Annu. Rev. Pharmacol. Toxicol. 2006. 46:277–300
doi: 10.1146/annurev.pharmtox.46.120604.141207

First published online as a Review in Advance on September 21, 2005

REGULATION OF PLATELET FUNCTIONS BY P2 RECEPTORS

Christian Gachet
Institut National de la Santé et de la Recherche Médicale, Unité 311, Etablissement Français du Sang-Alsace, Strasbourg 67065, France;
email: christian.gachet@efs-alsace.fr

Key Words hemostasis, thrombosis, ADP, $P2Y_1$, $P2Y_{12}$, $P2X_1$

■ **Abstract** The main role of blood platelets is to ensure primary hemostasis, which is the maintenance of vessel integrity and cessation of bleeding upon injury. While playing a major part in acute arterial thrombosis, platelets are also involved in inflammation, atherosclerosis, and angiogenesis. ADP and ATP play a crucial role in platelet activation, and their receptors are potential targets for antithrombotic drugs. The ATP-gated cation channel $P2X_1$ and the two G protein–coupled ADP receptors, $P2Y_1$ and $P2Y_{12}$, selectively contribute to platelet aggregation and formation of a thrombus. Owing to its central role in the growth and stabilization of a thrombus, the $P2Y_{12}$ receptor is an established target of antithrombotic drugs such as clopidogrel. Studies in $P2Y_1$ and $P2X_1$ knockout mice and selective $P2Y_1$ and $P2X_1$ antagonists have shown that these receptors are also attractive targets for new antithrombotic compounds. The potential role of platelet P_2 receptors in the involvement of platelets in inflammatory processes is also discussed.

INTRODUCTION

Blood platelets are anucleated cell fragments released from the bone marrow into the blood stream by fragmentation of megakaryocytes (1). Under normal conditions, platelets circulate freely in the blood at a concentration of 150 to 300 $\times$ 10^8/L and do not adhere to each other or to the vessel wall. Their main role is to ensure primary hemostasis, which means the maintenance of blood vessel integrity and the rapid cessation of bleeding in the event of vascular injury. The same mechanisms are involved when platelets are activated at the site of an atherosclerotic plaque rupture, leading to vessel occlusion and, depending on the vascular bed involved, ischemic complications such as myocardial infarction, stroke, or peripheral artery disease (2). Platelets are themselves involved in the progression of the atherosclerotic lesions as well as in angiogenesis through release of inflammatory mediators and growth factors such as RANTES, sCD40L, PF4, TGFβ, PDGF, and VEGF. Platelets have also been shown to play a role in cancer metastasis (3–8). Thus, this thin cell particle has a pivotal role in physiology and is involved in

the pathogenesis of many diseases, of which atherosclerosis and accompanying thrombosis is the leading cause of mortality and morbidity in Western countries.

Extracellular nucleotides and their receptors play important roles in the cardiovascular system, including platelet activation; vasorelaxation or vasoconstriction, depending on the presence or the absence of the endothelium, respectively; and the control of vascular tone by perivascular nerves (9). Forty-five years ago, ADP was identified as a factor derived from erythrocytes that influenced platelet adhesiveness to glass (10) and induced platelet aggregation (11). Its presence in large amounts in platelets (12) and its crucial role in physiological hemostasis and in the development and extension of arterial thrombosis was rapidly recognized (13, 14), but molecular identification of its receptors was elusive. These membrane receptors belong to the P2 receptor family, which consists of two classes: P2X ligand-gated cation channels and G protein–coupled P2Y receptors (15). To date, seven subtypes of mammalian P2X receptor ($P2X_{1-7}$) (16) and eight subtypes of P2Y receptor ($P2Y_{1,2,4,6,11-14}$) (17) have been cloned and characterized. It took approximately five years from the first reported cloning of a P2 receptor (18) to the complete identification of the platelet P2 receptors repertoire (19). From the concept of a unique P2T receptor (T for thrombocyte), originally postulated on the basis of pharmacological data (20), a model of three P2 receptors emerged (19, 21, 22): the $P2X_1$ cation channel, which is activated by ATP, and two G protein–coupled receptors, $P2Y_1$ and $P2Y_{12}$, both activated by ADP. We now know the role of each of these receptors during platelet activation and aggregation, which, of course, has implications for their role in thrombosis. Recent extensive reviews on the role of the P2 receptors in platelet function and in thrombosis have been published (23, 24). In the present review I highlight the most recent findings concerning these receptors and present the current views about pending questions. In addition, their involvement in atherosclerosis, angiogenesis, inflammation, immunity, and cancer metastasis is discussed.

General Mechanisms of Platelet Activation and Arterial Thrombus Formation

In the flowing blood, the endothelial cell monolayer provides an antithrombotic surface by separating blood from the subendothelial matrix proteins and by the synthesis and release of prostacyclin and nitric oxide, which both inhibit platelet activation via their ability to increase cAMP and cGMP, respectively. Endothelial cells also express CD39 at the luminal surface. CD39 is an enzyme (also called NTPDase1) that sequentially converts ATP into ADP and AMP, thus eliminating ATP and ADP from the vicinity of the vessel wall and preventing the interaction of the platelets with it (25, 26). In contrast, upon vessel wall injury, platelets are pushed to the vessel wall by the flowing red blood cells and encounter the exposed subendothelial matrix, which contains thrombogenic proteins that cause platelet adhesion, activation, and aggregation. The first step occurs through interaction of the glycoprotein GPIbα, a leucine-rich subunit of the GPIb-V-IX complex, with von Willebrand factor (vWF), a large multimeric glycoprotein bound onto subendothelial

collagen fibers (2). This first interaction is reversible and allows platelets flowing in the vasculature to slow down and roll on the injured vessel wall. A second step is the firm adhesion of platelets through their binding of collagen to its multiple receptors, including the $\alpha 2\beta 1$ integrin and the GPVI immunoglobulin-like receptor along with binding of vWF to the αIIb$\beta 3$ integrin (2). Concomitantly, platelet activation is triggered by multiple agonists, of whom the strongest are collagen, which acts via GPVI and thrombin, generated after exposure of tissue factor from the injured vessel wall, which activates platelets through protease-activated receptors (PARs) (27). Platelet activation also results from important rapid, positive feedback loops. One is the synthesis of thromboxane A_2 (TXA_2) through cyclooxygenase (COX) and TXA_2 synthase from phospholipase A_2–released arachidonic acid. TXA_2 is a potent platelet-aggregating and -vasoconstricting agent that activates the thromboxane prostanoid receptor (TP receptor) (28). Also released are large amounts of ADP and ATP, which are stored in the platelet-dense granules along with serotonin and calcium and which act on the P2 receptors. Despite being considered as secondary agonists of platelets, both TXA_2 and ADP greatly amplify the activation signals and enable robust platelet recruitment at the site of injury, thereby leading to the formation of the so-called hemostatic plug. All these pathways converge on the activation of the αIIb$\beta 3$ integrin to promote its conversion from an inactive to an active conformation at the platelet surface, which leads to the binding of soluble fibrinogen and vWF and subsequent platelet aggregation. Ultimately, thrombin, not only activates platelets but also triggers the formation of a fibrin clot that stabilizes the thrombus (Figure 1). The formation of a platelet thrombus is a dynamic process involving continuous remodeling and modification of its stability and size. The factors regulating this process include the rheological conditions, the strength of the stimuli, the equilibrium between inhibitory and activator systems, and the age of the thrombus, to cite a few. All the steps and molecular partners involved in platelet activation and thrombus formation are potential targets for antithrombotic drugs. Currently, the most widely used antiplatelet agents are the COX inhibitor aspirin, which inhibits the formation of TXA_2; blockers of fibrinogen binding to the platelet integrin αIIb$\beta 3$; and clopidogrel, a selective inhibitor of the platelet ADP receptor $P2Y_{12}$ (29, 30).

Current Model of Three P2 Receptors in Platelet Function

As noted above, two G protein–coupled P2Y receptors and one P2X ligand-gated cation channel are responsible for all the known effects of ADP and ATP, respectively, on platelets (Figure 2). Their identification, structure, and pharmacology have been extensively presented elsewhere (24, 31). The $P2Y_1$ receptor is widely distributed in many tissues, including heart, blood vessels, smooth muscle cells, neural tissue, testis, prostate, and ovary (15). ADP is its preferred natural agonist; ATP behaves as an antagonist in platelets (32) or as a poor partial agonist in heterologous transfected or reconstituted systems, depending on receptor density (33). Approximately 150 $P2Y_1$ receptor binding sites are expressed per platelet (34), which is very low as compared, for instance, with the TP receptors or with

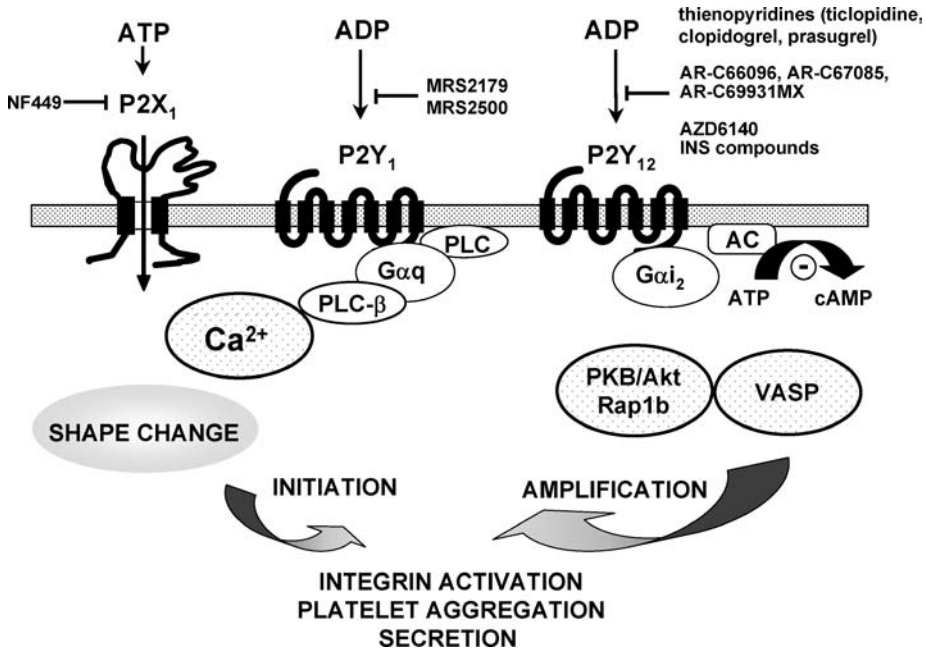

Figure 2 Current model of three platelet P2 receptors. The $P2X_1$ receptor is responsible for rapid calcium influx and platelet shape change in response to ATP and contributes to the platelet activation induced by low concentrations of collagen. The $P2Y_1$ and $P2Y_{12}$ receptors are essential for normal aggregation in response to ADP: the Gq-coupled $P2Y_1$ receptor is responsible for intracellular calcium mobilization, shape change and initiation of aggregation and the G_i-coupled $P2Y_{12}$ receptor for completion of the ADP-induced aggregation response and for potentiation of the aggregation and secretion induced by other agents through various intracellular pathways. Selective antagonists allow discrimination of the roles of the three receptors. $P2Y_{12}$ is the target of the antithrombotic drugs ticlopidine and clopidogrel while $P2Y_1$ and $P2X_1$ are potential targets for new antiplatelet compounds.

the thrombin receptor PAR-1 (1000 to 2000 receptors/platelet). Selective $P2Y_1$ receptor antagonists exist, among which MRS2179 (35) constitutes a valuable tool to investigate the role of the $P2Y_1$ receptor in platelet function in vitro. The newly described MRS2500 derivative (36) is the most potent and selective $P2Y_1$ antagonist for in vivo studies (B. Hechler, unpublished data). As it is coupled to Gαq, the $P2Y_1$ receptor triggers the mobilization of calcium from internal stores, which results in platelet shape change and weak, transient aggregation in response to ADP (37–40). It also participates in the aggregation response to collagen and plays a key role in collagen-induced shape change when TXA_2 formation is prevented (41, 42). Overall, the $P2Y_1$ receptor mediates weak responses to ADP but has, nevertheless, a crucial role in the initiation of platelet activation induced by ADP or collagen.

The $P2Y_{12}$ receptor, despite its being well known and characterized on the basis of both pharmacological and genetic evidence, was the last to be cloned (43). This receptor is deficient in patients with selective defects of ADP-induced platelet aggregation and as noted above, is the molecular target of the antiplatelet drug clopidogrel (44, 45). Its tissue distribution is very limited, although not entirely restricted to platelets as it is also present in the brain, endothelial cells, glial cells, and smooth muscle cells (23). ADP is the natural agonist of this receptor, whereas ATP and its triphosphate analogues are antagonists (46, 47). This receptor is coupled to G_{i2} and is responsible for completion of the platelet aggregation response to ADP. It plays a central role in amplification of the aggregation induced by all known platelet agonists, whatever their signaling pathway; these agonists include collagen, thrombin, immune complexes, TXA_2, adrenaline (epinephrine), and serotonin (24, 48). The $P2Y_{12}$ receptor is also involved in the potentiation of platelet secretion (49). All these features make the $P2Y_{12}$ receptor a pivotal player in sustaining platelet aggregation, and in turn, into thrombus growth and stabilization. The intracellular pathways through which $P2Y_{12}$ amplifies platelet responses include inhibition of cAMP production, vasodilator-stimulated phosphoprotein (VASP) dephosphorylation (50), phosphoinositide 3-kinase (PI 3-K) activity (51, 52), and activation of the small GTPase Rap1B (53, 54).

Coactivation of the $P2Y_1$ and $P2Y_{12}$ receptors is necessary for normal ADP-induced platelet aggregation because separate inhibition of either of them by selective antagonists results in a dramatic decrease in aggregation (38, 40, 55). However, the two receptors have differential roles because, except for collagen-induced activation, $P2Y_1$ has a minor contribution when platelet aggregation is induced by other agonists, whereas the $P2Y_{12}$ receptor supports amplification of response by those agonists. The $P2Y_1$ and $P2Y_{12}$ receptors are also differentially involved in the procoagulant activity of platelets, which is their ability to contribute to thrombin generation. Both receptors are indirectly involved in platelet P-selectin exposure and formation of platelet-leukocyte conjugates, which leads to leukocyte-tissue factor exposure (56, 57), whereas the $P2Y_{12}$ receptor also directly contributes to exposure of phosphatidylserine at the surface of platelets where the coagulation factors bind to concentrate and to localize thrombin generation (56, 58, 59).

The third component of the platelet P2 receptors is $P2X_1$, the ligand-gated cation channel responsible for the fast calcium entry induced by ATP (31). $P2X_1$ receptors are very quickly desensitized, thereby hampering the study of their function in platelet activation in vitro. However, when desensitization is prevented by addition of high concentration of apyrase (ATP-diphosphohydrolase E.C.3.6.1.5.) to hydrolyze ATP, the selective $P2X_1$ receptor agonist, alpha, beta-methylene-ATP ($\alpha\beta$MeATP), induces a rapid calcium influx associated with a transient shape change in human platelets (60). Although unable to trigger platelet aggregation by itself, the $P2X_1$ receptor has been shown to participate to collagen- and shear-induced aggregation (61–63). Because of the issue of desensitization and owing to its weak effect on platelet aggregation in stirred platelet suspensions, its role was unknown, and even denied, for a long time (64). Moreover, considerable time and

effort was required to show that this receptor is not activated by ADP but instead by ATP (65, 66). Synergistic interplay between $P2X_1$ and $P2Y_1$ has been reported, suggesting the $P2X_1$ receptor plays a priming role in the subsequent activation of $P2Y_1$ (67, 68). Interestingly, the $P2X_1$ component of the calcium signal resulting from simultaneous activation of both receptors is increased when the intracellular cAMP concentration is high. This could be relevant to the in vivo situation where platelets are in contact with the endothelium of the healthy vessel wall, which generates prostacyclin and nitric oxide (68). Whether $P2X_1$ also synergizes $P2Y_{12}$ responses remains to be investigated.

Importance of Shear Forces and Flow Conditions

Most of our knowledge of the roles of the P2 receptors in platelet activation and aggregation comes from studies using classical light transmission aggregometry. However, hemostasis and arterial thrombosis occur on surfaces and under flow conditions. Several systems have been used to monitor platelet activation and thrombus formation under flow with controlled shear rates. These include the cone and plate viscometer, which allows the measurement of shear-induced platelet aggregation, and flow chambers or glass capillaries coated with various substrate proteins to which platelets can attach and form a thrombus (69). Such systems were pivotal in defining the role of the $P2X_1$ receptor in thrombus formation. Indeed, shear-induced platelet aggregation has been found to depend on the $P2X_1$ receptor (62, 63). Furthermore, on collagen-coated surfaces, at shear rates above 3000 s^{-1} (which are those found in small arteries), thrombus formation is severely inhibited in $P2X_1$-deficient mice (61). The suramin analog NF449 (70), which is a $P2X_1$-selective antagonist when used at appropriate concentrations, similarly inhibits thrombus formation under high shear conditions (71). In vivo studies in $P2X_1$-deficient mice or wild-type mice treated with NF449 have further established the role of this receptor in the thrombosis of small arteries (61, 71). These results clearly indicate the relevance of flow systems as experimental systems that complement use of classical aggregometry. They also establish $P2X_1$ as a putative target for new antiplatelet compounds (see below).

Concerning the P2Y receptors, it has been known for a long time that shear-induced platelet aggregation initiated by the interaction of vWF with GPIbα is highly dependent on released ADP (72). This was further demonstrated by Cattaneo et al., in a patient with a congenital deficiency of ADP-induced platelet aggregation and in healthy individuals treated with ticlopidine (73, 74). It was subsequently confirmed that $P2Y_{12}$ is the ADP receptor involved in mediating vWF-dependent stable platelet aggregation occurring under high shear rate (75, 76), an effect also found to be dependent on PI 3-K activation (77). Turner et al. (75), demonstrated a role of $P2Y_1$ in this system, and showed that combined inhibition of the $P2Y_1$ and $P2Y_{12}$ receptors resulted in a stronger reduction of shear-induced platelet aggregation than blockade of either receptor alone.

In flow studies on collagen-coated capillaries, the $P2Y_{12}$ receptor was reported to mediate the stabilization of platelet aggregates. Early work using blood from

individuals treated with clopidogrel showed the formation of loosely packed thrombi on collagen under various flow conditions (78), consistent with the known role of $P2Y_{12}$ in sustaining activation of the $\alpha IIb\beta 3$ integrin. More recently, Goto et al. (79) reported that $P2Y_{12}$ inhibition was sufficient to inhibit platelet thrombus formation on collagen during perfusion of anticoagulated whole blood at a shear rate of 1500 s^{-1}. On the other hand, Remijn et al. (80) showed that the $P2Y_1$ and $P2Y_{12}$ receptors both participate in collagen-induced platelet thrombus formation in a similar system. However, Turner et al. (75) found that inhibition of both receptors was required for efficient inhibition of thrombus formation on collagen-coated capillaries at a shear rate of 3000 s^{-1}. These discrepancies concerning the respective contributions of $P2Y_1$ and $P2Y_{12}$ to thrombus formation on collagen under flow conditions might be attributable to the duration of perfusion or to the type of anticoagulant, which has been reported to influence the action of $P2Y_{12}$ (48). An important factor is the shear rate applied to the system. All the studies reported to date were performed under shearing conditions of 1500 to 3000 s^{-1}. However, increasing the shear rate to 6000 s^{-1} revealed a greater contribution of the $P2Y_1$ as compared to the $P2Y_{12}$ receptor, suggesting differential roles of these receptors as a function of shear rate (B. Hechler, unpublished data).

A selective role of the $P2Y_1$ receptor has also been highlighted in the early steps of the stable adhesion of platelets to a vWF surface. Thus, an $\alpha IIb\beta 3$ integrin-dependent calcium entry signal, concomitant with firm attachment of platelets to the substrate, is selectively inhibited by $P2Y_1$ receptor antagonists (81). In line with such findings, Goncalves et al. (82) reported that $P2Y_1$ was involved in calcium influx in an $\alpha IIb\beta 3$-dependent manner, under conditions of temporal flow gradients that would mimic the turbulence and local modifications in the flow regimen occurring at sites of stenosis.

Altogether, these data are consistent with the view of a key role of the $P2Y_1$ receptor in the early steps of thrombus formation after the initial tethering of platelets to the subendothelial matrix by GPIb and vWF. On the other hand, the $P2Y_{12}$ receptor would be involved in a later stage of thrombus growth and in ensuring the stability of the platelet plug before fibrin formation/transformation.

Desensitization

An important phenomenon in controlling thrombus growth is the regulation of platelet reactivity after stimulation and receptor desensitization is a general mechanism used by cells to adapt their responsiveness. Once initially activated by ADP, platelets become unresponsive to a second stimulation with the same agonist. This so-called refractory state of platelets to ADP is transient and full ADP-dependent responses recover within 15 to 30 min. This refractoriness is caused by selective desensitization of the $P2Y_1$ receptor with a resultant loss of shape change and aggregation (83). Conversely, the $P2Y_{12}$ receptor remains functional, suggesting that the two receptors are differentially regulated upon agonist activation. After stimulation of platelets with ADP or related compounds, the $P2Y_1$ receptor-stimulated calcium signal is abolished and the receptor internalized, whereas the $P2Y_{12}$

receptor conserves its ability to inhibit cAMP formation and to amplify the platelet aggregation induced by other agonists (84). This could be of major consequence in vivo because even in platelets refractory to stimulation by ADP the $P2Y_{12}$ receptor would still be able to ensure their reactivity at sites of injury where additional agonists might be present, thus preventing loss of haemostatic function. The matter will, nevertheless, require further study because others have reported contradictory results regarding desensitization of the $P2Y_{12}$ receptor (85). A key point which is not yet fully resolved is the intracellular traffic of these receptors upon cellular activation and in the course of their biosynthesis in the megakaryocyte. The $P2X_1$ receptor is also desensitized very quickly and requires lower concentrations of nucleotides as compared with the metabotropic receptor $P2Y_1$. The physiological implication of this is not yet well understood but may be related to the need of thrombus growth confinement at the site of a lesion and prevention of uncontrolled extension of the platelet aggregates. The impact of desensitization in flow systems has not yet been studied. In vivo, CD39 knockout mice have reduced thrombosis owing to persistent $P2Y_1$ desensitization (86).

Congenital Deficiencies

Comprehensive reviews have been written recently on this subject (see for example Reference 45). Briefly, no $P2X_1$- and $P2Y_1$-deficient patients have been reported so far. Doubt persists concerning a mutation in the $P2X_1$ sequence that has been reported in a patient with a severe bleeding disorder, as the clinical features do not correspond to the known characteristics of $P2X_1$ (45, 87).

In contrast, four families with congenital deficiency of platelet activation by ADP have been described, all with defects in the coding sequence of $P2Y_{12}$, resulting either in the absence of receptor expression or the expression of non-functional receptors (43, 45, 88–90). Interestingly, N-glycosylation of the $P2Y_{12}$ receptor is essential for signal transduction but not for ligand binding or cell surface expression (91). Whether differences in receptor glycosylation exist in the general population has not been investigated.

Polymorphisms of the P2Y Receptors

$P2Y_1$ and $P2Y_{12}$ have been shown to display gene sequence variations that have been associated with variable platelet responsiveness to ADP. The $P2Y_{12}$ polymorphisms are in the intronic part of the gene with no obvious impact on the coding sequence. Two haplotypes have been described, H1 and H2, where H2 was proposed to be associated with increased platelet reactivity to ADP (92). Furthermore, the H2 haplotype was associated with peripheral arterial disease in a case-control study (93). With respect to $P2Y_1$, a silent polymorphism was found at position 1622 (A/G) that was associated with increased platelet aggregation to a low concentration of ADP (0.1 μM) in subjects carrying the G allele (94). Interestingly, these authors also reported the polymorphisms of the $P2Y_{12}$ gene described by Fontana et al. (92), but did not confirm the increased platelet response associated with the

H2 haplotype. Further studies are required to address the questions raised by such observations. In particular, one would like to see how these polymorphisms result in levels of receptor expression, which can be measured by radioligand binding (34, 95). It would also be important to assess in each individual the spectrum of platelet receptor polymorphisms, including αIIbβ3, GPIbα, $\alpha 2\beta 1$, and PAR-1, because most of them have been reported to have an impact on platelet reactivity (96). Finally, because atherosclerosis and its thrombotic sequelae are known to be multifactorial and influenced by environment, one should be careful in proposing that such variants, especially single nucleotide polymorphisms, necessarily increase individual susceptibility to this disease and its complications.

The Platelet P2 Receptors as Molecular Targets for Antithrombotic Drugs

Owing to its central role in the formation and stabilization of a thrombus, the $P2Y_{12}$ receptor is a well-established target of antithrombotic drugs like clopidogrel, which has proved efficacy in many clinical trials and experimental models of thrombosis. Studies in $P2Y_1$ and $P2X_1$ knockout mice and experimental thrombosis models using selective $P2Y_1$ and $P2X_1$ antagonists have shown that, depending on the conditions, these receptors could also be potential targets for new antithrombotic drugs.

THE $P2Y_{12}$ RECEPTOR The restricted tissue distribution of this receptor makes it a most attractive target for selective antiplatelet drugs (43). Long before its molecular cloning, the pharmacological importance of this receptor in hemostasis and thrombosis was well-recognized. This was due to the fact that the potent antithrombotic thienopyridine compounds ticlopidine and clopidogrel, of which an active liver metabolite selectively targets the $P2Y_{12}$ receptor (43, 97), were used as molecular tools to characterize platelet responses to ADP and the role of the latter in thrombosis (14, 44). Conversely, patients with a congenital defect of ADP-induced platelet aggregation were shown to display a clopidogrel-like syndrome, and later found to carry $P2Y_{12}$ mutations (43, 45, 95). Clopidogrel treatment leads to inhibition of platelet aggregation in response to ADP with conserved shape change but blockade of the ability of ADP to inhibit cAMP production. Platelet aggregation in response to other agents is also affected through the effect on released ADP, which normally amplifies their responses and stabilizes the aggregates (see above). The active metabolite of clopidogrel covalently binds cysteine residues of the $P2Y_{12}$ receptor (97, 98), thus precluding the binding of ADP (95, 99, 100). Comprehensive reviews have been published emphasizing the clinical relevance of the $P2Y_{12}$ receptor as a target for antiplatelet drugs (48, 101) and surveying $P2Y_{12}$ targeting compounds (44, 102, 103). Large-scale clinical trials have demonstrated the beneficial effects of thienopyridines in the secondary prevention of major vascular events in patients with a history of cerebrovascular, coronary, or peripheral artery diseases, and of major cardiac events after coronary artery stent insertion (44, 104). Clopidogrel was approved for clinical applications in 1997 after a large phase III clinical trial

"Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events" (CAPRIE) involving 19,185 patients with a history of symptomatic atherosclerotic disease. The CAPRIE trial demonstrated an overall benefit of clopidogrel over aspirin in the prevention of vascular ischemic events, stroke, myocardial infarction, and vascular death (105). The Clopidogrel in Unstable angina to prevent Recurrent ischemic Events (CURE) trial showed a sustained, incremental benefit when clopidogrel was added to standard therapy (including aspirin) in patients with unstable angina and non-Q-wave MI. The Clopidogrel for the Reduction of Events During Observation (CREDO) trial demonstrated the benefit of continuing clopidogrel (plus aspirin) for 12 months, as opposed to 1 month, after percutaneous coronary intervention. For a complete review of past and ongoing trials, see Savi & Herbert (44).

Clopidogrel Resistance

One of the disadvantages of clopidogrel is that it has to be metabolized in the liver to generate an active metabolite. At the "classical" dosage of 75 mg/day, the steady state is achieved only after three to four days. Moreover, for safety reasons, i.e., in terms of bleeding risk, this dosage was chosen to achieve approximately 50% inhibition of platelet aggregation induced by ADP in citrated platelet-rich plasma. Several alternative protocols have been tested, in particular in acute situations, with loading doses of clopidogrel ranging from 300 mg to 900 mg so as to achieve a more rapid effect, followed by the chronic dose; this approach has resulted in improved clinical outcomes (44). Despite appropriate protocols and improved procedures, important interindividual variability has been observed in the response to clopidogrel (106). The concept of clopidogrel resistance has been put forward on the basis of the observation that 5%–10% of patients under treatment experienced acute or subacute thrombosis after a coronary event or implantation of a coronary stent (107–111). Many studies have been conducted to measure the biological response to clopidogrel, raising the question of the appropriate laboratory tests to define such responses. It would appear from these studies that classical light transmission aggregometry using ADP as the inducer, flow cytometric measurements of fibrinogen binding and flow cytometric evaluation of platelet reactivity based on the VASP phosphorylation state are well correlated and reveal that $\sim$ 30% of patients who receive clopidogrel treatment show responses that do not differ from those of untreated subjects, raising the question of their risk for a second ischemic event (109, 111–113). Although strongly suspected (114), the true clinical impact of low biological responsiveness to clopidogrel remains to be demonstrated in large prospective clinical trials. However, in a recent study (115) that assessed 60 consecutive patients classified in quartiles according to the inhibition of ADP-induced platelet aggregation after clopidogrel treatment, the patients in the first quartile (no inhibition) had a 40% recurrence of cardiovascular events during a six-months follow-up, whereas this percentage was null in the other quartiles, clearly indicating a relationship between the biological parameters and the clinical outcome. On the other hand, the bleeding tendency was increased in the fourth quartile. Thus, one may envisage a need for good laboratory tests

	Compounds	$P2Y_1$	$P2Y_{12}$	$P2X_1$	References
Agonists:	ADP	+	+	−	−
	2MeSADP	+	+	−	
	$\alpha\beta$MeATP	−	−	+	(16)
Antagonists:	MRS2179	+	−	+	(35)
	MRS2279	+	−	−	(33)
	MRS2500	+	−	−	(36)
	ATP	+	+	−	
	2MeSATP	+	+	−	
	2ClATP	+	+	−	
	AR-C66096MX	−	+	−	(119)
	AR-C67085MX	−	+	−	(119)
	AR-C69931MX	−	+	−	(58, 103, 119–122)
	C 1330–7	−	+	−	(43)
	INS compounds	−	+	−	(123)
	AZD6140	−	+	−	(103, 124)
	NF449	−	−	+	(70, 71)
Inhibitors:	Ticlopidine	−	+	−	(43, 44)
	Clopidogrel	−	+	−	(43, 44, 97)
	CS-747 (LY640315)	−	+	−	(102)

to monitor and adjust the treatment in individual patients treated with clopidogrel so as to achieve efficacy but limit the risk of bleeding. The mechanisms responsible for the inter-individual variability and the so-called resistance are not yet clearly defined. Poor compliance to the treatment, variable metabolization of the prodrug in the liver (116), drug-drug interactions (117), genetic polymorphisms of the platelet $P2Y_{12}$ receptor (92), a greater extent of $P2Y_1$-dependent platelet aggregation (118), or upregulation of other pathways are hypotheses that have been put forward but not yet demonstrated (107, 108). If the interindividual variability is the consequence of metabolic differences between individuals, one could envision simply adapting the dosage of clopidogrel. In contrast, if increase in dosage does not improve the response, then the antiplatelet drug should be changed in patients with low response to clopidogrel. Clinical trials are currently underway to address this question. In the case that a possible adjustment of the treatment is demonstrated, the term resistance will then be wrong and should no longer be used.

New thienopyridine compounds, such as prasugrel (CS-747, LY640315), have been described recently (Table 1), with apparent higher efficacy, faster onset, and longer duration of action as compared with clopidogrel (102). Whether prasugrel will display less variability remains to be demonstrated.

Competitive $P2Y_{12}$ Antagonists

The fact that ATP was known to be an antagonist of ADP-induced platelet aggregation first prompted the synthesis of ATP analogs with higher potency and better

stability toward ectonucleotidases. P2T receptor antagonists were discovered in the mid-1990s and turned out to be competitive $P2Y_{12}$ antagonists. These are the AR-C compounds (AR-C66096, AR-C67085, and AR-C69931MX), among which AR-C69931MX or cangrelor has been the most extensively studied in both animals and humans (58, 103, 119). In vitro, these compounds inhibit ADP-induced platelet aggregation with high selectivity for $P2Y_{12}$ relative to $P2Y_1$ and $P2X_1$ and cangrelor is currently used in many laboratories as the standard pharmacological tool to monitor the contribution of $P2Y_{12}$ to platelet functions (103). These compounds have been shown to be effective in several models of arterial thrombosis, as would be expected for $P2Y_{12}$ antagonists. Cangrelor and the more recent compound AZD6140 are under clinical evaluation, the latter being orally active, whereas cangrelor requires intravenous administration (103, 120–122). Other competitive $P2Y_{12}$ antagonists are currently in preclinical development (123) (Table 1).

Theoretically, use of such molecules would have an advantage mainly in acute situations, such as myocardial infarction, where fast blockade of the ADP ($P2Y_{12}$) receptor would be beneficial as compared to the delayed action of thienopyridine compounds. The rapid cessation of activity would also be beneficial in terms of safety. In fact, inhibition of the $P2Y_{12}$ receptor results in marked prolongation of the bleeding time in both animals and humans, which could represent a limitation of the use of $P2Y_{12}$ targeting compounds in a context of bleeding risk. Whether competitive antagonists will have a lesser effect on the bleeding time than the thienopyridine molecules remains to be assessed. This does not seem to be the case for AZD6140, which displays dose-dependent inhibition of platelet aggregation to ADP along with dose-dependent prolongation of the bleeding time (124). A second theoretical advantage of using competitive $P2Y_{12}$ antagonists could be if there is less interindividual variability in the response to the treatment. However, further studies will be needed to explore this possibility.

The $P2Y_1$ Receptor as a Target for New Antiplatelet Compounds

A consideration of the role of $P2Y_1$ in platelet aggregation and experimental thrombosis provides the rationale for suggesting this receptor to be a relevant target for new antiplatelet compounds. Thus, $P2Y_1$-knockout mice and animals treated with selective $P2Y_1$ antagonists display mild prolongation of the bleeding time and an absence of ADP-induced platelet shape change and aggregation at concentrations of ADP below 10 μM (34, 42, 125). These animals also display resistance to the systemic thromboembolism induced by infusion of a mixture of collagen and adrenaline, as evidenced by their reduced mortality and platelet consumption (42, 125). Lower levels of mortality, platelet consumption, and thrombin generation were likewise observed when thromboembolism was induced by infusion of tissue factor (59). A role of the $P2Y_1$ receptor has also been demonstrated in localized thrombosis, using intravital microscopy after ferric chloride injury of mouse mesenteric arteries (126). The above results clearly indicate that the $P2Y_1$ receptor

should be regarded as an attractive target for antiplatelet compounds. Moreover, a combination of $P2Y_1$ deficiency and clopidogrel treatment has been found to confer better thromboresistance than either condition alone, raising the possibility that a combination of P2 receptor antagonists could improve antithrombotic strategies (126). In a more precisely calibrated model of laser-induced vessel wall injury (127), $P2Y_1$ was found to be involved in the formation of an arterial thrombus but in a different way than $P2Y_{12}$ (128). The thrombus formed in $P2Y_1$-deficient mice seemed to be less stable than in wild-type mice, pointing to a role of the $P2Y_1$ receptor in stabilization of the thrombus at the vessel wall (128). Similar observations have been reported by Van Gestel et al. (129). Stronger $P2Y_1$ antagonists, such as MRS2500 (36), which shows higher potency and stability in vivo as compared with MRS2179, are currently under evaluation in experimental thrombosis in our laboratory. Of note, as observed with the $P2Y_1$-deficient mice, pharmacological inhibition of the $P2Y_1$ receptor results in only moderate prolongation of the bleeding time, which could be advantageous in terms of safety (42) (B. Hechler, unpublished data).

The $P2X_1$ Receptor as a Target for New Antiplatelet Compounds

Because the $P2X_1$ receptor plays an important role in thrombus formation only under high shear conditions, it might represent the ideal target for an antithrombotic drug. $P2X_1$-deficient mice have in fact no prolongation of their bleeding time as compared to the wild type, indicating that they conserve normal hemostasis. In contrast, they display resistance to the systemic thromboembolism induced by injection of a mixture of collagen and adrenaline, and to localized laser-induced injury of the vessel wall of mesenteric arteries (61). Conversely, increased systemic thrombosis has been reported in mice overexpressing the human $P2X_1$ receptor (130). Moreover, the new $P2X_1$ antagonist NF449 (70) has an inhibitory effect on platelet activation ex vivo and on thrombosis in vivo (71). These results clearly indicate that the $P2X_1$ receptor should be considered as a potential target for antiplatelet strategies, with the interesting feature that it would be effective only at sites of severe stenosis where shear forces are very high without a deleterious effect on normal hemostasis—a feature thought to be unique to $P2X_1$. However, in view of our recent data in vitro under flow conditions, the same may also be true for $P2Y_1$ (see above).

Currently, other platelet receptors are being evaluated as targets for anti-platelet drugs, including the TP receptors (131), the PARs (27), GPVI (132), or intracellular pathways such as the $G_{\alpha q}$ subunit (133) and PI3Kβ (52). Although each of those targets has advantages and disadvantages, all must be explored. However, owing to the central role of ADP and ATP in hemostasis and thrombosis, there is no doubt that their receptors are relevant targets for antiplatelet drugs, and research is very active in this field. There is no additional proof of concept required for $P2Y_{12}$ antagonists that are either irreversible, e.g., clopidogrel, or competitive, e.g., cangrelor. The

combination of clopidogrel with aspirin is much more efficient than each drug alone, demonstrating that inhibition of multiple pathways of platelet activation may be critical. Both $P2Y_1$ and $P2X_1$ receptors appear to be promising targets. Whether combined inhibition of the P2 receptors would also be beneficial requires further investigation. The tools now exist to allow progress in preclinical studies, including better animal models and new antagonists. When—and if—$P2Y_1$ or $P2X_1$ or mixed compounds will be tried in humans is an open question.

Future Directions: The Platelet P2 Receptors in Inflammation, Atherosclerosis, and Angiogenesis

Inflammation plays a major role in the progression of atherothrombosis and angiogenesis. Hence inflammatory markers are elevated in patients with stable or unstable ischemic diseases (6, 29, 134). Among all blood cell types, monocytes, T-lymphocytes, and platelets are the key agents and the contribution of platelets to the development of atherosclerosis has been established in many studies. Upon activation, platelets release active compounds, including growth factors such as platelet-derived growth factor (PDGF), which stimulates vascular smooth muscle cell migration and proliferation, proinflammatory cytokines, and chemoattractants (Il-1β or RANTES) (135), and express P-selectin (136) and CD40L (CD40 ligand or CD154, a member of the TNF family) (137). These inflammatory mediators promote the expression of adhesion molecules on endothelial cells and the recruitment and extravasation of monocytes (137–139), thereby contributing to the inflammatory and procoagulant responses and the exacerbation of atherosclerosis (4). CD40L is also directly involved in platelet activation and the stabilization of thrombi (140). Exposure of P-selectin results in the formation of platelet-leukocyte aggregates (141) and activation of the complement system, thus further propagating the inflammation (142). Platelets also interact with dendritic cells (143), which express several P2 receptors (144). Because the purinergic/nucleotide ligand-receptor system exists in all cell types and tissues involved in inflammation and atherosclerosis, and because they are so important in platelet physiology, they should be considered also as important partners in atherosclerosis (145). In addition to their short-term effects on vascular tone and platelet activation, nucleotides and P2 receptors are involved in long-term trophic effects on cell growth, proliferation, and death, which have important implications for atherosclerosis as well as angiogenesis (9, 145).

What do we know about the contribution of the individual platelet P2 receptors in these processes? So far, most of our knowledge comes from observed effects of thienopyridines or $P2Y_{12}$ antagonist effects in vitro, in animal models, or in patients. Decreased exposure of P-selectin, diminished formation of platelet-leukocyte aggregates, and subsequent tissue factor exposure have been documented (56, 121). Inhibition of CD40L exposure and release (146) and diminished circulating levels of CRP (C reactive protein) (29) clearly indicate a prominent role of the $P2Y_{12}$ receptor. Thus, in addition to their antiaggregating effect, the efficacy of

$P2Y_{12}$ antagonists might also be a consequence of the blockade of the contribution of platelets to inflammation (29, 44). In most cases, aspirin does not achieve such inhibition. It is assumed that the role of the $P2Y_{12}$ receptor not only in platelet aggregation but also in activation of multiple inflammatory and trophic processes should translate into a role of this receptor in the progression of atherosclerosis. However, this remains to be experimentally demonstrated.

The involvement of the $P2Y_1$ or $P2X_1$ receptors is less well documented. The $P2Y_1$ receptor has a role in P-selectin exposure, formation of platelet-leukocyte aggregates, and tissue factor exposure when platelets are stimulated with ADP, collagen, or low concentrations of thrombin receptor agonist peptides, as has been shown in vitro (56, 58). So far, no ex vivo data from animal models are available in terms of markers of inflammation. Using $P2Y_1$-knockout mice crossed with apolipoprotein E (ApoE)-knockout mice, we observed an inhibition of the size of plaques as compared with the controls (B. Hechler, unpublished data). Whether this effect is entirely due to platelet inhibition or to involvement of the endothelial or leukocyte $P2Y_1$ receptor requires further studies.

The hemostatic system and platelets are known to play a key role in angiogenesis (5, 7, 8). On the other hand, the role of ATP and ADP as mitogenic/apoptotic factors for vascular cells is known, and the involvement of their receptors is increasingly studied (9). More specifically, with respect to platelet P2 receptors, early studies showed the beneficial effect of ticlopidine in the treatment of the diabetic retinal angiopathy at the non-proliferative stage (147, 148). The precise mechanism is not yet fully understood, but part of the in vivo effect is probably attributable to their antiplatelet properties, i.e., inhibition of the $P2Y_{12}$ receptor. Goepfert et al. have shown that angiogenesis is impaired in an in vivo model (matrigel™ invasion) in CD39-deficient mice, which was tentatively attributed to desensitized $P2Y_1$ or $P2Y_2$ receptors (149). Although inhibition of macrophage migration has been proposed to explain the results, the role of blood platelets, known to display reduced reactivity by desensitization of $P2Y_1$, has not been ruled out. All these studies are thus preliminary. Further work should tell us the importance of the P2 receptor system and its relevance as a pharmacological target to modulate vascular remodeling and angiogenesis in an inflammatory context.

CONCLUSIONS

Among the platelet P2 receptors, only $P2Y_{12}$ has as yet been clearly demonstrated to be a target of clinical relevance, and this demonstration occurred prior to the receptor's identification by molecular cloning. However, there is evidence that $P2Y_1$ and $P2X_1$ could become relevant clinical targets, alone or in combination with those of other antiplatelet drugs. Pharmacological tools now exist to probe the efficacy of $P2Y_1$ and $P2X_1$ antagonists in various settings, such as in established experimental thrombosis porcine and canine models, where their use needs to be compared with responses to $P2Y_{12}$ acting drugs. Finally, both gene-targeted

animals and emerging pharmacological tools should provide new information to aid in determining the role of the platelet P2 receptors when platelets are involved in atherosclerosis, angiogenesis, and inflammation.

ACKNOWLEDGMENTS

I thank most sincerely Béatrice Hechler for her help in establishing the list of references and for critically reviewing the manuscript, François Lanza for his stimulating discussions and revision of the text and Juliette Mulvihill for revising the English.

LITERATURE CITED

1. Hartwig J, Italiano J Jr. 2003. The birth of the platelet. *J. Thromb. Haemost.* 1:1580–86
2. Ruggeri ZM. 2002. Platelets in atherothrombosis. *Nat. Med.* 8:1227–34
3. Massberg S, Brand K, Gruner S, Page S, Muller E, et al. 2002. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. *J. Exp. Med.* 196:887–96
4. Huo Y, Ley KF. 2004. Role of platelets in the development of atherosclerosis. *Trends Cardiovasc. Med.* 14:18–22
5. Browder T, Folkman J, Pirie-Shepherd S. 2000. The hemostatic system as a regulator of angiogenesis. *J. Biol. Chem.* 275: 1521–24
6. Ross R. 1999. Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* 340: 115–26
7. Carmeliet P. 2003. Angiogenesis in health and disease. *Nat. Med.* 9:653–60
8. Trikha M, Nakada MT. 2002. Platelets and cancer: implications for antiangiogenic therapy. *Semin. Thromb. Hemost.* 28:39–44
9. Burnstock G. 2002. Purinergic signaling and vascular cell proliferation and death. *Arterioscler. Thromb. Vasc. Biol.* 22:364–73
10. Gaarder A, Jonsen J, Laland S, Hellem A, Owen PA. 1961. Adenosine diphosphate in red cells as a factor in the adhesiveness of human blood platelets. *Nature* 192:531–32
11. Born GVR. 1962. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* 194:27–29
12. Reimers H. 1985. Adenine nucleotides in blood platelets. In *The Platelets: Physiology and Pharmacology*, ed. G Longenecker, pp. 85–98. Orlando, FL: Academic
13. Born GV. 1985. Adenosine diphosphate as a mediator of platelet aggregation in vivo: an editorial view. *Circulation* 72: 741–46
14. Maffrand JP, Bernat A, Delebassee D, Defreyn G, Cazenave JP, Gordon JL. 1988. ADP plays a key role in thrombogenesis in rats. *Thromb. Haemost.* 59:225–30
15. Ralevic V, Burnstock G. 1998. Receptors for purines and pyrimidines. *Pharmacol. Rev.* 50:413–92
16. Khakh BS, Burnstock G, Kennedy C, King BF, North RA, et al. 2001. International union of pharmacology. XXIV. Current status of the nomenclature and properties of P2X receptors and their subunits. *Pharmacol. Rev.* 53:107–18
17. Abbracchio MP, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, et al. 2003.

Characterization of the UDP-glucose receptor (re-named here the P2Y14 receptor) adds diversity to the P2Y receptor family. *Trends Pharmacol. Sci.* 24:52–55

18. Webb TE, Simon J, Krishek BJ, Bateson AN, Smart TG, et al. 1993. Cloning and functional expression of a brain G-protein-coupled ATP receptor. *FEBS Lett.* 324:219–25
19. Gachet C. 2001. ADP receptors of platelets and their inhibition. *Thromb. Haemost.* 86:222–32
20. Gordon JL. 1986. Extracellular ATP: effects, sources and fate. *Biochem. J.* 233: 309–19
21. Gachet C, Hechler B, Leon C, Vial C, Leray C, et al. 1997. Activation of ADP receptors and platelet function. *Thromb. Haemost.* 78:271–75
22. Kunapuli SP. 1998. Multiple P2 receptor subtypes on platelets: a new interpretation of their function. *Trends Pharmacol. Sci.* 19:391–94
23. Gachet C. 2005. The platelet P2 receptors as molecular targets for old and new antiplatelet drugs. *Pharmacol. Ther.* doi: 10.1016/j.pharmthera.2005.03.009
24. Hechler B, Cattaneo M, Gachet C. 2005. The P2 receptors in platelet function. *Semin. Thromb. Hemost.* 31:150–61
25. Robson SC, Wu Y, Sun X, Knosalla C, Dwyer K, Enjyoji K. 2005. Ectonucleotidases of CD39 family modulate vascular inflammation and thrombosis in transplantation. *Semin. Thromb. Hemost.* 31:217–33
26. Marcus AJ, Broekman MJ, Drosopoulos JH, Olson KE, Islam N, et al. 2005. Role of CD39 (NTPDase-1) in thromboregulation, cerebroprotection, and cardioprotection. *Semin. Thromb. Hemost.* 31:234–46
27. Brass LF. 2003. Thrombin and platelet activation. *Chest* 124:18S–25
28. Narumiya S, FitzGerald GA. 2001. Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108:25–30
29. Bhatt DL, Topol EJ. 2003. Scientific and therapeutic advances in antiplatelet therapy. *Nat. Rev. Drug Discov*. 2:15–28
30. Jackson SP, Schoenwaelder SM. 2003. Antiplatelet therapy: in search of the 'magic bullet.' *Nat. Rev. Drug Discov.* 2:775–89
31. Mahaut-Smith MP, Tolhurst G, Evans RJ. 2004. Emerging roles for P2X1 receptors in platelet activation. *Platelets* 15:131–44
32. Léon C, Hechler B, Vial C, Leray C, Cazenave JP, Gachet C. 1997. The P2Y1 receptor is an ADP receptor antagonized by ATP and expressed in platelets and megakaryoblastic cells. *FEBS Lett.* 403:26–30
33. Waldo GL, Harden TK. 2004. Agonist binding and Gq-stimulating activities of the purified human P2Y1 receptor. *Mol. Pharmacol.* 65:426–36
34. Baurand A, Raboisson P, Freund M, Léon C, Cazenave JP, et al. 2001. Inhibition of platelet function by administration of MRS2179, a P2Y1 receptor antagonist. *Eur. J. Pharmacol.* 412:213–21
35. Boyer JL, Mohanram A, Camaioni E, Jacobson KA, Harden TK. 1998. Competitive and selective antagonism of P2Y1 receptors by N6-methyl 2′- deoxyadenosine 3′,5′-bisphosphate. *Br. J. Pharmacol.* 124:1–3
36. Cattaneo M, Lecchi A, Ohno M, Joshi BV, Besada P, et al. 2004. Antiaggregatory activity in human platelets of potent antagonists of the P2Y 1 receptor. *Biochem. Pharmacol.* 68:1995–2002
37. Jin J, Daniel JL, Kunapuli SP. 1998. Molecular basis for ADP-induced platelet activation. II. The P2Y1 receptor mediates ADP-induced intracellular calcium mobilization and shape change in platelets. *J. Biol. Chem.* 273:2030–34
38. Hechler B, Eckly A, Ohlmann P, Cazenave JP, Gachet C. 1998. The P2Y1 receptor, necessary but not sufficient to support full ADP- induced platelet aggregation, is not the target of the drug clopidogrel. *Br. J. Haematol.* 103:858–66
39. Hechler B, Léon C, Vial C, Vigne P,

Frelin C, et al. 1998. The P2Y1 receptor is necessary for adenosine 5′-diphosphate-induced platelet aggregation. *Blood* 92:152–59
40. Savi P, Beauverger P, Labouret C, Delfaud M, Salel V, et al. 1998. Role of P2Y1 purinoceptor in ADP-induced platelet activation. *FEBS Lett.* 422:291–95
41. Mangin P, Ohlmann P, Eckly A, Cazenave JP, Lanza F, Gachet C. 2004. The P2Y receptor plays an essential role in the platelet shape change induced by collagen when TxA2 formation is prevented. *J. Thromb. Haemost.* 2:969–77
42. Léon C, Hechler B, Freund M, Eckly A, Vial C, et al. 1999. Defective platelet aggregation and increased resistance to thrombosis in purinergic P2Y(1) receptor-null mice. *J. Clin. Invest.* 104:1731–37
43. Hollopeter G, Jantzen HM, Vincent D, Li G, England L, et al. 2001. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* 409:202–7
44. Savi P, Herbert JM. 2005. Clopidogrel and ticlopidine: P2Y12 adenosine diphosphate-receptor antagonists for the prevention of atherothrombosis. *Semin. Thromb. Hemost.* 31:174–83
45. Cattaneo M. 2005. The P2 receptors and congenital platelet function defects. *Semin. Thromb. Hemost.* 31:168–73
46. Kauffenstein G, Hechler B, Cazenave JP, Gachet C. 2004. Adenine triphosphate nucleotides are antagonists at the P2Y12 receptor. *J. Thromb. Haemost.* 2:1980–88
47. Bodor ET, Waldo GL, Hooks SB, Corbitt J, Boyer JL, Harden TK. 2003. Purification and functional reconstitution of the human P2Y12 receptor. *Mol. Pharmacol.* 64:1210–16
48. Conley PB, Delaney SM. 2003. Scientific and therapeutic insights into the role of the platelet P2Y12 receptor in thrombosis. *Curr. Opin. Hematol.* 10:333–38
49. Cattaneo M, Lecchi A, Lombardi R, Gachet C, Zighetti ML. 2000. Platelets from a patient heterozygous for the defect of P2CYC receptors for ADP have a secretion defect despite normal thromboxane A2 production and normal granule stores: further evidence that some cases of platelet 'primary secretion defect' are heterozygous for a defect of P2CYC receptors. *Arterioscler. Thromb. Vasc. Biol.* 20:E101–6
50. Geiger J, Brich J, Honig-Liedl P, Eigenthaler M, Schanzenbacher P, et al. 1999. Specific impairment of human platelet P2Y(AC) ADP receptor-mediated signaling by the antiplatelet drug clopidogrel. *Arterioscler. Thromb. Vasc. Biol.* 19: 2007–11
51. Trumel C, Payrastre B, Plantavid M, Hechler B, Viala C, et al. 1999. A key role of adenosine diphosphate in the irreversible platelet aggregation induced by the PAR1-activating peptide through the late activation of phosphoinositide 3-kinase. *Blood* 94:4156–65
52. Jackson SP, Schoenwaelder SM, Goncalves I, Nesbitt WS, Yap CL, et al. 2005. PI 3-kinase p110beta: a new target for antithrombotic therapy. *Nat. Med.* 11:507–14
53. Lova P, Paganini S, Sinigaglia F, Balduini C, Torti M. 2002. A Gi-dependent pathway is required for activation of the small GTPase Rap1B in human platelets. *J. Biol. Chem.* 277:12009–15
54. Woulfe D, Jiang H, Mortensen R, Yang J, Brass LF. 2002. Activation of Rap1B by G(i) family members in platelets. *J. Biol. Chem.* 277:23382–90
55. Jin J, Kunapuli SP. 1998. Coactivation of two different G protein-coupled receptors is essential for ADP-induced platelet aggregation. *Proc. Natl. Acad. Sci. USA* 95:8070–74
56. Léon C, Ravanat C, Freund M, Cazenave JP, Gachet C. 2003. Differential involvement of the P2Y1 and P2Y12 receptors in platelet procoagulant activity. *Arterioscler. Thromb. Vasc. Biol.* 23:1941–47
57. Léon C, Alex M, Klocke A, Morgenstern E, Moosbauer C, et al. 2004. Platelet ADP

receptors contribute to the initiation of intravascular coagulation. *Blood* 103:594–600

58. Storey RF, Sanderson HM, White AE, May JA, Cameron KE, Heptinstall S. 2000. The central role of the P(2T) receptor in amplification of human platelet activation, aggregation, secretion and procoagulant activity. *Br. J. Haematol.* 110: 925–34
59. Léon C, Freund M, Ravanat C, Baurand A, Cazenave JP, Gachet C. 2001. Key role of the P2Y(1) receptor in tissue factor-induced thrombin-dependent acute thromboembolism: studies in P2Y(1)-knockout mice and mice treated with a P2Y(1) antagonist. *Circulation* 103:718–23
60. Rolf MG, Brearley CA, Mahaut-Smith MP. 2001. Platelet shape change evoked by selective activation of P2X1 purinoceptors with alpha,beta-methylene ATP. *Thromb. Haemost.* 85:303–8
61. Hechler B, Lenain N, Marchese P, Vial C, Heim V, et al. 2003. A role of the fast ATP-gated P2X1 cation channel in thrombosis of small arteries in vivo. *J. Exp. Med.* 198:661–67
62. Cattaneo M, Marchese P, Jacobson KA, Ruggeri Z. 2002. New insights into the role of P2X1 in platelet function. *Haematologica* 87:13–14
63. Oury C, Toth-Zsamboki E, Thys C, Tytgat J, Vermylen J, Hoylaerts MF. 2001. The ATP-gated P2X1 ion channel acts as a positive regulator of platelet responses to collagen. *Thromb. Haemost.* 86:1264–71
64. Takano S, Kimura J, Matsuoka I, Ono T. 1999. No requirement of P2X1 purinoceptors for platelet aggregation. *Eur. J. Pharmacol.* 372:305–9
65. Mahaut-Smith MP, Ennion SJ, Rolf MG, Evans RJ. 2000. ADP is not an agonist at P2X(1) receptors: evidence for separate receptors stimulated by ATP and ADP on human platelets. *Br. J. Pharmacol.* 131:108–14
66. Vial C, Pitt SJ, Roberts J, Rolf MG, Mahaut-Smith MP, Evans RJ. 2003. Lack of evidence for functional ADP-activated human P2X1 receptors supports a role for ATP during hemostasis and thrombosis. *Blood* 102:3646–51
67. Vial C, Rolf MG, Mahaut-Smith MP, Evans RJ. 2002. A study of P2X(1) receptor function in murine megakaryocytes and human platelets reveals synergy with P2Y receptors. *Br. J. Pharmacol.* 135:363–72
68. Sage SO, Yamoah EH, Heemskerk JW. 2000. The roles of P(2X1)and P(2T AC)receptors in ADP-evoked calcium signalling in human platelets. *Cell Calcium* 28:119–26
69. Kroll MH, Hellums JD, McIntire LV, Schafer AI, Moake JL. 1996. Platelets and shear stress. *Blood* 88:1525–41
70. Kassack MU, Braun K, Ganso M, Ullmann H, Nickel P, et al. 2004. Structure-activity relationships of analogues of NF449 confirm NF449 as the most potent and selective known P2X1 receptor antagonist. *Eur. J. Med. Chem.* 39:345–57
71. Hechler B, Magnenat S, Zighetti M, Kassack M, Ullmann H, et al. 2005. Inhibition of platelet functions and thrombosis through selective or non selective inhibition of the platelet P2 receptors with increasing doses of NF449. *J. Pharmacol. Exp. Ther.* 314:232–43
72. Moake JL, Turner NA, Stathopoulos NA, Nolasco L, Hellums JD. 1988. Shear-induced platelet aggregation can be mediated by vWF released from platelets, as well as by exogenous large or unusually large vWF multimers, requires adenosine diphosphate, and is resistant to aspirin. *Blood* 71:1366–74
73. Cattaneo M, Zighetti ML, Lombardi R, Mannucci PM. 1994. Role of ADP in platelet aggregation at high shear: studies in a patient with congenital defect of platelet responses to ADP. *Br. J. Haematol.* 88:826–29
74. Cattaneo M, Lombardi R, Bettega D,

Lecchi A, Mannucci PM. 1993. Shear-induced platelet aggregation is potentiated by desmopressin and inhibited by ticlopidine. *Arterioscler. Thromb.* 13: 393–97

75. Turner NA, Moake JL, McIntire LV. 2001. Blockade of adenosine diphosphate receptors P2Y(12) and P2Y(1) is required to inhibit platelet aggregation in whole blood under flow. *Blood* 98:3340–45
76. Goto S, Tamura N, Eto K, Ikeda Y, Handa S. 2002. Functional significance of adenosine 5′-diphosphate receptor (P2Y(12)) in platelet activation initiated by binding of von Willebrand factor to platelet GP Ibalpha induced by conditions of high shear rate. *Circulation* 105:2531–36
77. Resendiz JC, Feng S, Ji G, Francis KA, Berndt MC, Kroll MH. 2003. Purinergic P2Y12 receptor blockade inhibits shear-induced platelet phosphatidylinositol 3-kinase activation. *Mol. Pharmacol.* 63:639–45
78. Roald HE, Barstad RM, Kierulf P, Skjorten F, Dickinson JP, et al. 1994. Clopidogrel—a platelet inhibitor which inhibits thrombogenesis in non-anticoagulated human blood independently of the blood flow conditions. *Thromb. Haemost.* 71:655–62
79. Goto S, Tamura N, Handa S. 2002. Effects of adenosine 5′-diphosphate (ADP) receptor blockade on platelet aggregation under flow. *Blood* 99:4644–45; author reply 45–46
80. Remijn JA, Wu YP, Jeninga EH, IJsseldijk MJ, van Willigen G, et al. 2002. Role of ADP receptor P2Y(12) in platelet adhesion and thrombus formation in flowing blood. *Arterioscler. Thromb. Vasc. Biol.* 22:686–91
81. Mazzucato M, Cozzi MR, Pradella P, Ruggeri ZM, De Marco L. 2004. Distinct roles of ADP receptors in von Willebrand factor-mediated platelet signaling and activation under high flow. *Blood* 104:3221–27
82. Goncalves I, Nesbitt WS, Yuan Y, Jackson SP. 2005. Importance of temporal flow gradients and integrin alphaIIbbeta3 mechanotransduction for shear activation of platelets. *J. Biol. Chem.* 280:15430–37
83. Baurand A, Eckly A, Bari N, Léon C, Hechler B, et al. 2000. Desensitization of the platelet aggregation response to ADP: differential down-regulation of the P2Y1 and P2cyc receptors. *Thromb. Haemost.* 84:484–91
84. Baurand A, Eckly A, Hechler B, Kauffenstein G, Galzi JL, et al. 2005. Differential regulation and relocalization of the platelet P2Y receptors after activation: a way to avoid loss of hemostatic properties? *Mol. Pharmacol.* 67:721–33
85. Hardy AR, Conley PB, Luo J, Benovic JL, Poole AW, Mundell SJ. 2005. P2Y1 and P2Y12 receptors for ADP desensitize by distinct kinase-dependent mechanisms. *Blood* 105:3552–60
86. Enjyoji K, Sevigny J, Lin Y, Frenette PS, Christie PD, et al. 1999. Targeted disruption of cd39/ATP diphosphohydrolase results in disordered hemostasis and thromboregulation. *Nat. Med.* 5:1010–17
87. Oury C, Toth-Zsamboki E, Van Geet C, Thys C, Wei L, et al. 2000. A natural dominant negative P2X1 receptor due to deletion of a single amino acid residue. *J. Biol. Chem.* 275:22611–14
88. Cattaneo M, Lecchi A, Randi AM, McGregor JL, Mannucci PM. 1992. Identification of a new congenital defect of platelet function characterized by severe impairment of platelet responses to adenosine diphosphate. *Blood* 80:2787–96
89. Nurden P, Savi P, Heilmann E, Bihour C, Herbert JM, et al. 1995. An inherited bleeding disorder linked to a defective interaction between ADP and its receptor on platelets. Its influence on glycoprotein IIb-IIIa complex function. *J. Clin. Invest.* 95:1612–22
90. Cattaneo M, Zighetti ML, Lombardi R, Martinez C, Lecchi A, et al. 2003. Molecular bases of defective signal transduction in the platelet P2Y12 receptor of a

patient with congenital bleeding. *Proc. Natl. Acad. Sci. USA* 100:1978–83

91. Zhong X, Kriz R, Seehra J, Kumar R. 2004. N-linked glycosylation of platelet P2Y12 ADP receptor is essential for signal transduction but not for ligand binding or cell surface expression. *FEBS Lett.* 562:111–17
92. Fontana P, Dupont A, Gandrille S, Bachelot-Loza C, Reny JL, et al. 2003. Adenosine diphosphate-induced platelet aggregation is associated with P2Y12 gene sequence variations in healthy subjects. *Circulation* 108:989–95
93. Fontana P, Gaussem P, Aiach M, Fiessinger JN, Emmerich J, Reny JL. 2003. P2Y12 H2 haplotype is associated with peripheral arterial disease: a case-control study. *Circulation* 108:2971–73
94. Hetherington SL, Singh RK, Lodwick D, Thompson JR, Goodall AH, Samani NJ. 2005. Dimorphism in the P2Y1 ADP receptor gene is associated with increased platelet activation response to ADP. *Arterioscler. Thromb. Vasc. Biol.* 25:252–57
95. Gachet C, Cattaneo M, Ohlmann P, Hechler B, Lecchi A, et al. 1995. Purinoceptors on blood platelets: further pharmacological and clinical evidence to suggest the presence of two ADP receptors. *Br. J. Haematol.* 91:434–44
96. Kunicki TJ, Head S, Salomon DR. 2004. Platelet receptor structures and polymorphisms. *Methods Mol. Biol.* 273:455–78
97. Savi P, Pereillo JM, Uzabiaga MF, Combalbert J, Picard C, et al. 2000. Identification and biological activity of the active metabolite of clopidogrel. *Thromb. Haemost.* 84:891–96
98. Ding Z, Kim S, Dorsam RT, Jin J, Kunapuli SP. 2003. Inactivation of the human P2Y12 receptor by thiol reagents requires interaction with both extracellular cysteine residues, Cys17 and Cys270. *Blood* 101:3908–14
99. Savi P, Laplace MC, Herbert JM. 1994. Evidence for the existence of two different ADP-binding sites on rat platelets. *Thromb. Res.* 76:157–69
100. Mills DC, Puri R, Hu CJ, Minniti C, Grana G, et al. 1992. Clopidogrel inhibits the binding of ADP analogues to the receptor mediating inhibition of platelet adenylate cyclase. *Arterioscler. Thromb.* 12:430–36
101. Dorsam RT, Murugappan S, Ding Z, Kunapuli SP. 2003. Clopidogrel: interactions with the P2Y12 receptor and clinical relevance. *Hematology* 8:359–65
102. Niitsu Y, Jakubowski JA, Sugidachi A, Asai F. 2005. Pharmacology of CS-747 (prasugrel, LY640315), a novel, potent antiplatelet agent with in vivo P2Y12 receptor antagonist activity. *Semin. Thromb. Hemost.* 31:184–94
103. van Giezen JJ, Humphries RG. 2005. Preclinical and clinical studies with selective reversible direct P2Y12 antagonists. *Semin. Thromb. Hemost.* 31:195–204
104. Cazenave JP, Gachet C. 2002. Pharmacology of ticlopidine and clopidogrel. In *Platelets in Thrombotic and Non-Thrombotic Disorders*, ed. P Gresele, C Page, V Fuster, J Vermylen, pp. 929–39. Cambridge, UK: Cambridge Univ. Press
105. CAPRIE Steering Committee. 1996. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). *Lancet* 348: 1329–39
106. Serebruany VL, Steinhubl SR, Berger PB, Malinin AI, Bhatt DL, Topol EJ. 2005. Variability in platelet responsiveness to clopidogrel among 544 individuals. *J. Am. Coll. Cardiol.* 45:246–51
107. Cattaneo M. 2004. Aspirin and clopidogrel: efficacy, safety, and the issue of drug resistance. *Arterioscler. Thromb. Vasc. Biol.* 24:1980–87
108. Nguyen TA, Diodati JG, Pharand C. 2005. Resistance to clopidogrel: a review of the evidence. *J. Am. Coll. Cardiol.* 45:1157–64
109. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. 2003. Clopidogrel for coronary stenting: response variability,

drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 107:2908–13

110. Jaremo P, Lindahl TL, Fransson SG, Richter A. 2002. Individual variations of platelet inhibition after loading doses of clopidogrel. *J. Intern. Med.* 252:233–38
111. Muller I, Besta F, Schulz C, Massberg S, Schonig A, Gawaz M. 2003. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thromb. Haemost.* 89:783–87
112. Aleil B, Ravanat C, Cazenave JP, Rochoux G, Heitz A, Gachet C. 2005. Flow cytometric analysis of intraplatelet VASP phosphorylation for the detection of clopidogrel resistance in patients with ischemic cardiovascular diseases. *J. Thromb. Haemost.* 3:85–92
113. Geiger J, Teichmann L, Grossmann R, Aktas B, Steigerwald U, et al. 2005. Monitoring of clopidogrel action: comparison of methods. *Clin. Chem.* 51:957–65
114. Barragan P, Bouvier JL, Roquebert PO, Macaluso G, Commeau P, et al. 2003. Resistance to thienopyridines: clinical detection of coronary stent thrombosis by monitoring of vasodilator-stimulated phosphoprotein phosphorylation. *Catheter. Cardiovasc. Interv.* 59:295–302
115. Matetzky S, Shenkman B, Guetta V, Shechter M, Bienart R, et al. 2004. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation* 109:3171–75
116. Lau WC, Gurbel PA, Watkins PB, Neer CJ, Hopp AS, et al. 2004. Contribution of hepatic cytochrome P450 3A4 metabolic activity to the phenomenon of clopidogrel resistance. *Circulation* 109:166–71
117. Lau WC, Waskell LA, Watkins PB, Neer CJ, Horowitz K, et al. 2003. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction. *Circulation* 107:32–37
118. Jefferson BK, Foster JH, McCarthy JJ, Ginsburg G, Parker A, et al. 2005. Aspirin resistance and a single gene. *Am. J. Cardiol.* 95:805–8
119. Ingall AH, Dixon J, Bailey A, Coombs ME, Cox D, et al. 1999. Antagonists of the platelet P2T receptor: a novel approach to antithrombotic therapy. *J. Med. Chem.* 42:213–20
120. Storey RF, Oldroyd KG, Wilcox RG. 2001. Open multicentre study of the P2T receptor antagonist AR-C69931MX assessing safety, tolerability and activity in patients with acute coronary syndromes. *Thromb. Haemost.* 85:401–7
121. Storey RF, Wilcox RG, Heptinstall S. 2002. Comparison of the pharmacodynamic effects of the platelet ADP receptor antagonists clopidogrel and AR-C69931MX in patients with ischaemic heart disease. *Platelets* 13:407–13
122. Jacobsson F, Swahn E, Wallentin L, Ellborg M. 2002. Safety profile and tolerability of intravenous AR-C69931MX, a new antiplatelet drug, in unstable angina pectoris and non-Q-wave myocardial infarction. *Clin. Ther.* 24:752–65
123. Boyer JL, Patel RI, Douglass JG, Cowlen M, Crean CS, et al. 2004. Development of reversible P2Y12 receptor antagonists. *Haematologica* 89:13
124. Peters G, Robbie G. 2004. Single dose pharmacokinetics and pharmacodynamics of AZD6140- an oral reversible ADP receptor antagonist. *Haematologica* 89:14–15
125. Fabre JE, Nguyen M, Latour A, Keifer JA, Audoly LP, et al. 1999. Decreased platelet aggregation, increased bleeding time and resistance to thromboembolism in P2Y1-deficient mice. *Nat. Med.* 5:1199–202
126. Lenain N, Freund M, Léon C, Cazenave JP, Gachet C. 2003. Inhibition of localized thrombosis in P2Y1-deficient mice and rodents treated with MRS2179, a P2Y1 receptor antagonist. *J. Thromb. Haemost.* 1: 1144–49
127. Nonne C, Lenain N, Hechler B, Mangin P, Cazenave JP, et al. 2005. Importance

of platelet phospholipase Cγ2 signaling in arterial thrombosis as a function of lesion severity. *Arterioscler. Thromb. Vasc. Biol.* 25:1293–98

128. Lenain N, Freund M, Hechler B, Léon C, Evans R, et al. 2003. The role of the P2Y1, P2Y12, and P2X1 platelet receptors in a laser induced model of arterial thrombosis in vivo. J. Thromb. Haemost. (Suppl. 1), Abstr. No. OC425, XIX Int. ISTH Congr., Birmingham, UK
129. van Gestel MA, Heemskerk JW, Slaaf DW, Heijnen VV, Reneman RS, oude Egbrink MG. 2003. In vivo blockade of platelet ADP receptor P2Y12 reduces embolus and thrombus formation but not thrombus stability. *Arterioscler. Thromb. Vasc. Biol.* 23:518–23
130. Oury C, Kuijpers MJ, Toth-Zsamboki E, Bonnefoy A, Danloy S, et al. 2003. Overexpression of the platelet P2X1 ion channel in transgenic mice generates a novel prothrombotic phenotype. *Blood* 101:3969–76
131. Dogne JM, Hanson J, de Leval X, Masereel B, Kolh P, Pirotte B. 2004. New developments on thromboxane modulators. *Mini Rev. Med. Chem.* 4:649–57
132. Kahn ML. 2004. Platelet-collagen responses: molecular basis and therapeutic promise. *Semin. Thromb. Hemost.* 30: 419–25
133. Offermanns S, Toombs CF, Hu YH, Simon MI. 1997. Defective platelet activation in G alpha(q)-deficient mice. *Nature* 389:183–86
134. Libby P. 2002. Inflammation in atherosclerosis. *Nature* 420:868–74
135. Gawaz M, Brand K, Dickfeld T, Pogatsa-Murray G, Page S, et al. 2000. Platelets induce alterations of chemotactic and adhesive properties of endothelial cells mediated through an interleukin-1-dependent mechanism. Implications for atherogenesis. *Atherosclerosis* 148:75–85
136. André P. 2004. P-selectin in haemostasis. *Br. J. Haematol.* 126:298–306
137. Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, et al. 1998. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature* 391:591–94
138. Lindemann S, Tolley ND, Dixon DA, McIntyre TM, Prescott SM, et al. 2001. Activated platelets mediate inflammatory signaling by regulated interleukin 1beta synthesis. *J. Cell Biol.* 154:485–90
139. Gawaz M, Neumann FJ, Dickfeld T, Koch W, Laugwitz KL, et al. 1998. Activated platelets induce monocyte chemotactic protein-1 secretion and surface expression of intercellular adhesion molecule-1 on endothelial cells. *Circulation* 98:1164–71
140. Prasad KS, Andre P, Yan Y, Phillips DR. 2003. The platelet CD40L/GP IIb-IIIa axis in atherothrombotic disease. *Curr. Opin. Hematol.* 10:356–61
141. Ley K. 2003. The role of selectins in inflammation and disease. *Trends Mol. Med.* 9:263–68
142. Del Conde I, Cruz MA, Zhang H, Lopez JA, Afshar-Kharghan V. 2005. Platelet activation leads to activation and propagation of the complement system. *J. Exp. Med.* 201:871–79
143. Hagihara M, Higuchi A, Tamura N, Ueda Y, Hirabayashi K, et al. 2004. Platelets, after exposure to a high shear stress, induce IL-10-producing, mature dendritic cells in vitro. *J. Immunol.* 172:5297–303
144. Marteau F, Communi D, Boeynaems JM, Suarez Gonzalez N. 2004. Involvement of multiple P2Y receptors and signaling pathways in the action of adenine nucleotides diphosphates on human monocyte-derived dendritic cells. *J. Leukoc. Biol.* 76:796–803
145. Di Virgilio F, Solini A. 2002. P2 receptors: new potential players in atherosclerosis. *Br. J. Pharmacol.* 135:831–42
146. Hermann A, Rauch BH, Braun M, Schror K, Weber AA. 2001. Platelet CD40 ligand (CD40L)—subcellular localization, regulation of expression, and inhibition by clopidogrel. *Platelets* 12:74–82

147. De La Cruz P, Arrebola M, Gonzalez-Correa A, Martinez-Cerdan E, Moreno A, de la Cuesta FS. 2003. Effects of clopidogrel and ticlopidine on experimental diabetic ischemic retinopathy in rats. *Naunyn Schmiedebergs Arch. Pharmacol.* 367:204–10
148. 1990. Ticlopidine treatment reduces the progression of nonproliferative diabetic retinopathy. The TIMAD Study Group. *Arch. Ophthalmol.* 108:1577–83
149. Goepfert C, Sundberg C, Sevigny J, Enjyoji K, Hoshi T, et al. 2001. Disordered cellular migration and angiogenesis in cd39-null mice. *Circulation* 104:3109–15

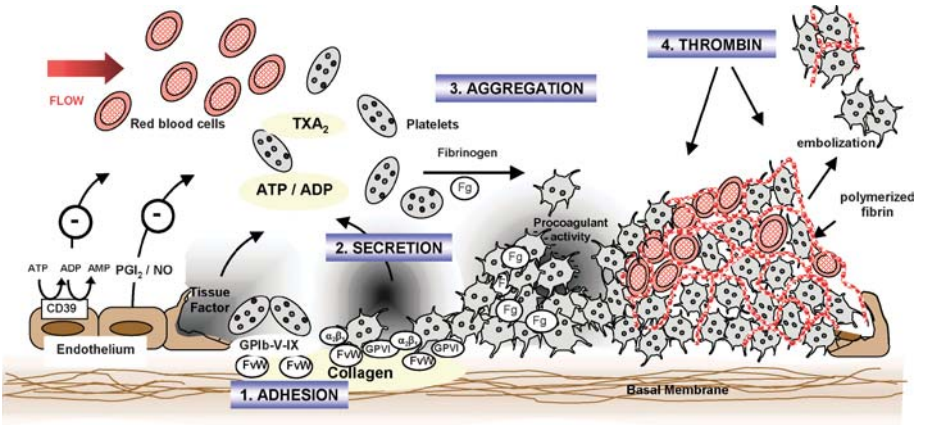

Figure 1 General mechanisms of platelet activation and arterial thrombus formation. Platelets first adhere to the subendothelial matrix where they become activated and release secondary agonists such as ATP and ADP secreted from the dense granules and TXA_2 synthesized from arachidonic acid. All these processes lead to the activation of the αIIbβ3 integrin to promote its conversion from an inactive to an active conformation at the platelet surface, which leads to the binding of soluble fibrinogen and vWF and subsequent platelet aggregation. Ultimately, thrombin not only activates platelets but also triggers the formation of a fibrin clot that stabilizes the thrombus.

Annual Review of Pharmacology and Toxicology
Volume 46, 2006

CONTENTS