P2Y₁₂ receptors in platelets and other hematopoietic and non-hematopoietic cells

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Abstract The P2Y₁₂ receptor is a Gi-coupled ADP receptor first described in blood platelets where it plays a central role in the complex processes of activation and aggregation. Platelet granules store important amounts of ADP which are released upon stimulation by interaction of platelets with the damaged vessel wall. Therefore, the P2Y₁₂ receptor is a key player in primary hemostasis and in arterial thrombosis and is an established target of antithrombotic drugs like the thienopyridine compounds ticlopidine, clopidogrel, and prasugrel or the direct, reversible antagonists ticagrelor and cangrelor. Beyond the platelet physiology and pharmacology, recent studies have revealed the expression of the P2Y₁₂ receptor in other hematopoietic cells including leukocyte subtypes and microglia in the central nervous system as well as in vascular smooth muscle cells. These studies indicate putative roles of the P2Y₁₂ receptor in inflammatory states and diseases of the brain, lung, and blood vessels. The selective role of P2Y₁₂ among other P2 receptors as well as the possible impact of P2Y₁₂ targeting drugs in these processes remain to be evaluated.

Keywords P2Y₁₂ receptors · Blood platelets · Hemostasis · Thrombosis · Inflammation

Introduction

The molecular identification of the P2Y₁₂ receptor was reported by Hollopeter et al. in *Nature* in 2001 [1], while

Zhang et al. [2] previously had reported that adenosine 5'-disphosphate (ADP) was the cognate ligand of the orphan receptor SP1999, a Gi-coupled receptor present in brain, spinal cord, and platelets. Later on, many other groups reported its sequence [3–5]. It was the last of the platelet P2 receptors to be identified. Long before its cloning, this receptor was pharmacologically described as being an ADP receptor, expressed by platelets and the molecular target of the antiplatelet drugs ticlopidine and clopidogrel, two thienopyridine compounds [6–8].

The P2Y₁₂ receptor is a G Protein Coupled Receptor (GPCR) composed of 342 amino acids. It contains four extracellular cysteines at positions 17, 97, 175, and 270 which are important sites for its function and expression [9]. The P2Y₁₂ gene is located on chromosome 3q25.1, together with the gene coding for P2Y₁ (3q25.2), P2Y₁₃ (3q24), and P2Y₁₄ (3q21–25) [9, 10]. The tissue distribution of the P2Y₁₂ receptor seemed to be restricted to platelets and subregions of the brain including the amygdala, caudate nucleus, corpus callosum, hippocampus, substantia nigra, and thalamus [1]. Further studies revealed its expression and roles in microglial cells [11], in vascular smooth muscle cells, [12, 13] as well as in dendritic cells [14], in macrophages [15], and in yet unspecified leukocytes [16, 17].

ADP is the natural agonist of this receptor, while ATP and a wide range of its triphosphate analogues behave as antagonists [18, 19]. It is the molecular target of the antiplatelet drugs clopidogrel and prasugrel, two thienopyridine compounds, of which the active metabolites formed in the liver covalently bind to the receptor [20, 21] and of ticagrelor (AZD6140), cangrelor (AR-C69931MX), and elinogrel (PRT060128), which are direct, reversible antagonists of the receptor [22]. Ticagrelor has been reported to have non-competitive interaction with the receptor suggesting its binding to occur at a site distinct from the ADP binding site [23].

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Two P2Y receptors regulate platelet activation by ADP

The main role of blood platelets is to ensure primary hemostasis, which means the maintenance of blood vessel integrity and the rapid cessation of bleeding in the event of loss of vascular integrity. They are also responsible for the formation of pathogenic thrombi at sites of rupture or erosion of an atherosclerotic plaque, promoting atherothrombotic diseases including acute coronary syndromes, ischemic stroke, and peripheral artery disease [24]. Platelets also play an important role in inflammation and can influence the phenotype of other blood and vascular cells, thereby contributing to other non-hemostatic disorders, from cystic fibrosis and arthritis to diabetes, atherosclerosis, and cancer [25–29].

ADP plays crucial roles in the physiological process of hemostasis and in the development and extension of arterial thrombosis [30]. As compared to strong agonists such as thrombin or collagen, ADP is, by itself, a weak agonist of platelet aggregation inducing only reversible responses. However, ADP, stored at a very high concentration along with ATP and other adenine nucleotides in platelet dense granules and released upon activation at sites of vascular injury, constitutes an important so-called secondary agonist which greatly amplifies most of the platelet responses and contributes to the stabilization of the thrombus. Addition of ADP to washed platelets results in shape change, reversible aggregation at physiological concentrations of calcium (2 mM), and finally desensitization [31, 32]. ADP induces a transient rise in free cytoplasmic calcium, due to mobilization of internal stores and secondary store-mediated influx, and a concomitant inhibition of adenylyl cyclase activity [6]. These effects of ADP are mediated by two distinct P2 receptors, namely P2Y₁ and P2Y₁₂ [33, 34]. Each of these receptors has a specific function during platelet activation and aggregation, which has implications for their involvement in thrombosis.

The P2Y₁ receptor is widely distributed in many tissues including heart, blood vessels, and blood cells, neural tissue, testis, prostate, and ovary [10]. About 150 P2Y₁ receptor binding sites are expressed per platelet [35, 36], which is very low as compared for instance to the TP receptors or the thrombin receptor PAR-1 (1,000 to 2,000 sites/platelet). As it is coupled to G α_q , the P2Y₁ receptor triggers the mobilization of calcium from internal stores, which results in platelet shape change and weak, transient aggregation in response to ADP [37–39]. The P2Y₁ receptor is absolutely required for ADP-induced platelet aggregation. Its pharmacological inhibition or genetic deficiency results in complete absence of platelet aggregation and shape change when ADP is the sole agonist. As a consequence, at the intracellular level, the calcium signal is abolished while the ability of ADP to inhibit cAMP formation is preserved [37, 40]. Overall, the P2Y₁ receptor mediates weak responses to ADP but is nevertheless a crucial factor in the initiation of the platelet activation induced by ADP or collagen [41].

The P2Y₁₂ receptor is responsible for completion of the platelet aggregation response to ADP initiated by P2Y₁ [42] and for the ADP-dependent amplification of platelet aggregation induced by any other agents such as Gq-coupled serotonin receptors [39], Gq and G12/13-coupled TXA₂ and PAR-1 receptors [43, 44], immune complexes [45, 46], or when platelets are activated by collagen through the GPVI/tyrosine kinase/PLC γ 2 pathway [47]. The P2Y₁₂ receptor is also responsible for the ability of ADP to restore collagen-induced aggregation in G α_q -deficient mouse platelets [48]. The P2Y₁₂ receptor is involved in potentiation of platelet secretion independently of TXA₂ generation and macroaggregate formation [49, 50] and mediates the stabilization of platelet aggregates induced by thrombin [51–53] or TXA₂ [54]. The requirement of this receptor for completion of aggregation in response to ADP but also for the ADP-dependent amplification of aggregation induced by other agents was confirmed in P2Y₁₂^{–/–} mice [3]. The bleeding time is markedly prolonged in these mice as it is in patients with severe P2Y₁₂ deficiency [55, 56] as well as in animals treated with high doses clopidogrel or other P2Y₁₂ antagonists.

Studies of ten healthy subjects showed that the selective P2Y₁₂ radioligand [3H]PSB-0413 [57] bound to 425±51 sites/platelet with a K_d of 3.3±0.6 nM [58]. How the expression of the P2Y₁₂ receptor is regulated is not really well known. MicroRNA possibly plays a key role as platelets have been shown to contain abundant and diverse array of miRNA. The detection of the P2Y₁₂ receptor mRNA in immunoprecipitates of proteins involved in the processing of miRNA suggests that its expression may be controlled by such mechanisms. Whether this could occur during platelet activation or before their release as mature platelets by megakaryocytes remains to be established [59]. Co-activation of the P2Y₁ and P2Y₁₂ receptors is necessary for normal ADP-induced platelet aggregation since separate inhibition of either of them with selective antagonists results in a dramatic decrease in aggregation [39, 42, 60]. The P2Y₁ and P2Y₁₂ receptors are differentially involved in platelet aggregation induced by other agonists, with the P2Y₁ playing only a minor role, except in the case of collagen-induced activation [41], while P2Y₁₂ supports amplification of these responses. This is also the case in the procoagulant activity of platelets. While both receptors are indirectly involved through their role in platelet P-selectin exposure and in the formation of platelet–leukocyte conjugates leading to leukocyte tissue factor exposure [61, 62], the P2Y₁₂ receptor is also directly implicated in the exposure of phosphatidylserine at the surface of platelets [62–64]. The specific involvement of the P2Y₁₂ receptor in the procoagulant role of platelets has been further studied and confirmed as it has been found to have a major role in the formation of the so-called COAT-platelets, namely platelets expressing phosphatidylserine and activated factor V [65, 66].

P2Y₁₂ receptor triggered intracellular signaling

The P2Y₁₂ receptor is coupled to inhibition of adenylyl cyclase activity through activation of a G α i2 G protein subtype [67, 68]. However, adenylyl cyclase inhibition and lowering cAMP levels are not sufficient to cause platelet aggregation [69, 70] and other signaling events are required for full activation of the α IIB β 3 integrin and subsequent aggregation. One important intracellular pathway which regulates Gi-dependent integrin α IIB β 3 activation is constituted by phosphoinositide 3-kinases (PI 3-Ks) [53, 71, 72]. PI 3-K isoform p110 β regulates integrin activation through a classical lipid kinase-dependent mechanism, involving the small GTPase Rap1 and the serine-threonine protein kinase B/Akt (PKB/Akt) [73–77], whereas p110 γ appears to regulate integrin principally through a non-catalytic signaling mechanism [78, 79]. Whether other PI3K class I isoforms such as the p110 α or PI3K class II or III isoforms, which are highly expressed in blood platelets, play a role in integrin α IIB β 3 activation is under investigation. Another way by which P2Y₁₂ contributes to modulate aggregation through G α i2 may involve inhibition of the cAMP-dependent protein kinase (PKA)-mediated phosphorylation of the vasodilator-stimulated phosphoprotein (VASP), an intracellular actin regulatory protein [80]. This protein does not play a key role in integrin activation but is used as an important marker of the P2Y₁₂ receptor activation state especially to monitor the effect of P2Y₁₂ targeting antiplatelet drugs [81]. Where exactly the crosstalk between Gq and Gi most likely occurs seems to be at the level of diacylglycerol (DAG) metabolism. Indeed, Guidetti et al. [82] have shown that activation of the P2Y₁₂ receptor is required for pleckstrin phosphorylation upon activation through a Gq coupled receptor, either P2Y₁ or the TP receptor. These authors also reported that inhibition of DAG kinase bypasses the need of P2Y₁₂ stimulation for agonist induced PKC activation and platelet aggregation which indicates that DAG is a key second messenger of the P2Y₁₂ dependent amplification loop [82]. The rap1B-GEF CalDAG-GEFI is also involved in P2Y₁₂ signaling as in CalDAG-GEFI deficient mice, signaling by PKC/P2Y₁₂ is not sufficient to promote thrombus formation [83]. Further work is required to fully understand all the signaling steps downstream of P2Y₁₂ and the complex interplay with other pathways to promote amplification and stabilization of the platelet responses to various stimuli.

Desensitization

An important phenomenon in controlling thrombus growth is the regulation of platelet reactivity after stimulation and receptor desensitization is one general

mechanism used by cells to adapt their responsiveness. It has long been known that after being exposed to ADP, platelets become unresponsive to a second stimulation with ADP with a resultant loss of shape change and aggregation. This so-called refractory state of platelets to ADP is transient and, depending on the experimental conditions, lasts 15 to 30 min provided an enzymatic system degrades ADP in the medium. In the absence of such a system, platelets do not recover responsiveness to ADP. The molecular mechanisms of this phenomenon have been studied in detail but consensus has not been reached, and two different views have not yet been completely reconciled. On the one hand, it is thought that the phenomenon of platelet refractoriness to ADP is due to selective desensitization and internalization of the P2Y₁ receptor, while the P2Y₁₂ receptor remains functional with the ability of ADP to induce amplification of the platelet aggregation induced by other agonists [84, 85]. Desensitization of the P2Y₁ receptor has been shown to be dependent on receptor C-terminal phosphorylation sites, β -arrestin-2 interaction, and protein kinase C (PKC) activity [86–89]. The *in vivo* consequence is that under conditions of platelets refractory to stimulation by ADP, the P2Y₁₂ receptor remains functional and able to promote their reactivity at sites of injury, thus preventing loss of hemostatic function. On the other hand, it is reported that both P2Y₁ and P2Y₁₂ receptors undergo desensitization, and that P2Y₁₂ desensitization is mediated by G protein-coupled receptor kinases (GRK) [87, 88, 90]. Recent studies in beta-Arrestin 1 and in beta-Arrestin 2 deficient mice indicate that neither P2Y₁ nor P2Y₁₂ function are altered by deficiency of these regulatory proteins [91, 92] pointing to possible redundancy between arrestins or only minor roles of these proteins in receptor desensitization. Now, while it appears clearly that the P2Y₁₂ receptor is internalized upon activation, as all authors agree, the functional relevance of this ability remains to be established in platelets as well as in other cells and tissues. A very recent study reports on a patient with heterozygous mutation in the PDZ domain of P2Y₁₂ (P341A) that is associated with reduced expression of the receptor at the platelet surface related to compromised receptor recycling [93]. Although not clearly related to a question of desensitization, it points to the importance of receptor recycling in platelet physiology.

Genetic polymorphisms of the P2Y receptors

The P2Y₁₂ receptor has been shown to display gene sequence variations which have been proposed to be associated with increased platelet responsiveness to ADP. These

polymorphisms are in the intronic part of the gene and have no obvious impact on the coding sequence. Two haplotypes have been identified, designated as H1 and H2, the latter being proposed to be linked to enhanced platelet reactivity to ADP [94] and to a diminished response to clopidogrel [95] and associated with increased risks for peripheral arterial disease [96] and coronary artery disease [97]. However, these results were not confirmed in latter studies [98–100]. It thus appears that polymorphisms of the non-coding region of the P2Y₁₂ receptor gene do not have any impact on the receptor function, or on the individual responsiveness to clopidogrel. Thus, whether polymorphisms of P2Y₁₂ receptor exist, their impact on the platelet physiology or in clinical pharmacology, probably requires further studies. Interestingly, N-glycosylation of the P2Y₁₂ receptor is essential for signal transduction but not for ligand binding or cell surface expression [101]. Whether differences in receptor glycosylation exist in the general population has not been investigated so far.

P2Y₁₂ deficient patients

Congenital P2Y₁₂ deficiency is an autosomal recessive disorder first described in Italy by Cattaneo et al. [102] followed by a second family described in France by Nurden et al. [103]. These patients experience mild to severe bleedings. Comprehensive reviews have been written recently on this subject (see for example [55, 56, 104]). So far, 13 patients with congenital deficiency of platelet activation by ADP have been described, all with defects in the coding sequence of P2Y₁₂, resulting either in the absence of receptor expression (six patients) or partial deficiency or the expression of non-functional receptors with normal radioligand binding sites (seven patients) [102, 103, 105–108]. Whether these patients have other disorders than bleeding, related to the role of the P2Y₁₂ receptor in leukocytes, smooth muscle cells or glial cells (see below) is not known.

The P2Y₁₂ receptor as a molecular target for antithrombotic drugs

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 and irreversibly targets the P2Y₁₂ receptor, were used as molecular tools to characterize platelet responses to ADP and the role of the latter in thrombosis [109]. The thienopyridine compounds are prodrugs which have to be metabolized by the liver in order to generate active metabolites.

The active metabolite of clopidogrel [110] covalently binds cysteine residues of the P2Y₁₂ receptor, thus precluding the binding of ADP [111–113]. Moreover, it has been reported that clopidogrel's active metabolite disrupts homopolymers of the P2Y₁₂ receptor expressed in lipid rafts and partitions them out of lipid rafts [20], pointing to the importance of oligomerization and membrane localization on the function of this receptor. Further studies are however required to confirm these findings. The fact that lipid raft integrity is required for P2Y₁₂ signaling has been reported earlier [114]. Clopidogrel treatment leads to a dose-dependent inhibition of platelet aggregation in response to ADP with conserved shape change and transient weak aggregation driven by P2Y₁. At the intracellular level, P2Y₁₂ blockade results in the inhibition of the ability of ADP to inhibit cyclic AMP production while calcium signaling is preserved [42]. Platelet aggregation in response to strong activators is also strongly inhibited through the effect on released ADP. Large scale clinical trials have demonstrated the beneficial effects of thienopyridines in the prevention of major cardiac events after coronary artery stent insertion and in the secondary prevention of major vascular events in patients with a history of cerebrovascular, coronary, or peripheral artery disease [115, 116]. Prasugrel (CS-747, LY640315) is a third generation thienopyridine compound which has higher efficacy and faster onset of action than clopidogrel. This is due to a slightly different metabolic pathway and better rate of active metabolite generation as compared to clopidogrel [22, 116]. A large scale clinical trial, TRITON-TIMI 38, including 13,609 patients planned for percutaneous coronary intervention (PCI) demonstrated the overall superiority of prasugrel (60 mg loading dose followed by 10 mg maintenance dose) in comparison to clopidogrel (300 mg loading dose, 75 mg maintenance dose) with a total of 19 % reduction of ischemic events with, particularly, 52 % decreased Stent thrombosis [117], but with a 32 % increase of major bleeding, including fatal bleeding. Although not really surprising, these results had an important impact in the practices of interventional cardiologists [118].

The competitive intravenous P2Y₁₂ antagonist cangrelor (AR-C69931MX) is under investigation in phase 3 clinical trials while the orally active compound ticagrelor (AZD6140) has recently been approved by the FDA for the treatment of ischemic diseases. Ticagrelor demonstrated improved cardiovascular outcomes, including a reduction in myocardial infarctions and vascular events as compared to clopidogrel in the PLATO trial [119]. The main adverse events with ticagrelor are bleeding and dyspnea, the latter of which is of unclear etiology and of unknown long-term clinical concern. Theoretically, use of competitive antagonists would have an advantage mainly in acute situations like myocardial infarction, where fast

blockade of the ADP receptor should be beneficial as compared to the delayed action of thienopyridine compounds. The rapid cessation of activity would also be beneficial in terms of safety. A second theoretical advantage of using competitive P2Y₁₂ antagonists could be if there is less inter-individual variability in the response to the treatment (for reviews, see [22, 116]).

The platelet P2Y₁₂ receptor beyond hemostasis

Vascular inflammation plays a central role in both the progressive and acute components of atherothrombotic disease. It is now appreciated that activated platelets contribute to inflammation since platelets are an important source of inflammatory mediators, compounds with trophic activity such as PDGF, expose P-selectin, CD40, and CD40 ligand (CD40L) which allow interaction with leukocytes and subsequent leukocyte activation and release of a range of inflammatory cytokines and exposure of tissue factor [26]. Thus, the clinical efficacy of antiplatelet drugs might also be related to blockade of the contribution of platelets to inflammation [120]. The role of the P2Y₁₂ receptor not only in platelet aggregation but also in the activation of multiple inflammatory and trophic processes may be expected to result in its direct involvement in the progression of atherosclerosis and restenosis, which has been reported recently in rabbit and in mice [121–125]. One particular type of atherosclerosis is transplant atherosclerosis. Abele and colleagues have shown that the P2Y₁₂ inhibitor clopidogrel reduced experimental transplant atherosclerosis in mice [126] which was confirmed in P2Y₁₂ deficient mice [127]. However, from these studies, it was difficult to distinguish the effects of the platelet P2Y₁₂ receptor from those of the receptor expressed in other cell types, namely dendritic cells, macrophages, or smooth muscle cells. Indeed, selective platelet depletion or inhibition did not inhibit transplant atherosclerosis strongly suggesting the P2Y₁₂ receptor of other cell types to be involved in this process [126]. Interestingly, migration properties of smooth muscle like cells and of leukocytes dependent on the P2Y₁₂ receptor independently of platelets have been observed in transplant atherosclerosis [128], further indicating the expression of the receptor in these cells.

In addition to vascular inflammation, through their capability of interacting with many other cells, platelets are involved in many physiological and pathological processes including allergic asthma [129]. They are necessary for lung leukocyte recruitment in a murine model of allergic inflammation, and platelet–leukocyte aggregates are formed in circulating blood of patients with asthma after allergen exposure [130]. It has been reported that the P2Y₁₂ receptor is required for proinflammatory actions of the stable

abundant mediator LTE4 in allergic asthma and has been suggested to be a novel potential therapeutic target for this pathology since clopidogrel showed efficacy in experimental allergen-induced pulmonary inflammation [131]. Although not fully unravelled, the mechanisms suggested to explain the key role of the P2Y₁₂ receptor was its association with an as yet unidentified coreceptor, of the cysteinyl leukotriene family. Where this association occurs is not known but platelets are required as their depletion abrogated the process [131].

The P2Y₁₂ receptor in smooth muscle cells

Smooth muscle cells are important regulators of vessel tone and are involved in vascular inflammation, atherosclerosis, and restenosis following angioplasty. The first report of the presence of the P2Y₁₂ receptor in smooth muscle cells was in 2004 when Wihlborg and colleagues published a paper showing that P2Y₁₂ mRNA was found in human smooth muscle cells and that human vessel contraction induced by ADP was blocked by a direct P2Y₁₂ antagonist, ARC67085 [13]. However, the authors also reported that patients treated by clopidogrel did not display inhibition of contraction [13]. The same team further studied human and murine vessel contraction and reported that AZD6140 (ticagrelor) was able to inhibit contraction in both systems, adding evidence that direct P2Y₁₂ antagonists may have beneficial effects on vessel tone and patency [12] in contrast to clopidogrel which, even at high dose, did not inhibit vascular contraction. The reason for this discrepancy is not known, although the authors speculate that the active metabolite of clopidogrel may not reach enough amounts in the circulation. This can be discussed as these metabolites can be dosed and their dosage is strongly correlated to their antiplatelet effect. Other authors, using bone marrow transplanted P2Y₁₂^{−/−} chimeric mice failed to demonstrate a direct effect of these drugs on smooth muscle cells but observed an effect related to inhibition of platelet functions [122], highlighting the role of initial platelet thrombus formation to later restenosis. Increased transcriptional expression of the P2Y₁₂ receptor in smooth muscle cells has recently been found in response to thrombin where it increases IL6 production and smooth muscle cells mitogenesis, an effect inhibited by the active metabolite of prasugrel [132].

The P2Y₁₂ receptor in leukocytes

High amounts mRNA of the P2Y₁₂ receptor has been found previously in lymphocytes as well as in CD34+ progenitor cells [133]. More recently, it has been proposed that P2Y₁₂ receptors expressed on leukocytes may directly be affected

by P2Y₁₂ antagonists with implications in terms of anti-inflammatory action [16]. In sharp contrast with that, Kunapuli's group showed proinflammatory action of clopidogrel as a consequence of inhibition of leukocyte P2Y₁₂ receptors [17]. Obviously, there is a need for further investigations to clearly establish the functional expression of the P2Y₁₂ receptor in the various subpopulations of white blood cells and distinguish its role in these cells from its role in platelets and the interplay between platelets and other blood and vascular cells. One limitation is the lack of selective antagonists as the most popular P2Y₁₂ antagonist, ARC69931MX (cangrelor) also inhibits P2Y₁₃ [134]. Thus, combinations of agonists and antagonists, along with siRNA studies are required to clearly distinguish the functional expression of the various P2Y receptor subtypes in these cells. In addition, the functional assay to use is not trivial as these receptors are coupled to Gi and most studies report on calcium signaling.

The P2Y₁₂ receptor in dendritic cells

Dendritic cells are obviously of great importance in many aspects of inflammation and immune reactions and the interplay between platelets and the immune system as a whole is actively studied [135]. A wide range of adenosine and purinergic receptors are expressed in these cells and play a role in their maturation and orientation of the immune response [134]. ADP has been found to stimulate calcium mobilization and ERK phosphorylation in a pertussis toxin-dependent manner and to inhibit IL12 production [136]. As ARC69931MX inhibited only part of these effects and as it inhibits both P2Y₁₂ and P2Y₁₃, further studies were conducted using P2Y₁₂ and P2Y₁₃ knockout mice. It was concluded that the P2Y₁₂ receptor is expressed in murine dendritic cells and that its activation increases antigen endocytosis with subsequent enhancement of specific T cell activation [14]. These latter observations along with the studies of experimental transplant atherosclerosis clearly demonstrate a role for the P2Y₁₂ receptor in murine dendritic cells. Whether the human dendritic cells behave similarly is not yet firmly demonstrated. The use of clopidogrel in million patients with ischemic diseases over the years has not yet reported on selective immune disorders related to the drug. Similarly, P2Y₁₂ defective patients do not suffer from overt disorders of their immune system. However, as often in science and medicine, hidden phenomena have to be revealed.

P2Y₁₂ and the CNS

Microglial cells are primary immune sentinels in the central nervous system. Extracellular nucleotides function as

regulators of microglial behavior in vivo. Microglial cells express several subtypes of P2 receptors including the ionotropic P2X₄ and P2X₇ subtypes and the metabotropic P2Y₁, P2Y₂, and P2Y₁₂ receptors [137]. The P2Y₁₂ receptor has been shown to play a key role in microglial activation especially in the early stages of the activation process [11]. Interestingly, upon activation, microglial cells change their shape to an amoeboid state where the P2Y₁₂ receptors are down regulated. Haynes et al. also showed that the lack of the P2Y₁₂ receptor delays, but does not abolish, the ability of microglial cells to respond to local tissue damage. Further studies showed that TGFβ and LPS modulate ADP-induced migration of microglial cells through both P2Y₁ and P2Y₁₂ receptor expression [138]. Finally, the P2Y₁₂ receptor has been found to contribute to neuropathic pain [139, 140]. The presence of this receptor has been established in oligodendrocytes and myelin sheaths of rat cerebral cortex, subcortical areas, and periventricular white matter [141]. A recent post mortem study of cerebral cortex from patients with multiple sclerosis found a strong correlation between the extent of lesions and decreased expression of the P2Y₁₂ receptor at the axon–myelin interface [142] suggesting that loss of P2Y₁₂ receptor expression might be detrimental to tissue integrity. Whether this decreased expression is the cause or the consequence of the disease is not established. It does not seem that patients with severe P2Y₁₂ deficiency experience overt neurological problems (Cattaneo, personal communication).

Conclusion

The P2Y₁₂ receptor is mostly a hematopoietic cell receptor which has a key role in platelet physiology and is a major target for antithrombotic drugs. It is also strongly involved in many inflammatory manifestations either through its role in platelets or through direct activation of various immune cells by ADP. Whether the currently used P2Y₁₂ targeting drugs could also provide therapeutic efficacy in inflammatory diseases remains to be tested in experimental models and in clinical trials.

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