Chapter

Crosstalk between the Purinergic and Immune Systems: Implications for the Glutathione Antioxidant System in Health and Disease

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Abstract

Glutathione (GSH) represents the major nonprotein thiol in cells and, alongside with glutathione-dependent enzymes such as glutathione reductase (GR), glutathione peroxidase (GPx), and glutathione S-transferase (GST), exerts several biological functions including the protection against free radicals and other essential metabolic reactions within the body. Disturbances in the homeostasis of this complex glutathione antioxidant system may damage cells and have been implicated with the development and progression of several human diseases. In this context, the immune and purinergic systems are also essential, since the dysregulation in both systems may also be correlated with numerous diseases. These two networks are closely related and control inflammatory responses, especially by the crosstalk of signaling molecules, receptors, and enzymes; thus, they can exacerbate or slow down the progression of diseases. Based on this background, we aimed to provide a general scenario of the purinergic and immune systems and the connection between both and the modulation of glutathione and glutathione-dependent enzyme expression and activity in the context of health and disease.

Keywords: ectonucleotidases, receptors, cytokines, inflammation, reactive species, oxidative stress, signaling, activity, metabolism

1. Introduction

Glutathione (GSH) represents the major nonprotein thiol in cells and exerts numerous biological functions including the defense against reactive oxygen species and reactive nitrogen species (ROS and RNS, respectively). Moreover, GSH participates in countless cellular and metabolic processes in the body, and, thus, changes in its homeostasis can cause irreversible cellular damage and influence the etiology and evolution of several human diseases, such as cardiovascular, inflammatory, neurodegenerative, and metabolic diseases and cancers, among others [1, 2].

However, in addition to the direct action of GSH, a second line of defense against oxidation is carried out by glutathione-dependent enzymes that counteract the negative effects of free radicals. These enzymes are (a) glutathione reductase (GR), which regenerates reduced GSH from its oxidized form, glutathione disulfide (GSSG); (b) glutathione peroxidase (GPx), which detoxifies the cell from organic and inorganic peroxides; and (c) glutathione S-transferase (GST), which catalyzes the conjugation of GSH with diverse compounds that are produced in the presence of oxidative stress detoxification [3].

Oxidative stress and inflammation are closely linked, and cells elicit antioxidant defenses against free radicals, as well as pathogens, and other foreign substances by activating immune responses [4]. This results in a sophisticated interaction between immune system cells and several molecules released by them to defend the organism against microorganisms or damaged cells from injured tissues and maintain tissue homeostasis [5]. Nevertheless, when the inflammatory responses are exacerbated and the mechanisms of homeostatic control do not work properly, this may trigger further tissue damage which is associated with several diseases [6]. Furthermore, purinergic signaling, comprised by an intricate network of receptors, enzymes, and signaling molecules, has been shown to participate in numerous cellular functions in the context of health and disease, especially immunomodulatory functions, since the components of the purinergic system are widely expressed in immune cells of several tissues [7–9].

In this chapter, we provide a general scenario of the purinergic and immune systems and how they interplay by modulating glutathione and glutathione-antioxidant enzymes in the context of health and disease.

2. Purinergic system

The formulation of a purinergic neurotransmission hypothesis was firstly proposed by Geoffrey Burnstock back in 1972 [10]. Burnstock, in his search for answers about "what molecule could be the transmitter released during non-cholinergic/non-adrenergic inhibitory transmission in the gut," suggested that perhaps adenosine triphosphate (ATP) could fill the criteria based on the following conditions needed by a neurotransmitter: (a) the substance must be present within the presynaptic neuron; (b) it must be inactivated by ectoenzymes and/or neuronal uptake; (c) it must be released by a Ca^{2+}-dependent mechanism; and (d) specific receptors for the substance must be present on the postsynaptic cell [10–12]. Although some other researchers had already highlighted the role of purines in blood vessels and the heart, and the action of ATP in the autonomic ganglia, the ATP molecule had its role as a neurotransmitter discredited in the beginning [11].

Nowadays, however, the existence of a purinergic signaling system is well-accepted and widely studied because its constituents are found in all tissues of the body and associated with immune, nervous, cardiac, hepatic, renal, metabolic, and digestive functions, among others. Besides, the purinergic system shows all the criteria needed for ATP to be considered a neurotransmitter. In the following sections, the purinergic system components will be discussed in more detail.

2.1 Nucleotides and nucleosides

Nucleotides have three characteristic components: a nitrogenous base (containing nitrogen), a pentose (sugar), and one or more phosphates. The molecule without the phosphate group is called nucleoside. Nitrogen bases are derived from two related compounds, pyrimidine and purine. The purine bases are adenine

(A) and guanine (G), and the pyrimidine bases are cytosine (C), thymine (T), and uracil (U). Nucleotides and nucleosides have important roles described in the literature such as (a) energy currency in metabolic reactions; (b) chemical bonds in cellular responses to hormones and other extracellular stimuli; (c) components of an ordered structure of enzymatic cofactors and metabolic intermediates; and (d) components in DNA and RNA structures. However, although these are the most known properties, purine and pyrimidine nucleotides have other signaling functions described below [13].

The biological properties of pyrimidine and purine nucleotides and nucleosides are mainly linked with their binding (or not) to specific receptors. Uridine nucleotide is known by its action in the metabolism of carbohydrates as uridine diphosphate (UDP)-glucose and glycogen synthesis. Besides, cUMP is related alongside with cCMP as intracellular second messengers. Cytidine is known to form cCMP, which has been associated with the control of cell growth and blood cell function; however, the intracellular signal transduction pathways are not well-defined [14]. Some authors proposed that its mechanism is related to the use of the cGMP signal transduction pathway [15]. Furthermore, cytidine (as cytidine triphosphate (CTP)) and uridine (which is converted to uridine triphosphate UTP and then to CTP) contribute to brain phosphatidylcholine and phosphatidylethanolamine synthesis [16]. Thymidine and its associated nucleotides have a role as modulators of active anticancer drugs, especially antimetabolites [17].

Guanosine and adenine nucleotides are the most commonly known. For example, GTP and cGMP are associated with intracellular signaling in physiological events of hormonal regulation; GTP and ATP (as well as CTP) are involved with the regulation of allosteric enzymes [18]. It is known that the suppression of GTP concentrations could be related to the invasion of melanoma cells and cells from other cancer types [19, 20]. Moreover, guanosine nucleotides have a role in immune response, cardioprotection, and memory formation, among others [18, 21, 22]. Adenine nucleotides, besides being part of the energy metabolism (mainly ATP), are also neurotransmitters or signaling molecules that act in the control of cellular responses, for example: (a) While ADP stimulates platelet aggregation, adenosine (Ado) inhibits this process; (b) While ATP is an excitatory neurotransmitter in the central nervous system (CNS), Ado acts in neuroprotection; (c) ATP is a pro-inflammatory molecule known as a damage-associated molecular pattern (DAMP); (d) On the other hand, Ado is an anti-inflammatory molecule. Besides, Ado nucleotides are found in all tissues of the body presenting many cellular modulatory effects [23–25].

2.2 Purinergic receptors

ATP, ADP, UTP, and UDP bind to P2 receptors, while Ado binds to P1 receptors (**Figure 1**). P2 receptors are subdivided into P2X and P2Y families [26]. P2Y receptors are metabotropic G protein-coupled receptors (GPCRs), and its eight subtypes can be divided into two groups, depending on the type of G protein-coupled receptor: P2Y1, P2Y2, P2Y4, and P2Y6 are Gq protein-coupled receptors and activate the protein phospholipase Cβ, while P2Y12, P2Y13, and P2Y14 are Gi protein-coupled receptors which act by inhibiting adenylate cyclase. P2Y11 is a peculiar receptor because it is coupled to Gq and Gs and, thus, causes an increase in the intracellular levels of 3′,5′-cyclic adenosine monophosphate (cAMP) and Ca^{2+} [27, 28].

GPCRs are the largest and most diverse group of membrane receptors. The activation of a single G protein can affect the production of second messenger molecules such as cyclic AMP, diacylglycerol (DAG), and inositol 1,4,5-trisphosphate (IP3). Furthermore, there are mainly three subtypes of G proteins, Gs, Gi,

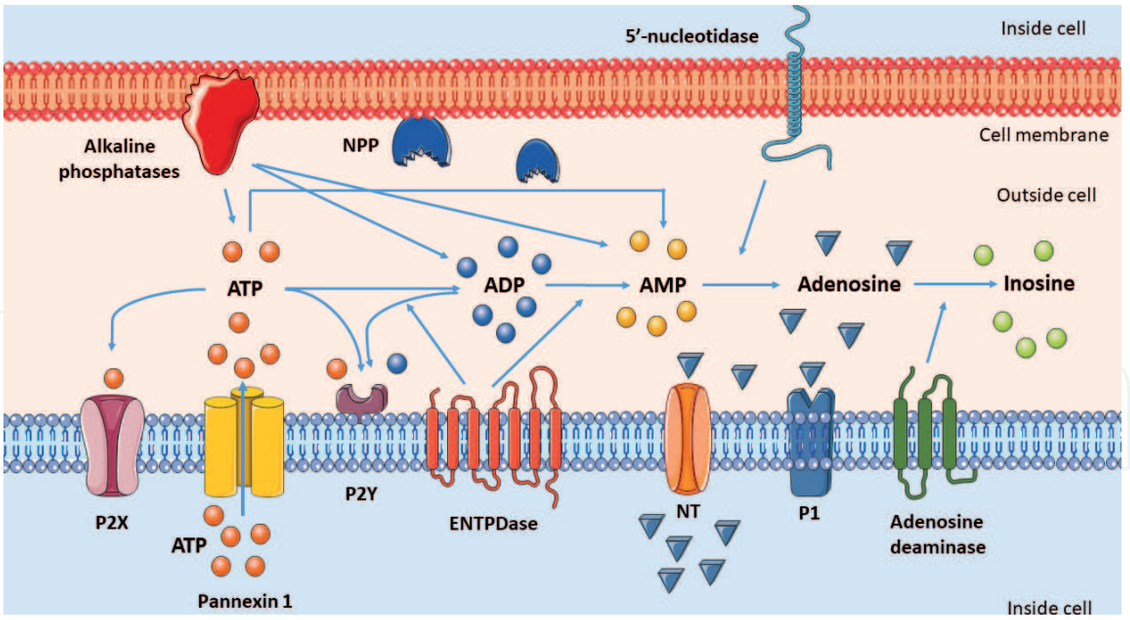

Figure 1.
Purinergic system components. P1 and P2 receptors, enzymes: ENTPDase, alkaline phosphate, NPP, 5′-nucleotidase, and adenosine deaminase. Nucleotides and adenosine. Nucleoside transporter (NT) and pannexin 1 (channel is an integral component of the P2X/P2Y purinergic signaling). (Authors' artwork).

and Gq. The Gs (stimulatory) protein activates adenylate cyclase, which catalyzes the formation of cAMP from ATP, being involved in the signaling of many receptors such as glucagon, epinephrine, and calcitonin, among others. The Gq protein is involved in the activation of the phospholipase C (PLC) enzyme that participates in the formation of second messengers. Once activated, it degrades phosphatidylinositol 4,5-bisphosphate (PIP2) present in the membrane into IP3, and 1,2-DAG. Gp protein has important functions in the brain, such as neuronal transmission, synaptic plasticity, and neuronal survival. Taking this information into account, studies have shown that Gq protein plays an important role in the processes of neurodegeneration in Alzheimer's disease. The Gi (inhibitory) protein inhibits the activity of adenylate cyclase enzyme. The Gi isoform, related to the decrease in cellular response, is responsible for mediating the inhibitory effects of receptors [29, 30]. The biological effects mediated by PY2 purinergic receptors are associated with these types of G proteins.

P2X receptors are ionotropic receptors linked to channels in the plasma membrane; they have ATP as an agonist. There are seven different P2X receptor subtypes (P2X1–7) that form trimeric receptors [27]. The extracellular domain of these receptors contains binding sites for ATP, competitive antagonists, and modulatory metal ions. The transmembrane domains form a nonselective cation channel. The opening properties of ion channels differ greatly according to the receptor subtype: while the homomeric P2X2, P2X4, and P2X7 receptors exhibit slow desensitization, the P2X1 and P2X3 receptors exhibit rapid desensitization [31].

P1 receptors, which have Ado as an agonist, are divided into four subtypes, A1, A2A, A2B, and A3, and all are G protein-coupled receptors. The A1 receptor mediates signaling responses which may be caused by its coupling to different proteins within the Gi/o family. The known pathway of action of this receptor is through the inhibition of adenylate cyclase, which causes a decrease in cAMP. Moreover, the A1 receptor mechanism of action is through the activation of PLC, which leads to an increase in cytosolic calcium. In turn, A2A receptors are Gs protein-coupled receptors and activate adenylate cyclase. A2B receptor is coupled to different

signaling pathways, including guanylate cyclase activation, through PLC-mediated Gq coupling, and an increase in Ca^{2+} concentrations is dependent on IP3. A3 receptors are Gi protein-coupled and also Gq protein-coupled. A2B and A3 receptors are known as methylxanthine-insensitive receptors on the contrary of A1 and A2A receptors [26, 32, 33].

P1 receptors are widely distributed in metabolically active tissues, such as the pancreas, liver, and adipose tissues; they are also present in immune cells, indicating an important role in the regulation of immune system responses. The A1 receptor is an oxidative stress sensor, and it has shown to have pro- and anti-inflammatory effects as well as it is associated with the reduction of ischemic events [34, 35]. Moreover, A1 receptor activation has been shown during the initial phase of leukocyte recruitment, and the A2A receptor is expressed at the resolution phase [36]. On the other hand, the activation of the A2A receptor exacerbates neuronal damage as well as recruitment and activation of microglia in the CNS [35], although some anti-inflammatory effects by its stimulation in immune cells have been suggested [37]. A2B receptor has pro- and anti-inflammatory effects. Studies have demonstrated that A2B receptors stimulate pro-inflammatory cytokines like IL-4, IL-6, IL-8, IL-13, IL-19, and others; it has also been shown to activate human mast cells being involved in allergic and inflammatory disorders [33, 34]. A3 receptor is widely distributed in the immune tissue, and its functions are related to the release of allergic mediators including histamine by mast cells, suggesting a role in inflammation. In the brain, A3 and A1 receptors seem to be associated, as hippocampal A3 receptors have been shown to desensitize A1 receptors [38]. Moreover, A3 receptors were found to act in cardiovascular protection [33].

Both P1 and P2 receptors are located in several tissues, mainly the pancreas, vascular system, CNS, liver, kidney, and immune cells [39–42]. In the CNS, the expression of these receptors has been shown in many structures and proposed to be associated with the development of several pathologies, such as Alzheimer's disease [41, 43]. P2Y receptors, especially, are related to platelet function and thrombus promotion [42]. Platelets are known to express P2X1R, P2Y1R, and P2Y12R, where ADP signaling predominates through the activation of P2Y1R and P2Y12R, which is critical for initiating platelet aggregation [44, 45]. The P2X7 and P2X4 receptors are present in the kidney, and their expression can be increased in pathologies involving inflammatory processes of this tissue [46]. In the liver, the purinergic system is involved in the physiological regulation and also plays a role in the pathological processes of liver disease. Purinoceptors are also involved in bile secretion and glycogen and lipid metabolism. Moreover, the activation of P2Y1 receptors in human and rodent hepatocytes stimulates the glycogen phosphorylase enzyme [40].

It is important to highlight that when high concentrations of ATP bind to the P2X7 receptor, it can form pores in the membrane promoting inflammasome activation in macrophages and endothelial cells and, subsequently, promoting the release of cytokines, such as interleukin-1β (IL-1β), through a caspase-1-dependent process [47]. IL-1β is associated with autoinflammatory diseases as well as other inflammatory conditions such as hypoxia and hemorrhage. Furthermore, IL-1β causes a marked increase in the expansion of naïve and memory $CD4^+$ T cells in response to antigens and particularly when used with lipopolysaccharide (LPS) as a costimulant [48, 49].

Purinergic receptors, completely unknown 50 years ago, are nowadays widely studied as they participate in the modulation of many physiological processes and since their up- or downregulation is associated with many diseases. Besides, several nucleotides or nucleosides bind to these receptors and trigger their correspondent effects, inhibiting or stimulating downstream pathways. Furthermore,

there are also some enzymes whose function is to control the levels of these molecules, which will be presented below.

2.3 Ectonucleotidases

The ectonucleotidases are divided mainly into four gene families, which include pyrophosphate/phosphodiesterases (ENPPs), alkaline phosphatases, ectonucleoside triphosphate diphosphohydrolases (ENTPDases), and 5′-nucleotidases. ENPPs act on triphosphate nucleotide (ATP and UTP) hydrolysis into monophosphate nucleotides (AMP and UMP) and pyrophosphates. Seven enzymes are found in the ENPP family. Two isoforms are capable of hydrolyzing ATP, especially isoforms ENPP1 and ENPP3. Moreover, ENPP4 is involved in ADP hydrolysis in platelets, and the other isoenzymes hydrolyze phosphodiester bonds into phospholipids. Furthermore, ENPP1 is related to bone mineralization and tissue calcification and has been described for acting on insulin resistance in diabetic patients [50, 51]. NPP2 is expressed mainly in the brain, lung, kidney, endothelial cells, and also biological fluids, being associated with intracellular modulation through its binding to activated integrins on the target cells [52].

Alkaline phosphatases have a wide substrate specificity for different phosphomonoesters and other compounds containing phosphate, including adenine nucleotides, inorganic polyphosphates, and pyrophosphates. Three isoenzymes are tissue-specific and have 90–98% homology, which are the alkaline phosphatases of the intestine, the placenta, and those of germ cells. The last isoenzyme, tissue-non-specific alkaline phosphatase (TNAP), is approximately 50% identical to the others, and it is expressed mainly in the bones, liver, and kidney. TNAP is mostly known for its function in bone tissue mineralization [51, 53].

ENTPDases hydrolyze di- and triphosphate nucleotides into mononucleotides and inorganic phosphates. For their activity, they require Ca^{2+} and Mg^{2+} as cofactors. Eight different genes encode members of the ENTPDase family which differ in substrate specificity, cell location, and tissue distribution [53]. Four of them (NTPDases 1, 2, 3, and 8) are present on the extracellular surface of the membranes. NTPDases 5 and 6 exhibit cytoplasmic location, while NTPDases 4 and 7 are entirely located intracellularly, facing the lumen of cytoplasmic organelles [51]. Members of the membrane-bound NTPDase family show molecular masses of approximately 70–80 kDa, and they are proteins with glycosylated residues. They show sequence homology in special regions called “apyrase-conserved regions,” which are important for the catalytic activity. These enzymes may exist either in monomeric or in oligomeric states constituted by two transmembrane domains close to the amino and carboxyterminal groups [51, 54].

Concerning their catalytic activity, different isoenzymes have different substrates affinities. NTPDases 1 and 2 have a preference for hydrolyzing adenine nucleotides in the detriment of uracil nucleotides. All membrane-bound NTPDases hydrolyze ATP more quickly than ADP. NTPDase 1 is the enzyme that has more affinity for ATP; however, it hydrolyzes the ADP product to AMP in the same proportion [55]. NTPDase 2 has a great preference for ATP hydrolysis. NTPDases 3 and 8 hydrolyze ATP and UTP in a similar proportion [56]. Intracellular enzymes differ in substrate preference. NTPDases 4, 5, and 6 preferentially hydrolyze NTP and NDP, but to a lesser extent ATP and ADP. NTPDases 5 and 6 prefer to hydrolyze ATP, but not ADP, while NTPDase 7 preferentially hydrolyzes UTP, CTP, and GTP, but has a very low affinity for ATP [53, 57].

The family of NTPDases also differs in their tissue location. NTPDase 1 is mainly located in immune cells, for example, lymphocytes, monocytes, and blood vessel

endothelial cells, and in the CNS [58–60]. NTPDase 2 is also expressed in blood vessels and neuronal progenitor cells [53, 58]. Both NTPDases 1 and 2 are expressed in pancreas acinar cells. NTPDase 3 is mainly found in subsets of neurons, epithelial cells of the kidney, the upper respiratory system, and the digestive and reproductive systems [28, 61]. NTPDase 8 has a more restricted expression, being found in the liver, kidney, and intestine [62, 63]. Regarding the location of intracellular isoenzymes, they have a wider expression, due to their control of nucleotides inside the cell [54, 64]. For instance, in the CNS, different isoenzymes are expressed by neurons, astrocytes, and microglia [65]. Besides, this variation in isoenzymes may change according to distinct brain regions [60].

NTPDases can be coexpressed with another enzyme that continues with the nucleotide hydrolysis cascade, such as ecto-5′-nucleotidase (eN—CD73, E.C.3.1.3.5). eN is an enzyme anchored to the plasmatic membrane by glycophosphatidylinositol (GPI) with its catalytic site facing the extracellular medium, but it can also be found in the soluble form [53]. Mammalian eN consists of two glycoprotein subunits linked by non-covalent bonds. Zinc and other divalent metal ions bind to the end of the N-terminal domain. This ectoenzyme belongs to a large superfamily of metallophosphoesterases that act on different substrates, such as several nucleotides, serine/threonine phosphoproteins, and also sphingomyelins [66, 67].

eN is expressed in many tissues, being more abundant in the colon, kidney, and brain and less abundant in the liver, lung, and heart [68]. In the vascular system, eN is highly expressed in the endothelia and platelets. However, in immune cells, it is only present in some subpopulations of cells [67]. Besides, in the CNS, this enzyme can be found in different structures, including the cerebral cortex, hypothalamus, cerebellum, hippocampus, and olfactory bulb, among others [60].

Also, eN hydrolyzes ribo- and 5′-monophosphate deoxyribonucleotides to their respective nucleosides. Among these nucleotides, the prominent function of eN is the hydrolysis of AMP to Ado. According to Dunwiddie and Masino [69], Ado is considered a third "purinergic messenger." This nucleoside regulates many physiological processes, particularly in tissues involved with excitatory stimuli, such as the heart and the brain, by reducing their excitatory activity [69]. Ado effects are related to fluid transport, induced tolerance to ischemia and reperfusion in the cardiovascular system, immunity, and inflammation, among others [53].

Ado levels can be regulated by adenosine deaminase (ADA) enzyme activity, and in humans, two isoforms are expressed: ADA1 and ADA2. ADA1 is more relevant in the purinergic cascade because it catalyzes the irreversible Ado and 2′-deoxyadenosine deamination into inosine and 2′-deoxy-inosine, respectively. ADA1 is widely expressed in the intestine, thymus, spleen, and other lymphoid and nonlymphoid tissues; it is also involved in neurotransmission [70]. Moreover, liver, monocytes/macrophages, and serum also contain another isoenzyme, ADA2, which can be active at sites of inflammation during hypoxia and in areas of tumor growth [67]. Studies have shown that the ADA2 structure is precisely designed to act in the extracellular environment. ADA2 fits into the new family of adenosine deaminase-related growth factors (ADGFs), which play a role in tissue growth. Besides, Kaljas et al. [72], when analyzing $CD4^+$ T-cell subsets, showed that ADA2 particularly binds to regulatory T cells expressing CD39 and lacking the receptor for ADA1 [71, 72].

Understanding the regulatory mechanisms of purinergic signaling continues to be of great importance to several diseases since the overexpression or suppression of nucleotidase activities, receptor expression, and nucleotide/nucleoside levels are known to be involved in a variety of pathologies, including cancers and inflammatory, neurodegenerative, and cardiovascular diseases.

3. Involvement of purinergic signaling in immune responses

Immune responses are the result of a complex interaction between immune cells and several soluble factors, aimed to protect the host from the invasion by microorganisms or to eliminate apoptotic cells at sites of tissue injury, thus maintaining tissue homeostasis [5]. However, an intense inflammatory response, not properly balanced by endogenous mechanisms of homeostatic control, can lead to cell and tissue damage with the production of free radicals [6]. To avoid excessive oxidative stress, cells use different mechanisms to activate the immune system including antioxidant defenses and purinergic signaling [4]. It is worth mentioning that, since its discovery, purinergic signaling has been shown to mediate a wide range of functions in health and disease, especially immunomodulation and inflammation [9].

Immune cells recognize ATP, released from dying cells and damaged tissues, as a danger signal that elicits a variety of inflammatory responses. There is evidence that, following tissue injury, purinergic signaling response may be divided into three temporal phases [4]. First, an acute phase, when ATP is rapidly released into the extracellular space from damaged or stressed cells, accumulates to high levels and has chemotactic and excitatory effects on immune cells. Second, there is a decrease in the extracellular ratio of ATP/Ado responsible to limit the extent and duration of inflammation. Third, there is a chronic phase associated with a low extracellular ratio of ATP/Ado to promote tissue remodeling [4]. In the next sections, the functional role of purines in immune cell responses and the contribution of purinergic signaling to the mechanisms of inflammation will be highlighted.

3.1 How does ATP release promote inflammasome activation?

Necrotic and apoptotic cells release ATP, which works as a find-me signal to attract macrophages to phagocytose and remove dead or dying cells, a process that involves the activation of the NLRP3 inflammasome [73]. The NLRP3 inflammasome is a protein complex involved in IL-1β and IL-18 processing that senses a variety of signals including infection, tissue damage, and metabolic dysregulation [74]. The activation of the NLRP3 inflammasome results in the assembly of scaffold components: the cytoplasmic receptor NLRP3, the adaptor protein ASC, and the effector protein caspase-1. This association leads to the activation of caspase-1, allowing the processing of pro-IL-1β and pro-IL-18 to their mature and secreted forms which are biologically active. IL-1β production is a tightly controlled process playing a pivotal role in inflammation and the recruitment of neutrophils [75].

In pathological conditions, high levels of ATP (5 mM) are passively released from necrotic cells and act as a pro-inflammatory danger signal, activating the NLRP3 inflammasome through binding to the ionotropic P2X7 receptors [76]. Thus, the extracellular ATP (eATP) leads to K^+ efflux, membrane pore formation, and ROS-driven activation [77].

3.2 Purinergic receptors play a crucial role as stimuli for chemotaxis of inflammatory cells

Activation of purinergic receptors in immune cells can elicit either positive or negative feedback mechanisms and thus can tightly regulate immune responses [78]. All P1 and P2 receptor subtypes are expressed by immune cells, in a cell type- and differentiation-dependent manner (**Table 1**).

After an infection, leukocytes are programmed to exit the circulation and move toward epicenters of infection/inflammation, guided by chemical gradients of

Purinergic receptor	Ligand	Immune cell expression	Function
A1	Ado	Neutrophils and immature DCs	Chemotaxis
A2A	Ado	Most immune cells	Anti-inflammatory responses
A2B	Ado	Macrophages, DCs, and mast cells	Promotes IL-6 and VEGF release by macrophages and DCs, and drives mast cell degranulation
A3	Ado	Neutrophils and mast cells	Reduces neutrophil chemotaxis and stimulates mast cell degranulation
P2X7	ATP	$CD4^+$ T cells, $CD8^+$ T cells, Treg cells, iNKT cells, macrophages, and DCs	Activation of effector T cells, Treg cells, iNKT cells, monocytes, macrophages, and DCs
P2Y2	ATP/UTP	Phagocytes, DCs, monocytes, and lymphocytes	Chemotaxis and activation
P2Y6	UDP/UTP	Monocytes, macrophages, neutrophils, and lymphocytes	Activation

Adapted from Cekic et al. [4].

Table 1.
Principal purinergic receptors of immune cells: expression and functions.

different stimuli. Neutrophils are the most abundant leukocytes in the circulation, representing the first line of defense in the innate response. Neutrophils are characterized by a large phenotypic heterogeneity and functional versatility, placing these cells as important modulators of inflammatory responses [5]. Under adverse conditions, neutrophils release ATP via connexin or pannexin 1 hemichannels, and ATP undergoes rapid conversion to Ado via the CD39/CD73 axis expressed on the neutrophils surface [79].

Regarding P1 type of receptors, A1 and A3 receptors facilitate neutrophil chemotaxis, in part, by upregulating the neutrophil adhesion to tissue injury [80]. In particular, the stimulation of A1 receptors induces ROS production from activated neutrophils favoring bactericidal functions, whereas the activation of A2A receptors downregulates ROS generation [81]. Regarding P2 receptors, P2X1 receptors also mediate neutrophil chemotaxis negatively regulating systemic neutrophil activation, thereby limiting the oxidative response, coagulation, and organ damage [82]. However, P2Y11 receptors could retain the immune functions of neutrophils and reduce the injurious effects of increased neutrophil longevity during inflammation [83].

Phagocytes, such as macrophages, are innate immune cells that play an integral role in the defense of the host due to their ability to recognize, engulf, and kill pathogens while sending out danger signals via cytokines to recruit and activate inflammatory cells [84]. The P2X7 receptor has been suggested to play an important role in ATP-induced inflammation because it is mainly expressed on inflammatory cells. Furthermore, the role of P2X7 in the protection against neutrophil apoptosis has been reported as well as its association with the generation of ROS [85].

Some studies demonstrated the involvement of the P2X7 receptor in several responses of macrophages to danger, in particular the proinflammatory response mediated by IL-1β secretion, bacterial killing, and the associated macrophage death. ATP was shown to promote the maturation and release of IL-1β from macrophages, via P2X7 receptors [84]. Despite the dominant role of P2X7 in macrophages, evidence has supported the role of additional receptors. For example, the P2Y2 signaling on macrophages contributes to the clearance of apoptotic cells and also mediates the potentiation of prostaglandin E2 release involved in the induction

of nitric oxide synthase (NOS) [86]. On the other hand, A2B receptor activation by Ado was reported to inhibit tumor necrosis factor (TNF)-α expression from macrophages, whereas it potentiates NOS and interferon (IFN)-γ expression contributing to the inflammatory profile.

Like macrophages, dendritic cells (DCs) are professional antigen-presenting cells (APCs), whose main role is to activate adaptive immunity (second defense line), thereby maintaining immune homeostasis and tolerance [5]. DCs express almost all known P2 receptors; besides, extracellular nucleotides exert multiple effects on these cells ranging from chemotaxis to control of cytokine release and induction of cell death [87]. Mature DCs mainly express A2A and A2B receptors, which have pro-inflammatory effects on these cells [88–90].

Together, macrophages and DCs are APCs responsible for the cell-mediated immune response and interaction with T lymphocytes [5]. T cells recognize antigens through their T-cell receptors (TCR), located at the immune synapse, and physically interact with peptides that are presented on major histocompatibility complex (MHC) molecules by APCs. This immune interaction causes the activation of T-cell receptors on lymphocytes, therefore eliciting T-cell differentiation, cytokine production, and cytotoxic activity. Once activated, T cells orchestrate effector immune cell function by recruiting macrophages, neutrophils, eosinophils, and basophils to sites of infection and inflammation and by increasing the microbicidal activity and cytokine and chemokine production of these cells [8].

T cells express many members of the P2X, P2Y, and P1 receptor families, as well as the ENTPD1 ectonucleotidase. Purinergic signal amplification in T cells occurs mainly through P2X1, P2X4, and P2X7 receptors [8]. A2A receptors are the most important receptors in regulating lymphocyte activation, where the overall effect is suppressive [91]. A2A receptors inhibit both IL-4 and IFN-γ production by both naïve $CD4^+$ T cells and Th1 and Th2 cells.

However, ATP is known to boost the activation of T cells by amplifying the TCR-induced activation and by increasing IL-2 production by P2X1 and P2X4 receptors [92]. Thus, T cells promote strong positive purinergic feedback mechanisms, which are further amplified in the confined space of the synaptic cleft. Confinement of ATP in the immune synapse results in a powerful autocrine feedback mechanism that facilitates the signal amplification required for antigen recognition (**Figure 2**) [8].

3.3 Role of Ado as a regulator of immune responses

In general, Ado has opposite effects on inflammation compared to ATP, essentially acting as an anti-inflammatory molecule [23]. Ado, for instance, inhibits adhesion to endothelial cells, reduces superoxide anion production by neutrophils, and lowers the release of pro-inflammatory cytokines (**Figure 2**) [93]. Besides, Ado facilitates the release of IL-10, an anti-inflammatory cytokine, from monocytes [94]. Ado also induces the production of vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis and vascular permeability through its binding to the A2 receptors [93].

The role of Ado in regulating macrophage activation indicates that this molecule, by activating A2A, A2B, and A3 receptors, inhibits the production of several pro-inflammatory mediators such as TNF-α, IL-6, IL-12, nitric oxide (NO), and macrophage inflammatory protein (MIP)-1α by macrophages [95]. In parallel, extracellular Ado promotes the release of the anti-inflammatory cytokine IL-10 by monocytes and macrophages via A2A and A2B receptors exerting an anti-inflammatory effect. Moreover, Ado inhibits Th1 and Th2 differentiation by decreasing T-cell proliferation and IL-2 production [93, 95].

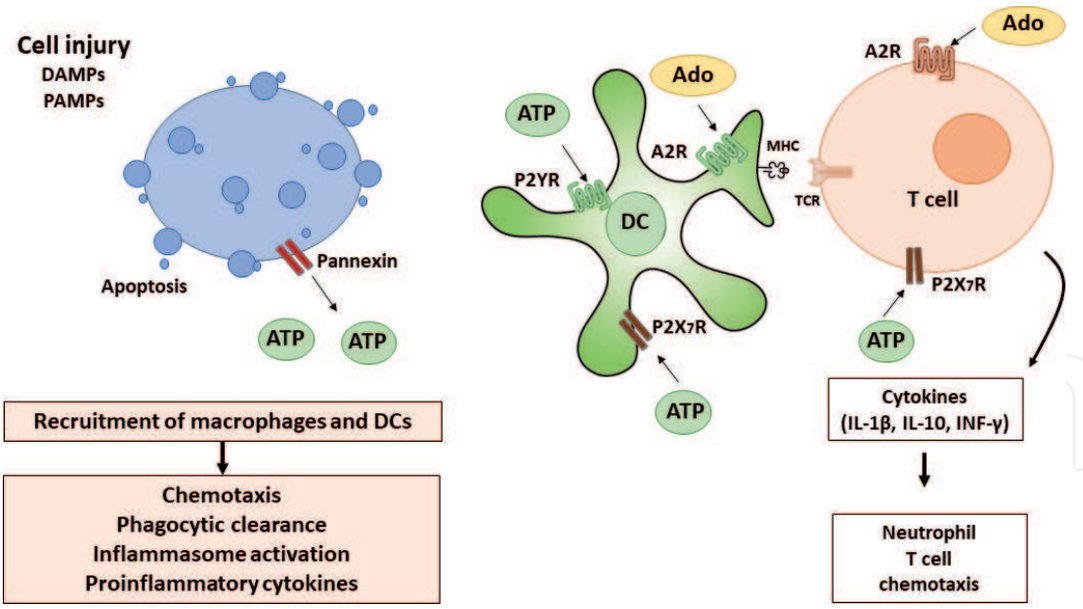

Figure 2.
Purinergic signaling during inflammation. After cell injury by DAMPs or pathogen-associated molecular patterns (PAMPs), apoptotic cells release ATP and other nucleotides by pannexin channels. ATP acts as "signal damage" for the recruitment of macrophages and DCs. Extracellular ATP ligates and activates the P2 purinergic receptors (P2Rs) and is then degraded by soluble and plasma membrane ectonucleotidases to generate ado, which acts at ado receptors (AR). DCs, as antigen-presenting cells, present antigens to the MHC of TCR, which promote the release of cytokines such as interleukin-1β (IL-1β), interleukin-10 (IL-10), and interferon-gamma (INF-γ), which then recruit neutrophil and T lymphocytes to injured tissues. Ado binds to A2 receptors and suppresses inflammation. (Authors' artwork).

As described above, inflammation appears to be oppositely regulated by extracellular ATP and Ado. At the initiation of inflammation, there are high levels of ATP, produced by damaged and stressed cells. High ATP levels promote the rapid migration of dendritic cells and macrophages through the activity of pannexin 1 channels and P1 and P2 receptors that trigger NLRP3 inflammasome activation.

Phagocytes and lymphocytes are recruited by chemoattractants and danger signals released from inflamed sites upregulating phagocytosis and other phagocyte-killing mechanisms, resulting in the clearance of the dying cells. At the end of the inflammatory process, increasing levels of Ado are induced by the breakdown of ATP by the ATP-dephosphorylating enzymes and the production of Ado by cells at the inflammatory site. This results in increased Ado levels and, consequently, inhibits the inflammatory processes.

Therefore, purinergic signaling represents the result of the activity of a complex and heterogeneous "molecular machinery" comprising nucleotide/nucleoside molecules, plasma membrane P1 and P2 receptors, and nucleotide-degrading enzymes, such as CD39 and CD73, cooperating in the inflammatory microenvironment and protecting tissues, particularly from immune-mediated excessive tissue damage.

4. The interplay of the purinergic and immune systems in the modulation of glutathione antioxidant enzymes

Besides knowing the importance of GPx, GST, and GR in several diseases, studies have drawn specific attention to the relationship between oxidative stress and purinergic signaling. Therefore, the connection between the activity and expression of glutathione antioxidant enzymes with the purinergic system is highlighted. Although many studies showed the relationship between the purinergic system and

oxidative stress, they did not directly assess the activity of glutathione-dependent enzymes. In this sense, the outcomes in glutathione and purinergic system modulation of some studies addressing different human diseases were summarized in **Table 2**.

One of the studies that addressed the direct relationship between GPx and the purinergic system showed that Ado administration upregulated GPx-1 expression and activity in endothelial cells [96]. It has been shown that polymorphisms in GPx and GST enzyme genes are related to increased risk of developing coronary heart disease and stroke and is associated with elevated inflammatory markers and increased risk of coronary heart disease in smokers, respectively [97]. Moreover, in another study, the administration of an Ado receptor agonist increased GPx and GR activities in the heart of rats, and the treatment with an Ado receptor antagonist blocked this augmentation, confirming the effect of Ado on glutathione antioxidant enzymes [98]. As the generation and release of Ado can increase during acute myocardial ischemia, its high concentrations may be sufficient to induce GPx-1 expression, which results in enhanced cellular tolerance to reactive species (RS) and contributes to the cardioprotective role of Ado [96]. In agreement with this, a study performed with acute myocardial infarction patients revealed an increase in NTPDase activity, with enhanced hydrolysis of adenine nucleotides, which promotes an increased Ado generation [99].

Concerning metabolic diseases, GSH levels in erythrocytes and blood plasma were observed to be lower in patients with diabetes or metabolic syndrome (MetS), and, consequently, the reduced GSH levels potentiate the effects caused by RS [1]. Recent studies have also demonstrated the depletion of the GPx and GST enzymes in the liver [100] and heart [101] from rats with MetS. Additionally, Martins et al. [102] revealed that subjects with MetS present an increase in NTPDase, 5′-nucleotidase, and NPP activities while decreased ADA activity in platelets. Moreover, an increase in ATP and ADP hydrolysis and a decrease in Ado deamination in lymphocytes of MetS patients were observed [103]. Further, Madec et al. [104] showed an increase in the P2X7 purinergic receptor in human adipocytes, which modulates the release of inflammatory cytokines and might contribute to the subclinical inflammatory status found and conferring increased cardiovascular risk.

In the same way, Cardoso et al. [105] showed an increase in the nucleotide hydrolysis, indicating an augment in NTPDase, 5′-nucleotidase, and ADA activities in platelets from hypertensive rats, suggesting that hypertension increases Ado generation, which acts through A2A receptors. Recently, an increase in NTPDase 1, NTPDase 3, and CD73 expression and activity in the cortex and in A2A expression in the hippocampus and cortex in hypertensive rats was also demonstrated [106]. Additionally, it has been shown that animals with hypertension induced by 1,3-dipropyl-8-sulfophenylxanthine (DPSPX), an antagonist of Ado receptors, present a redox dysfunction in the initial phase of hypertension, which may be explained by the blockade of Ado's protective effects and increased generation of RS. With the interruption of DPSXP administration, Ado seems to be involved in the adaptive response to enhance the activity of vascular antioxidant enzymes, such as GPx to counteract the increase in RS generation [107].

The purinergic network emerges as a central player in pathophysiological conditions particularly linked to immune system regulation including diabetes. It has been demonstrated that Ado affects insulin secretion, glucose homeostasis, and lipid metabolism through the activation of four Ado receptors [108]. In this context, studies demonstrated that activities of enzymes that hydrolyze adenine nucleotides and nucleosides were changed in diabetic rats [109, 110]. Moreover, the administration of an Ado receptor agonist in diabetic rats caused a decrease in the plasma glucose concentration and a decrease in medullary and cortical

hydrogen peroxide production, which was associated with a proportional increase in GPx activity, illustrating that the activation of Ado receptors may improve renal antioxidant capacity and glucose metabolism in diabetic rats. A review delineated a central role of purines, their receptors, and enzymes in diabetes by demonstrating that the manipulation of the purinergic axis at different levels can prevent or exacerbate the development and evolution of both type 1 and type 2 diabetes [111].

In the same line, a study with obese rats showed a decrease in GPx and GST activities, besides a decrease in GSH levels in hepatic and renal tissues. The decrease in these enzymatic activities may be due to their rapid consumption and exhaustion of stored GSH levels in fighting RS generated during the development of obesity, which possibly contributes to the progression of obesity-related problems [112].

Purinergic signaling can be exploited in the development of novel therapeutic approaches to treat obesity. Hall et al. [113] showed that ATP could mediate the long-term effects of leptin on blood pressure involved in obesity and hypertension, and high concentrations have been reported to induce inflammatory responses and insulin resistance generation in rat adipocytes [114].

Regarding other metabolic diseases, in a study with an animal model of thyroid disorders, GST and GPx activities and GST protein expression in red blood cells of hyperthyroid and hypothyroid rats were shown to be increased [115]. On the other hand, Baldissarelli et al. [116] demonstrated that the GST activity was decreased in patients with post-thyroidectomy hypothyroidism, probably to preserve high levels of GSH, which can be used by other reactions in the body, such as the neutralization of hydroxyl radicals. Furthermore, the authors also showed an increase in the activity and expression of NTPDase (CD39) and an increase in 5′-nucleotidase and ADA activities, besides a lower concentration of Ado in hypothyroid patients, which was positively correlated with RS levels.

The role of GSH in cancer has also been demonstrated since the decrease in the activity of antioxidant enzymes, such as GPx, and the increase in the levels of damaged DNA bases due to oxidative damage may lead to the formation of free radicals which could induce the appearance of malignant cells [117, 118]. Moreover, Li et al. [119] showed that GR inhibition generates oxidative stress and suppresses lung metastasis and subcutaneous growth of melanoma in vivo. The tumor microenvironment is characterized by unusually high concentrations of ATP and Ado. Ado is a major determinant of the immunosuppressive tumor milieu. In this sense, preclinical data show that targeting the Ado-generating pathway (CD73) or adenosinergic receptors (A2A) relieves immunosuppression and potently inhibits tumor growth [120, 121]. In this context, patients with lung cancer showed a decrease in ADA activity and an increase in A1 receptor expression in lymphocytes, which may contribute to Ado pro-tumor effects by promoting a profile of cytokine levels that favors tumor progression [122].

Oxidative stress, which is implicated in the pathophysiology of neurodegenerative diseases, also affects brain astrocytes. P2Y receptors, largely expressed in the CNS, are proposed to have a cytoprotective action. In the work of Förster and Reiser [123], the potential involvement of P2Y receptors in the antioxidant protection against hydrogen peroxide-induced toxicity in rat brain astrocytes was investigated. Cells were incubated with the wide range P2Y receptor agonist adenosine 5′-(3-thiotriphosphate) (ATPγS) and the particular P2Y1 receptor agonist 2-methylthio-ADP (2MeSADP), and findings showed that levels of GSH were augmented in the presence of both agonists. Moreover, the expression of genes involved in GSH metabolism also relied on the increase of intracellular Ca^{2+} mediated by the P2Y receptor. Taken together, the authors suggest the participation of P2Y receptors in the cytoprotection of astrocytes in the event of oxidative stress.

In the case of neurodegenerative diseases, studies addressing the relationship between glutathione antioxidant enzymes and purinergic signaling have also been performed. Recently, the effect of intracerebroventricular injection of streptozotocin (ICV-STZ), a model of sporadic dementia of the Alzheimer's type, and administration of berberine (BRB) on GSH levels and GST activity was investigated in the cerebral cortex and hippocampus of rats [124]. Both, in the cerebral cortex and hippocampus, the STZ-induced Alzheimer's model significantly decreased GSH levels and GST activity; however, treatment with BRB at the doses of 50 and 100 mg/kg was able to prevent these alterations induced by STZ in rats. Moreover, BRB at both doses also prevented the reduction in NTPDase, 5′-nucleotidase (EC-5′-Nt), and ADA activities in synaptosomes of the cerebral cortex and hippocampus. In this sense, the authors suggested that BRB could have a neuroprotective activity against oxidative stress and purinergic system damage in STZ-induced Alzheimer's model in rats.

Disease	Glutathione system	Reference	Purinergic system	Reference
Acute myocardial infarction	↑ GPx in whole blood	[125]	↑ NTPDase activity in platelets	[99]
	↓ GPx in serum	[126]	↑ ATP, ADP, and AMP hydrolysis ↑ ADA activity in platelets	[134]
	↓ GR in serum	[127]		
Metabolic syndrome (MetS)	↓ GPx and GST activities in liver	[100]	↑ NTPDase, 5'-NT, and NPP activities in platelets ↓ ADA activity in platelets	[102]
	↓ GPx and GST activities in the heart ↓ GSH levels in the heart	[101]	↑ ATP and ADP hydrolysis in lymphocytes ↓ ADA activity in lymphocytes	[103]
Diabetes	↓ GPx and GST activities in liver	[128]	↑ NTPDase, E-NPP, 5'-NT, and ADA activities in platelets	[135]
	↓ GST activity in liver	[129]	↓ NTPDase activity in the cerebral cortex ↓ A1R and ↑A2R in the cerebral cortex	[110]
			↑ ATP and ADP hydrolysis and ADA activity in lymphocytes ↑ NTPDase and ADA activities in platelets ↓ ATP and ↑ ADP and AMP hydrolysis in serum	[109]
Obesity	↓ GPx and GST activities in hepatic and renal tissues ↓ GSH levels in hepatic and renal tissues	[112]	↓ ATP, ADP, and AMP hydrolysis in serum	[136]
			↑ ADA activity in saliva	[137]
Hypothyroidism	↓ GPx activity in serum ↑ T-SH and NPSH concentrations in platelets	[116]	↑ NTPDase, 5'-NT, and ADA activities in platelets ↑ CD39 expression ↓ Ado levels in serum	[116]
	↑ T-SH and NPSH concentrations in serum	[130]	↓ 5'-NT activity in platelets ↑ NPP activity in platelets ↑ AMP and inosine levels in serum	[138]
			↑ CD73 in lymphocytes	[130]

Disease	Glutathione system	Reference	Purinergic system	Reference
Hyperthyroidism	↓ GPx activity in the hippocampus	[131]	↓ NTPDase and 5'-NT activities in platelets ↑ ADA activity in platelets ↑ ATP, ADP, AMP, and inosine in serum ↓ Ado levels in serum	[138]
	↑ GPx activity in the hippocampus	[132]		
Hypertension	↑ GPx activity in mesenteric arteries	[107]	↑ NTPDase, 5'-NT, and ADA activities in platelets	[105]
	↓ GST activity in the kidney	[133]	↑ NTPDase1, NTPDase3, and CD73 expression and activity in the cortex ↑ A2A expression in hippocampus and cortex	[106]
Alzheimer's disease	↓ GSH and GST activity in cortex and hippocampus	[124]	↓ NTPDase, 5'-NT, and ADA activities in cortex and hippocampus	[124]

Table 2.
Changes in glutathione and purinergic systems during diseases.

5. Conclusions

In summary, purinergic and immune systems, comprised mainly of receptors, signaling molecules, and also enzymes, play a key role in many pathologies and regulate the functions especially of the immune system. Besides, these two complex systems closely interact and may modulate GSH levels as well as the expression and activity of glutathione-dependent antioxidant enzymes in both scenarios, health and disease. Future studies will possibly provide more details into the mechanisms underlying the regulation of these enzymes and help to expand the current knowledge.

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Conflict of interest

The authors declare no conflict of interest.

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