Platelet Purinergic Receptors- Far from Home

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Abstract

Purinergic receptors are the ones that use nucleotides as their agonists or antagonists. They have crucial role in platelets. Their physiological role as well as pharmacological target for antiplatelet drugs are well established and they have immense applications in clinical practice. But today the same purinergic receptors over platelets were identified elsewhere and they have been implicated in various health conditions. So, this review focuses to explain the non-thrombotic uses of platelet purinergic receptors

Introduction:

Platelets are vital cells in our body homeostasis they play an important role in homeostasis which is well known. The coagulation or thrombotic effect caused by platelets is mainly mediated by platelet purinergic receptors namely P2Y1, P2Y12 and P2X1. P2Y1 and P2Y12 are G-protein coupled receptors but P2X1 is an adenosine gated nonselective cation channel [1]. P2Y12 activation causes platelet adenyl cyclase inhibition but in combination with P2Y1 it causes platelet aggregation [2]. Stimulation of P2X1 causes conformational changes in platelets and synergize the action mediated by former two receptors [3]. The role of these receptors in various other conditions have been identified. They are also identified in various other systems other than platelets. Their role apart from thrombotic and platelet related disorders are slowly getting broadened and they are being recognized as candidates in various disorders. So, the knowledge regarding their role in non-thrombotic diseases has to be given special emphasis and this could pave the way for sustained research in this area. This in turn can produce important discoveries that could ultimately serve as newer modalities in areas of diagnostics, drug discovery and therapeutics.

Platelets and Purinergic receptors:

The major class of receptors present over platelets is the purinergic receptors. They are of three subtypes P2Y1, P2Y12 and P2X1. The first two are G-Protein coupled receptors and the last one is Adenosine gated ion channel receptor. ADP is the physiological activator of platelets that mediate its effects via these receptors. P2Y1 is Gq coupled that act through phospholipase c system and P2Y12 is Gαi coupled that act by adenyl cyclase system [4]. P2X1 acts via influx of calcium functionally they produce only conformational changes in the platelets along with synergistic action with other 2 receptors [5]. There are several drugs that target these receptors. P2Y12 is the major receptor involved in activation of GPIIb/IIIa receptors This is blocked by a group of drugs called non-thienopyridines namely elinogrel, ticagrelor and cangrelor [6]. Other than these there are various drugs that target various other pathways of platelet aggregation, one among them is the class called ADP inhibitors. They act by inhibiting ADP which is the physiological activator of platelets. The drugs include clopidogrel and prasugrel [6]. So, the purinergic receptors not only play an important role in physiological homeostasis of platelets but also serve as important drug targets for treating various life threatening and common diseases. These antiplatelet drugs are one of the most common drugs used in clinical practice. These antiplatelet drugs are used in prophylaxis of various hypercoagulable states

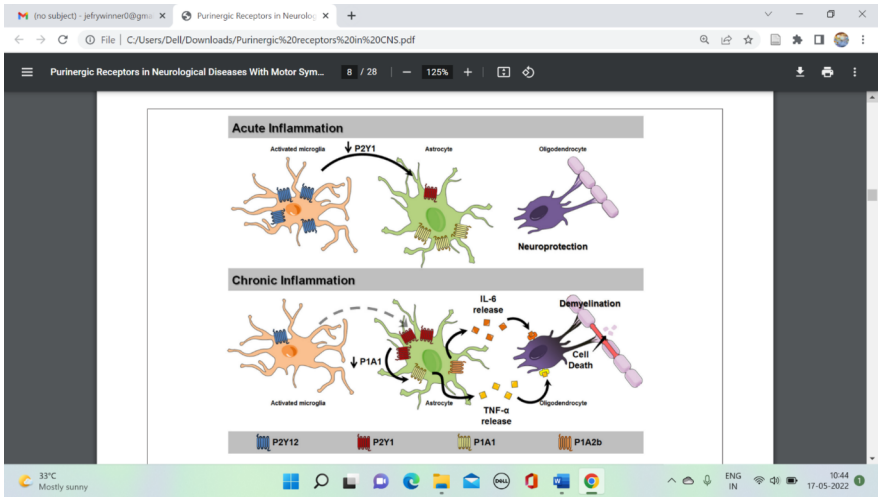
including Myocardial infarction and atherosclerosis. Clopidogrel is used along with atorvastatin and aspirin as loading dose in Myocardial infarction. This strategy is known to reduce the development of reinfarction substantially and provides benefits in patients with MI undoubtedly [7].

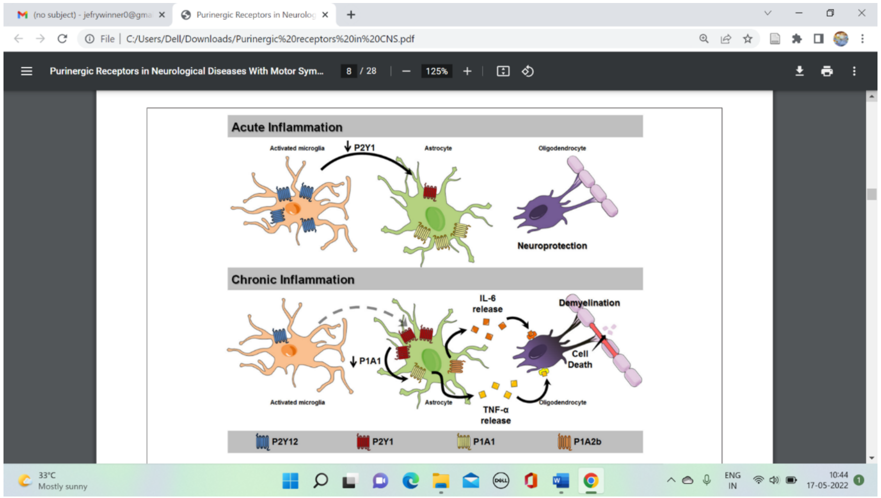
Platelet Purinergic receptors in CNS:

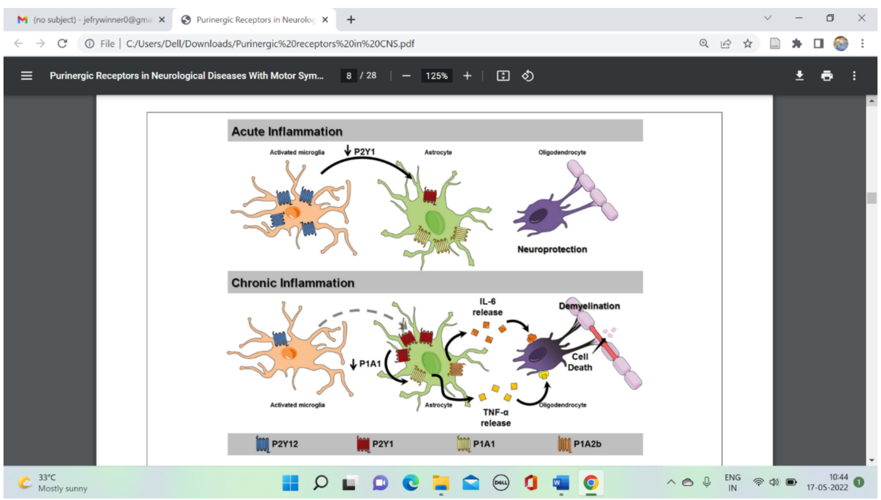
There are numerous types of purinergic receptors that constitute the so called purinergic system. They include the those in platelets that are P2Y1, P2Y12 and P2X1. They are in the initial stage of research. Some preclinical studies have shown effects in the early stages which are promising to carry them forward. P2Y12 receptors levels are associated with Multiple sclerosis. They were found to be lower in patients with MS near the demyelination areas [8]. This shows that there is some association between multiple sclerosis and its progression, but the nature or type of association has to be established and explained. Another study that involved Experimental Autoimmune Encephalomyelitis mouse models showed that the P2Y12 knockout mice developed severe EAE when compared to others [9]. This was proposed to be due to higher IL-23 release. Another reason given was due to imbalance between cytokines and Th cells (T-helper cells). These are the roles until now discovered for autoimmune and inflammatory diseases. Their role in neurodegenerative diseases is also well studied in preclinical models. The studies done for Parkinson's disease shows that P2X1 receptor is involved. Antagonism of this receptor leads to reduced α-synuclein in the brain of tested mouse models [10]. This effect was brought about in a dose dependent manner mediated by ATP inhibition. Other than these preclinical studies the purinergic receptors of other subtypes that is those that are non-platelet purinergic receptors have immense roles that have been studied in CNS disorders and these might influence the existing therapeutics in future including rare and fatal diseases. But the practicality of these can be established with only sustained research in future.

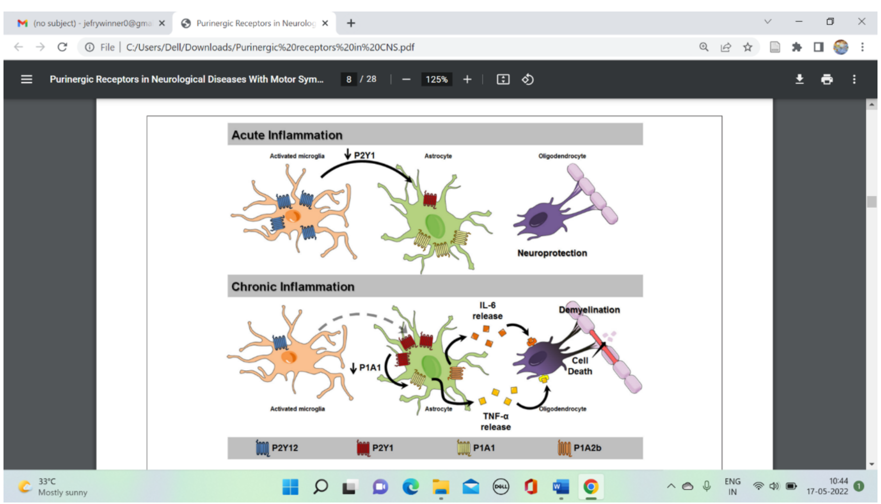
Figure 1:

A)Chronic inflammation B) Acute inflammation







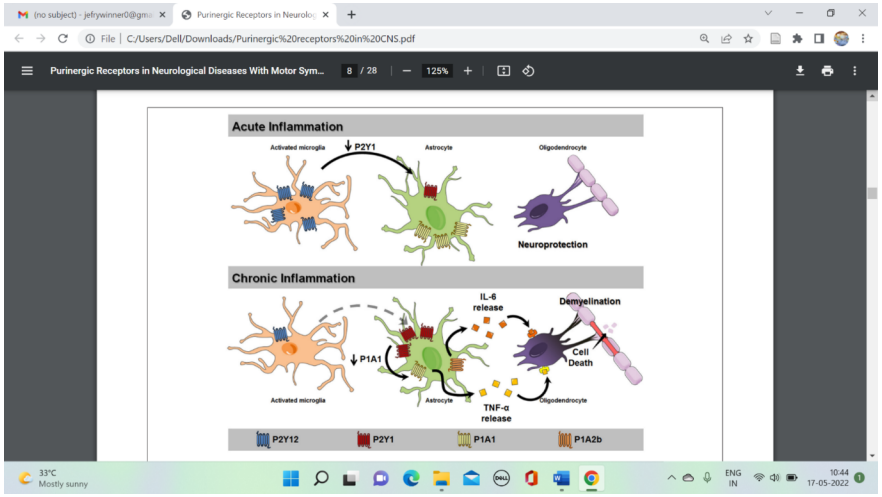


Proposed mechanism for glial purinergic dysfunction leading to loss of myelination and cell death. In acute inflammation scenarios, microglial activation upregulates P2Y12 receptor expression and activity (blue), stimulating microglial motility to the injury site. The activation of these receptors reduces P2Y1 receptor (red) expression in astrocytes, increasing reactive astrogliosis and promoting neuroprotection. Chronic inflammation, as observed in motor neuron diseases (MND), unable of upregulating microglial P2Y12 receptor expression results in constant astrocytic P2Y1 receptor activation and reduction of A1 receptor expression (yellow). These events result in stimulating tumour necrosis factor α (TNF-α) release, which in turn induces A2B receptor activation (orange) and release of IL-6. These detrimental factors induce oligodendrocyte death and neuron demyelination, aggravating the pathological scenario

Platelet Purinergic receptors in Inflammation:

After the advent in technological development with the various molecular biology techniques in place purinergic receptors were also identified in neutrophils apart from platelets [11]. In the studies conducted on this it was discovered that P2X1 in neutrophils causes activation of ROCK dependent MLC phosphorylation causing cytoskeletal deformation and also release of granules that help in neutrophil chemotaxis, Interestingly P2X1 deficiency also caused reduced NADPH oxidase activity [12]. In another study done in P2X1 knockout mice showed exaggerated inflammatory and ROS production when compared with controls after inflammatory ex vivo stimuli. This shows that they might have a role in negative feedback regulation of inflammation [13]. In agreement with this finding intraperitoneal injection of LPS shows increased MPO activity which is a marker of neutrophil activation [13]. This was also comparatively greater in P2X1 knockout mice to control mice. There was also an exaggerated drop in platelet and neutrophil count in case of P2X1 knockout mice when compared with controls. Again, lethality following LPS injection was also higher in the former compared to latter. These findings suggest that there could be a crucial role for P2X1 receptors in neutrophils that has to be further studied and explored. These might become drug targets for anti-inflammatory drugs in future with sustained good quality research.

Figure 2:



A role for platelet and neutrophil P2X1 receptors in thrombosis. Experimental data in mice indicate that activation of P2X1 receptors by extracellular ATP acts to maintain circulating neutrophil in a quiescent state (1), recruit neutrophil at the site of endothelial injury (2), and activate adhered neutrophils (3) and platelets (4), thereby promoting thrombus growth and fibrin generation. TF: tissue factor, ROS: reactive oxygen species.

Platelet Purinergic receptors in CVS diseases:

The role of purinergic receptors in heart is very well interlinked with platelets. These are discovered decades ago and they act as successful therapeutic targets for diseases like MI. These protective effects are mainly dependent on the drug actin over platelet purinergic receptors. But today studies and research has brought to light the various locations and roles of these platelet receptors other than the conventional thrombosis related pathologies.

Hypertension:

This is a very common disorder with most common being idiopathic and has good medications to combat it. Still there are cases who show poor response to existing antihypertensives. So, with the knowledge from preclinical studies showing the role of purinergic signaling via P2X1 receptors. ATP that acts as a co-transmitter with nor-adrenaline causes vasoconstriction of arteries in rats which could be reversed by guanethidine a drug that was proposed to act by ATP inhibitory effects [14, 15]. Another study in Spontaneously hypertensive rats (SHR) showed inhibition of tail arteries in rats by desensitization of P2X1 receptors. Again, P2Y1 receptors also play an important role in intimal proliferation of blood vessels and the drugs that antagonize these receptors are potential candidates to prevent neointimal formation [16]. These studies suggest that purinergic platelet receptors in blood vessels could be potential targets for drug discovery in treatment of hypertension and its complications.

Ischemic diseases of heart:

Ischemia is a major concern to almost all organs and heart is not an exception. The heart pumps the blood to whole body but the amount of blood it receives is very merger when compared to other organs. This accounts for only 4 to 5% of the total blood volume and major part of perfusion occurs during diastole. These factors make the heart vulnerable to ischemia. One of the most common causes of ischemia is MI. It ranges from milder form like Angina where ischemia is almost always reversible to the severe form MI were there is infarction due to ischemia. A newer concept in this field is the role of purines and pyrimidines released at the site of injury. These were initially thought to be detrimental but later on some studies also showed protective effect. ATP plays a detrimental role in Ischemic injury. P2Y12 receptor antagonists play a role in prevention of ischemic stroke and MI [17]. So, this might play a role in development of future therapeutics other than the existing ones.

Calcific Aortic valve disease:

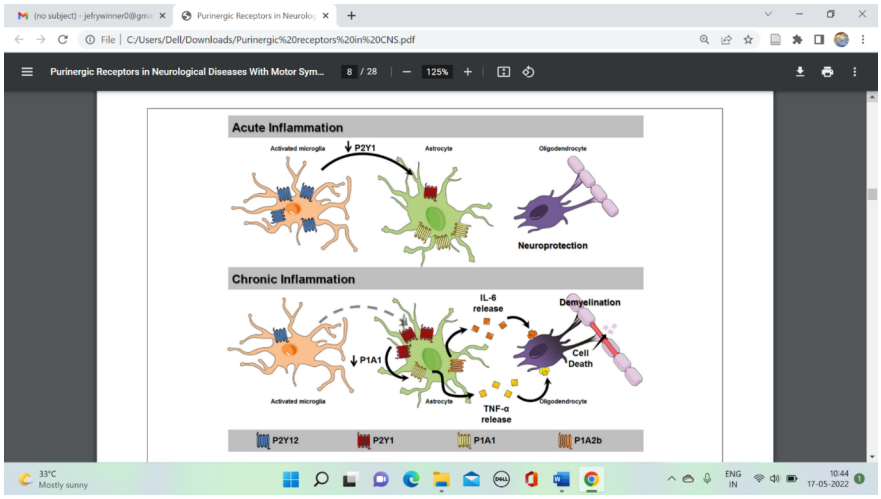
Valvular diseases of heart are another major concern in all parts of the world which means both the developing and developed world. The etiologies might vary but still they possess a major health related issue. P2Y2 receptors are known to play an important role in this. They are required for the normal survival of valvular tissue [18]. A high level of membrane-bound ecto-nucleotidase pyrophosphatase 1 present in the calcific aortic valve is responsible for the further progression of the disease. Treating the rats that are given warfarin with ARL67156 is found be effective in preventing the development of calcification of aortic valve. This is a commercially available nucleotide analog and CD39 inhibitor [19]. These studies suggest that the purinergic receptors or the nucleotides could be exploited for development of future therapeutics that could help in arresting the progress of calcification of aortic valve. This will be a great achievement in the field of therapeutics as only surgical and minimally invasive techniques are the mainstay in treatment with conflicting results.

Platelet Purinergic receptors in wound healing:

Any injury to our body is healed by the means of various intricate mechanisms and complex processes. Wound healing is a part of our body's normal homeostasis. Recent studies show there is a massive release of ATP and UTP in case of inflammation in an area of injury and also expression of platelet purinergic receptors namely P2Y1, P2Y12 and P2X1[20]. These are also present in normal human keratinocytes that are identified by in situ hybridization technique. Mast cells also play a major role in tissue healing and homeostasis. But these cells express a vast heterogeneity depending upon their location. Mast cells from lungs show P2X7 expression in higher amounts. But the activated human cord blood mast cells (CBMCs) are

rich in P2X1, P2Y1 and P2Y12 [21]. RT-PCR analysis of mRNA from endothelium-denuded human coronary arteries has demonstrated strong bands for P2Y2 and P2X1, although bands for P2Y1, P2Y4, and P2Y6 receptor mRNA could also be detected [22]. Endothelial cells, vascular smooth muscle cells, and fibroblasts are the structural elements of the blood vessel walls. Blood vessel regeneration is an important component of normal wound healing. This process occurs through both angiogenesis and vasculogenesis. Angiogenesis assays using Human Umbilical vein Endothelial Cells (HUVEC) have shown the expression of P2X4 in higher levels [23]. All cell types involved in wound healing differentially express P2X and P2Y receptors, and the receptor expression patterns vary between cellular types. Moreover, the differential expression of nucleotide receptor subtypes contributes to tissue cell heterogeneity which might be responsible for inducing a variety of responses by nucleotides present in the site of wounding. These studies are yet to prove any functional implications of purinergic receptors. These information with sustained research in future might revolutionize the therapeutics in an optimistic way it might even lead to development of successful antifibrotic agents as well.

Figure 3: Cellular events during different stages of wound healing



Conclusion:

The role of purinergic receptors at varied systems in our body is implicated in numerous physiological and pathological roles. This is in the stage of budding research that includes invitro and some preclinical studies. The roles of various receptors and their implications in various diseases has to be extensively researched and reported. Such good scientific works could throw more light in this topic. This review has exclusively focused on the platelet purinergic receptors and their role in other diseases apart from their role in platelets which is briefly touched upon. This new topic has been reviewed with the intent to provoke interest in the minds of young researchers to take this topic as an advanced research puzzle and try to fit in the pieces. This puzzle once solved might undoubtedly improve our understanding of physiology, pathogenesis which in turn could help in drug discovery and development and also aid in diagnostics and therapeutics which is our ultimate goal in any health research. Let's hope for the best here.

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References:

1. Daniel JL, Dangelmaier C, Jin J, Ashby B, Smith JB, Kunapuli SP. Molecular basis for ADP-induced platelet activation. I. Evidence for three distinct ADP receptors on human platelets. J Biol Chem. 1998; 273: 2024–9.

2. Jin J, Kunapuli SP. Coactivation of two different G protein-coupled receptors is essential for ADPinduced platelet aggregation. Proc Natl Acad Sci U S A. 1998; 95: 8070–74.

3. Kunapuli SP. P2 receptors and platelet activation. ScientificWorldJournal. 2002; 2: 424–33.

4. Kunapuli SP, Dorsam RT, Kim S, Quinton TM. Platelet purinergic receptors. Curr Opin Pharmacol. 2003; 3: 175–80.

5. Vial C, Rolf MG, Mahaut-Smith MP, Evans RJ. A study of P2X1 receptor function in murine megakaryocytes and human platelets reveals synergy with P2Y receptors. Br J Pharmacol. 2002; 135: 363–72.

6. Meadows TA, Bhatt DL. Clinical aspects of platelet inhibitors and thrombus formation. Circ Res. 2007;100(9):1261–75.

7. Patti, Giuseppe, Giuseppe Colonna, Vincenzo Pasceri, Leonardo Lassandro Pepe, Antonio Montinaro et al "Randomized Trial of High Loading Dose of Clopidogrel for Reduction of Periprocedural Myocardial Infarction in Patients Undergoing Coronary Intervention." *Circulation* 111, 16 (2005): 2099–106.

8. Amadio, S., Montilli, C., Magliozzi, R., Bernardi, G., Reynolds, R., and Volonté,C. (2010). P2Y12 receptor protein in cortical gray matter lesions in multiple sclerosis. Cereb. Cortex 20, 1263–73.

9. Zhang, J., Li, Z., Hu, X., Su, Q., He, C., Liu, J., et al. (2017). Knockout of P2Y 12 aggravates experimental autoimmune encephalomyelitis in mice via increasing of IL-23 production and Th17 cell differentiation by dendritic cells. Brain Behav Immun. 62, 245–55.

10. Gan, M., Moussaud, S., Jiang, P., and McLean, P. J. (2015). Extracellular ATP induces intracellular alpha-synuclein accumulation via P2X1 receptormediated lysosomal dysfunction. Neurobiol. Aging 36, 1209–20.

11. Lecut C, Frederix K, Johnson DM, Deroanne C, Thiry M, et al. P2X1 ion channels promote neutrophil chemotaxis through rho kinase activation. J Immunol 2009;183: 2801–9.

12. Lecut C, Faccinetto C, Delierneux C, van Oerle R, Spronk HM, et al. ATP-gated P2X1 ion channels protect against endotoxemia by dampening neutrophil activation. J Thromb Haemost 2012;10:453–65

13. Oury C, Lecut C, Hego A, Wéra O, Delierneux C. Purinergic control of inflammation and thrombosis: Role of P2X1 receptors. Comput Struct Biotechnol J. 2014 Nov 28;13:106-10.

14. 155. Brock JA, Van Helden DF. Enhanced excitatory junction potentials in mesenteric arteries from spontaneously hypertensive rats. Pflugers Arch. 1995; 430: 901–8.

15. Rodrigues JQ, da Silva ED Jr, de Magalhães Galvão K, MirandaFerreira R, Caricati-Neto A, Jurkiewicz NH, Garcia AG, Jurkiewicz A. Differential regulation of atrial contraction by P1 and P2 purinoceptors in normotensive and spontaneously hypertensive rats. Hypertens Res. 2014; 37:210–19.

16. Liu R, Ma S, Lu Z, Shen H, Sun L, Wei M. The ADP antagonist MRS2179 regulates the phenotype of smooth muscle cells to limit intimal hyperplasia. Cardiovasc Drugs Ther. 2015; 29: 23–9.

17. Liu F, Tantry US, Gurbel PA. P2Y12 receptor inhibitors for secondary prevention of ischemic stroke. Expert Opin Pharmacother. 2015; 16: 1149–65.

18. Côté N, El Husseini D, Pépin A, et al. ATP acts as a survival signal and prevents the mineralization of aortic valve. J Mol Cell Cardiol. 2012;5

19. Schäkel, Laura, Constanze C. Schmies, Riham M. Idris, Xihuan Luo, Sang-Yong Lee, Vittoria Lopez et al. "Nucleotide Analog ARL67156 as a Lead Structure for the Development of CD39 and Dual CD39/CD73 Ectonucleotidase Inhibitors." *Frontiers in Pharmacology* 11 (2020). 2:1191–202.

20. Inoue K, Hosoi J, Denda M (2007) Extracellular ATP has stimulatory effects on the expression and release of IL-6 via purinergic receptors in normal human epidermal keratinocytes. J Invest Dermatol 127:362–71

21. Bradding P, Okayama Y, Kambe N (2003) Ion channel gene expression in human lung, skin, and cord blood-derived mast cells. J Leukoc Biol 73:614–20

22. Malmsjo M, Hou M, Harden TK, Pendergast W, Pantev E, Edvinsson L et al (2000) Characterization of contractile P2 receptors in human coronary arteries by use of the stable pyrimidine's uridine 5'-O-thiodiphosphate and uridine 5'-O-3- thiotriphosphate. J Pharmacol Exp Ther 293:755–60

23. Yamamoto K, Korenaga R, Kamiya A, Qi Z, Sokabe M, Ando J (2000) P2X4 receptors mediate ATP-induced calcium influx in human vascular endothelial cells. Am J Physiol Heart Circ Physiol 279:H285–H92