet activation occurs at vulnerable sites, where the thin fibrous cap separating the lipid-rich core of the plaque from the lumen disintegrates, tears, or breaks.[83] The majority of such acute vascular lesions resolve spontaneously through a repair phenomenon akin to hemostasis. Hemorrhage into the fissured plaque and platelet-mediated sealing of the disrupted surface contribute to the unpredictable, nonlinear progression of coronary lesions.[83] However, in a substantial proportion of symptomatic episodes of acute coronary-plaque disruption, platelet activation progresses to persistent intraluminal thrombosis in the absence of antithrombotic treatment.[24]

PLATELET ACTIVATION IN ACUTE CORONARY SYNDROMES

Animal models of arterial thrombosis[84,85] have shown that the mechanical, photochemical, or thermal injury of healthy vessels exposes the native extracellular matrix, thereby triggering thrombus formation through mechanisms dependent on collagen and thrombin.[85] There are, however, differences between murine and human hemostatic systems, including a considerably higher number of circulating platelets in mice.[86] Spontaneous plaque rupture and secondary thrombosis have been described in apoE$^{-/-}$ mice and LDL-receptor$^{-/-}$ mice,[87] but these events usually occur in older mice after the prolonged feeding of a high-fat diet, and intraluminal occlusive thrombi occur only rarely. Several methods of triggering

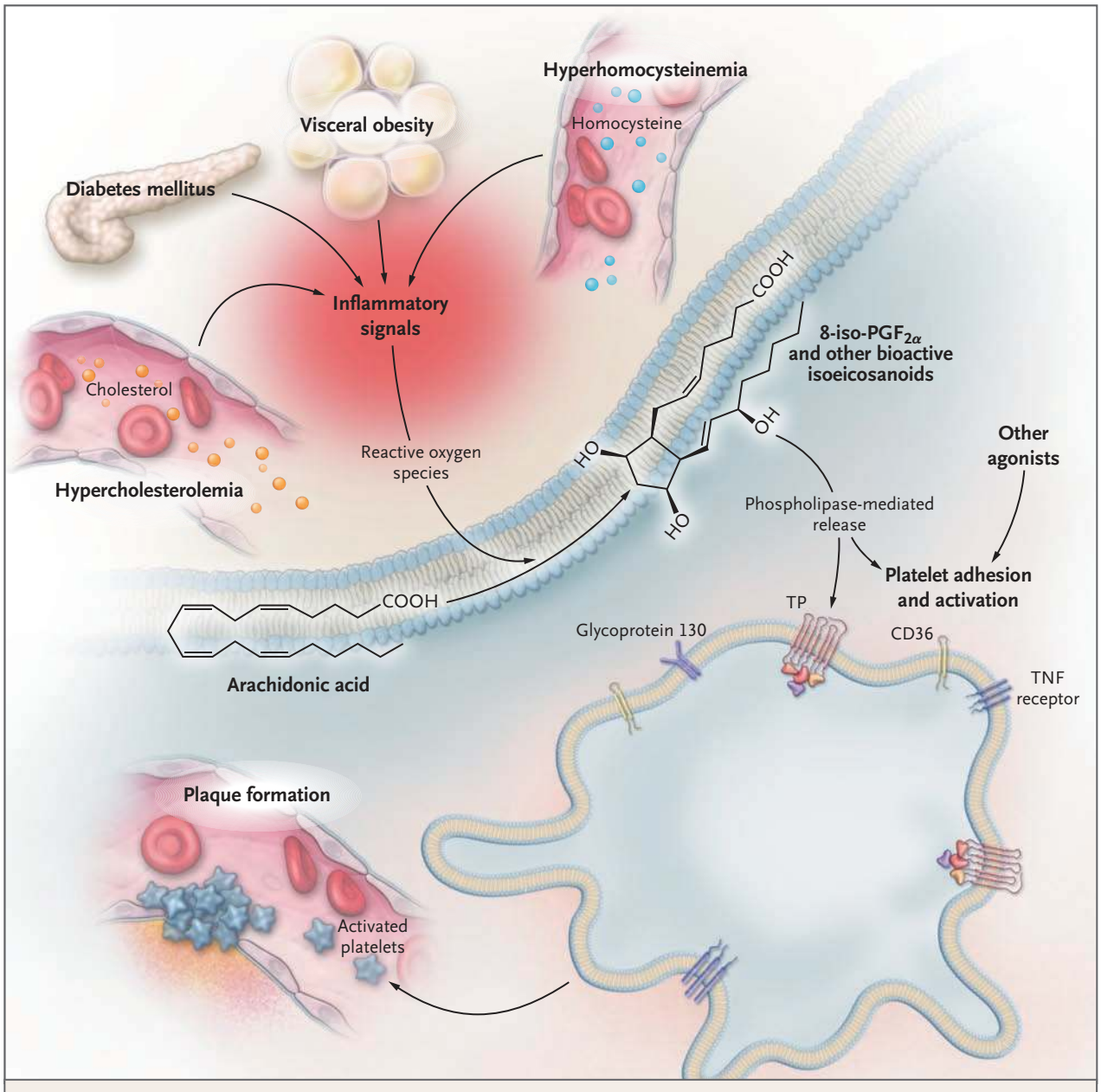


Figure 4. Isoprostane Formation as a Biochemical Link between Low-Grade Inflammation and Platelet Activation in Human Metabolic Disorders.

A number of human metabolic disorders — hypercholesterolemia, diabetes mellitus, visceral obesity, and hyperhomocysteinemia — are associated with inflammatory signals generated from the metabolism of lipid, carbohydrate, and protein. Enhanced formation of reactive oxygen species (through the mechanisms shown in Figure 3) leads to enhanced lipid peroxidation and free radical–catalyzed conversion of arachidonic acid into bioactive isoprostanes (e.g., 8-iso-prostaglandin $F_{2\alpha}$ [8-iso-$PGF_{2\alpha}$]) and other isoeicosanoids. Phospholipase-mediated release of these compounds from cell membranes and low-density lipoprotein particles triggers platelet adhesion and activation in the presence of low levels of other agonists. Several receptors for cytokines and oxidized lipids involved in the inflammatory response are also expressed on the platelet membrane. CD36, also known as glycoprotein IIIb, has been shown to interact with a variety of ligands. This receptor can take up oxidized LDL and thereby activate the platelet, causing the release of cytokines that amplify the inflammatory response and platelet activation. TP denotes thromboxane receptor, and TNF tumor necrosis factor.

plaque rupture in apoE$^{-/-}$ mice have been described,[88] but none seem to result in a phenotype similar to that of patients.

The most convincing evidence for the participation of platelets in arterial thrombosis in humans comes from studies of platelet activation in patients with acute ischemic syndromes and from trials of antiplatelet drugs. Repeated, transient increases in the excretion of thromboxane metabolites have been described in patients with acute coronary syndromes.[89,90] The episodic nature of platelet activation is consistent with the concept of coronary thrombosis as a dynamic process, in which repeated episodes of thrombus formation and fragmentation occur over a disrupted plaque.[83] Treatment with either aspirin or streptokinase, initiated within 24 hours after the onset of a suspected acute myocardial infarction, reduced the 5-week mortality by approximately 25%.[91] This finding strongly supports the concept that repeated episodes of platelet activation over the persistently thrombogenic surface of a disrupted plaque are important contributions to the risk of death from coronary causes.[92] The consistent finding of a 50% reduction in the risk of myocardial infarction or death from vascular causes among patients with unstable angina who take aspirin[24] highlights the importance of thromboxane A_2 as a platelet-mediated mechanism of the growth and stabilization of an intraluminal coronary thrombus.

Platelet Activation in Acute Ischemic Stroke

Platelet activation is also important in patients with ischemic stroke,[93] as suggested by biochemical measurements[94,95] and trials of platelet inhibitors.[93] Episodic increases in thromboxane biosynthesis have been described in the acute phase of ischemic stroke,[94,95] though with a lower frequency and shorter duration than in acute coronary syndromes.[89,90] This difference may reflect the heterogeneity of the mechanisms responsible for ischemic stroke — a thrombosis in a large artery accounts for only a fraction of the ischemic events.[93] The effect of aspirin therapy, started within 48 hours after the onset of symptoms of an acute ischemic stroke, on short-term rates of death and nonfatal outcomes[93] is correspondingly smaller than the benefit seen in the acute phase of myocardial infarction.[91]

Thromboxane A_2 and Prostacyclin

Prostacyclin is a vasodilator prostanoid that inhibits platelet activation in response to a variety of agonists.[22] In a murine model of catheter-induced carotid injury, vascular proliferation and platelet activation were enhanced in prostacyclin receptor$^{-/-}$ mice but were inhibited in thromboxane A_2 receptor$^{-/-}$ mice.[96] The enhanced response to vascular injury was abolished in mice deficient in both receptors.[96] These findings suggest that prostacyclin modulates platelet–vascular interactions and, specifically, limits the response to thromboxane A_2. Low-dose aspirin reduces platelet production of thromboxane A_2 by 97 to 99% in humans,[24] and this reduction has been mimicked in a mouse model with hypomorphic *COX-1* alleles (*COX-1* "knockdown").[97] The 97% decline in production of platelet thromboxane A_2 in these mice decreased platelet aggregation and prevented arterial thrombosis induced by photochemical injury.

In mice, the selective inhibition, knockout, or mutation of the *COX-2* gene or deletion of the prostacyclin receptor accelerates the thrombotic occlusion of a carotid artery induced by photochemical injury.[98] The effects of deletion of the prostacyclin receptor were dependent on the number of affected alleles; a thrombotic phenotype was associated with the deletion of just one copy of the gene.[98] Neither the disruption of *COX-2* nor the deletion of the prostacyclin receptor resulted in spontaneous thrombosis, but both augmented the response to thrombogenic stimuli.[98] These effects were attenuated, but not abolished, by the knockdown of *COX-1*.[98]

Aspirin is antithrombotic at a wide range of daily doses, from 30 mg to 1500 mg.[81] However, an apparent inverse relationship between the aspirin dose and the clinical benefit has been reported in indirect comparisons (i.e., comparisons of a range of doses with placebo) and in a limited number of randomized evaluations.[24] This finding is consistent with the dose-dependent inhibition of prostacyclin biosynthesis by aspirin in healthy volunteers.[99] The functional importance of prostacyclin in restraining the response to thromboxane A_2 is further supported by the approximately twofold increased risk of myocardial infarction associated with the use of selective COX-2 inhibitors in patients at low risk for atherothrombosis.[82]

ADP

The interaction of ADP with its platelet $P2Y_{12}$ receptor is essential for platelet activation.[100] ADP, which is stored in and secreted from dense granules in platelets, amplifies the response to other agonists (Fig. 1), thereby contributing to the growth and stability of a thrombus in an injured artery.[101] $P2Y_{12}^{-/-}$ mice have a prolonged bleeding time and are protected against chemically induced arterial thrombosis.[101] The importance of the ADP–$P2Y_{12}$ interaction is borne out by the effects of $P2Y_{12}$ blockers, such as ticlopidine and clopidogrel, in reducing the risk of major vascular events to an extent similar to that achievable with low-dose aspirin.[24] The clinical benefit associated with $P2Y_{12}$ blockade by clopidogrel in patients receiving aspirin is relatively modest[102,103] and inconsistent.[104] In contrast to aspirin, which inhibits the production of thromboxane by platelets virtually completely at low doses, ticlopidine and clopidogrel incompletely and variably inhibit ADP-induced platelet aggregation.[24] The role of ADP in amplifying the platelet response to plaque fissuring may have been underestimated on the basis of ticlopidine and clopidogrel trials. More effective $P2Y_{12}$ blockers are currently being developed, and they may help to define the role of ADP in arterial thrombosis.

THROMBIN

Thrombin is rapidly generated at sites of vascular injury and, in addition to cleaving fibrinogen, it is a very effective platelet activator (Fig. 1).[105] Moreover, thrombin induces procoagulant activity on the platelet surface, which supports additional thrombin generation.[106] Platelet responses to thrombin are largely mediated through G-protein–coupled protease-activated receptors (PARs) that convert an extracellular proteolytic cleavage event into a transmembrane signal. Platelets in humans express PAR1 and PAR4.[105] PAR1 carries its own ligand, which becomes active upon cleavage of the receptor by thrombin.[105] PAR1 is the main thrombin receptor on human platelets; PAR4 probably provides some redundancy in an important system.[105]

Studies of $PAR4^{-/-}$ mice clearly indicate that the cleavage of fibrinogen to fibrin is more important for hemostasis than is platelet activation by thrombin.[105] This finding is consistent with the clinical observation that inhibition of the catalytic activity of thrombin is associated with a substantial risk of bleeding.[107] But the finding in mice that the suppression of PAR3- or PAR4-mediated thrombin signaling in platelets causes only a mild hemostatic defect raises the possibility that the blockade of PAR1 in human platelets poses a lower risk of bleeding than does the inhibition of thrombin generation or activity.[105] PAR1 inhibition appears to be sufficient to achieve an antithrombotic effect in nonhuman primates, and PAR1 antagonists are currently being developed for the prevention and treatment of atherothrombosis.[108]

FUTURE DIRECTIONS

Platelets have emerged as key cellular determinants of physiologic vascular repair and its pathologic derangement.[1-4] They act not only through the immediate release of a variety of lipid and protein mediators but also through previously unrecognized time-dependent events such as signal-dependent pre-mRNA splicing[7,17] and the translation of constitutively expressed mRNA.[51] These post-transcriptional pathways are potential targets for molecular intervention in atherothrombosis.[109] The availability of new techniques for examining the platelet transcriptome, proteome, and lipidome in an integrated fashion[8] makes it feasible to analyze the determinants of the variability in platelet function among persons and the response to antiplatelet agents. Because the repertoire of platelet mRNA and proteins depends on the expression profile of their bone-marrow precursors and may change with platelet age,[110] it will be important to examine different subpopulations of the circulating platelet pool, which may vary among clinical settings. The regulation of platelet responses mediated by signaling to megakaryocytes and the alteration of the functions of these progenitor cells in vascular disease also appear to be biologically plausible and worth investigating.

New approaches are needed to bridge the gap between the large body of evidence supporting a role of platelets in the initiation and progression of experimental atherogenesis and the relatively modest evidence for a role of platelets in the disease in humans. Studies of the use of antiplatelet prophylaxis in relatively young persons at risk for accelerated atherogenesis, involving vascular

imaging end points, may provide proof-of-concept results to justify large-scale clinical trials with hard clinical end points. Given the growing concern over the cardiovascular consequences of obesity, people who are obese could constitute a suitable population for feasibility studies, in light of the evidence of persistent platelet activation in obese women who are otherwise healthy and relatively young.[68] Furthermore, the study of obesity could provide further insight into the mechanisms linking inflammatory mediators to platelet activation.

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