Emerging role of extracellular nucleotides and adenosine in multiple sclerosis

Marek Cieślak · Filip Kukulski · Michal Komoszyński

Abstract Extracellular nucleotides and adenosine play important roles in inflammation. These signaling molecules interact with the cell-surface-located P2 and P1 receptors, respectively, that are widely distributed in the central nervous system and generally exert opposite effects on immune responses. Indeed, extracellular ATP, ADP, UTP, and UDP serve as alarmins or damage-associated molecular patterns that activate mainly proinflammatory mechanisms, whereas adenosine has potent anti-inflammatory and immunosuppressive effects. This review discusses the actual and potential role of extracellular nucleotides and adenosine in multiple sclerosis (MS).

Keywords Neuroinflammation · Demyelination · Autoimmune encephalomyelitis · P2 receptor · P1 receptor

Introduction

Multiple sclerosis (MS) is a debilitating autoimmune disease of the central nervous system (CNS) that leads to progressive physical and cognitive disability. The complex etiology of MS includes the combination of genetic, environmental, and infectious factors. Wallerian degeneration, i.e., axonal injury and death resulting from demyelination, represents the major pathological symptom of MS. Axon degeneration is followed by the atrophy of dendrites and neuronal cell bodies, which altogether result in local loss of neurological function [1, 2]. Demyelination process observed in MS patients is heterogeneous and seems to result from either autoimmunity (i.e., the T cell-mediated or T cell-mediated plus antibody-mediated encephalomyelitis) or a dystrophy of the myelinating cell oligodendrocyte [3]. Prevalent evidence demonstrates that MS progression is associated with relapsing inflammation in disease lesions (called plaques) [4, 5]. In agreement, active plaques show the presence of various immune cells such as T and B lymphocytes, glial cells (microglia, oligodendroglia, and astrocytes), dendritic cells, monocytes/macrophages, and neutrophils. All these cells contribute to neuroinjury by secreting a wide array of proinflammatory cytokines that exacerbate inflammation and promote chronic astrogliosis [6, 7]. Interestingly, although activated B lymphocytes produce antibodies against oligodendrocytes and myelin which manifests in the presence of oligoclonal bands in the cerebrospinal fluid in 95% of MS patients [8], the role of these antibodies in the etiology of MS has not been demonstrated.

Neuroinflammation is associated with high levels of extracellular ATP which is released from activated cells or leaks from injured or dead cells [9]. The cells involved in MS, i.e., neurons, glia, and immigrated immune cells, can sense this molecule, as well as other extracellular nucleotides (e.g., ADP, UTP, and UDP), via specific P2 receptors. This family of receptors includes ionotropic P2X (P2X1-7) and metabotropic P2Y (P2Y<sub>1,2,4,6,11–14</sub>) receptors that differ...
in respect to specificity towards nucleotides: all P2X and P2Y₁₁ receptors are activated by ATP; P2Y₂ by ATP and UTP; P2Y₁, P2Y₁₂, and P2Y₁₃ by ADP; P2Y₄ by UTP; P2Y₆ by UDP; and P2Y₁₄ by UDP glucose [10]. Mounting evidence links the release of extracellular nucleotides with the induction of a myriad of proinflammatory responses such as the production of inflammatory mediators and the proliferation, differentiation, trafficking, and apoptosis of immune cells [6, 11]. Cells involved in MS also express P1 receptors, namely A₁, A₂₅, A₂₆, and A₃, that are activated by extracellular adenosine [12, 13]. This molecule is produced mainly from the degradation of extracellular ATP and ADP by the ectonucleotidases and ecto-5′-nucleotidase [14, 15]. In contrast to P2 receptors, P1 receptors generally exert anti-inflammatory and immunosuppressive responses.

This review discusses the actual and potential roles of extracellular nucleotides and adenosine in MS.

**Role of extracellular nucleotides and P2 receptors in MS**

The proinflammatory P2X7 receptor, previously known as a death receptor or P2Z, is one of the most abundant P2 receptors in the CNS. It is expressed either by resident cells (e.g., microglia, oligodendroglia, astrocytes, and Schwann cells) or leukocytes infiltrating the CNS during immune responses (lymphocytes, monocytes, and macrophages) [7, 16–18]. The biological role of P2X7 is closely associated with inflammatory process that increases the expression of this receptor and also generates large quantities of extra-cellular ATP and ADP by the ectonucleotidases nucleoside triphosphate diphosphohydrolases (NTPDases) and ecto-5′-nucleotidase [14, 15]. In contrast to P2 receptors, P1 receptors generally exert anti-inflammatory and immunosuppressive responses.

The cytokines induced by IL-1β also play a key role in MS. For example, IL-2 stimulates the proliferation, differentiation and survival of antigen-selected cytotoxic T cells. Moreover, this cytokine is also necessary for the maturation of regulatory T cells (Tregs) that prevent other T cells from recognizing and reacting against “self antigens” [35]. TNF-α is involved in the control of synaptic strength and mediates the alteration of excitatory transmission occurring in MS [36]. Together with IFN-γ and IL-6, this cytokine also controls immune cell recruitment by increasing the expression of adhesion molecules, namely intercellular adhesion molecule-1, vascular cell adhesion molecule-1 (VCAM-1), and E-selectin, at the surface of endothelium. Notably, natalizumab (Tysabri, Elan Pharma International Ltd.), a monoclonal antibody directed against α₄ integrin (binds endothelial VCAM-1) expressed at the lymphocyte surface is currently used in MS therapy. In addition, P2X7 is also implicated in N-formyl-Met-Leu-Phe (also known as fMLF or fMLP)-induced expression of CD11b (or α₅β₂, a subunit of MAC-1) in human neutrophils which regulates the transendothelial migration of these cells [37, 38].

P2X7 plays an important role in glial cells where, depending on the conditions, its activation can stimulate either proliferation or apoptosis. The former response was observed in the monoculture of microglial cells treated with exogenous ATP at milimolar concentration [39, 40] whereas the latter response was exerted by repetitive receptor activation by endogenous ATP released from astrocytes co-cultured with microglia [41]. Interestingly, IFN-γ that greatly potentiates ATP release from astrocytes further increased microglial apoptosis. In addition, it was demonstrated that the activation of P2X7 receptors can kill
oligodendrocytes in vitro [20]. This P2X7-induced response appears to have an important role in chronic autoimmune encephalomyelitis, where a sustained activation of P2X7 receptors leads to lesions resembling MS plaques in respect to demyelination, oligodendrocyte death, and axonal damage. In agreement with the role of P2X7 in this model, severity of encephalomyelitis was significantly reduced either in P2X7 knockout mice or in wild-type mice treated with P2X7 antagonists [20, 42]. In contrast, another study demonstrated an exacerbated experimental autoimmune encephalomyelitis in P2X7 knockout mice which was attributed to a loss of apoptotic activity in lymphocytes [43]. These stark differences in the outcome of encephalomyelitis between studies using P2X7 knockout mice may result from differences in phenotype of mice used. Indeed, these studies were done with P2X7 knockout mice generated by GlaxoSmithKline and Pfizer, respectively, which, to our knowledge, tend to give conflicting results. In addition to proliferation and apoptosis, P2X7 stimulation in microglia can also activate superoxide production and ATP release [40, 44]. The latter response subsequently increases microglia activation by triggering intracellular Ca\(^{2+}\) waves in an autocrine manner. Due to well-documented and important role of P2X7 in neuroinflammation, the antagonists of this receptor are currently tested as potential treatments against inflammatory diseases of the CNS [45–47].

Another ATP-activated P2 receptor involved in inflammation, that could therefore play a role in MS, is P2Y\(_{11}\). This receptor is expressed by granulocytes, dendritic cells and lymphocytes [48–50]. In neutrophils, the activation of P2Y\(_{11}\) protects against apoptosis and triggers chemotaxis [51, 52]. In dendritic cells, P2Y\(_{11}\) controls the production of cytokines responsive for the key innate and adaptive immune responses, i.e., it induces the release of IL-10 and IL-23 but inhibits the release of IL-12 and IL-27. IL-10 is a potent anti-inflammatory cytokine that inhibits the synthesis of multiple proinflammatory cytokines (IFN-\(\gamma\), IL-2, IL-3, TNF-\(\alpha\), and GM-CSF) and attenuates the antigen presentation capacity of antigen presenting cells. IL-23 stimulates the proliferation and increases cytotoxicity of T lymphocytes, and also induces the release of IL-17 by T-helper cells that subsequently triggers leukocyte recruitment. In addition, IL-23 is necessary for the generation of T-memory cells and autoimmunity, and thus plays a role in autoimmune diseases such as autoimmune encephalomyelitis. IL-12 and IL-27 promote Th1 response by stimulating the production of IFN-\(\gamma\) in natural killers (NK) cells and natural killer T (NKT) cells [49]. IL-12 also enhances the proliferation, activation and cytotoxicity of NK and cytotoxic T cells but inhibits their IFN-\(\gamma\) and TNF-\(\alpha\) secretion.

The P2Y\(_{2}\) receptor activated by ATP and UTP is expressed by neurons, astrocytes, microglia and leukocytes [53]. The expression of this receptor can be upregulated by proinflammatory cytokines such as IL-1\(\beta\) [54]. Interestingly, it is likely that P2Y\(_{2}\) may increase its own expression via a positive loop mechanism involving P2Y\(_{2}\)-dependent and metaloprotease-induced activation of IL-1\(\beta\) production in astrocytes and primary neurons. The major role of P2Y\(_{2}\) in inflammation seems to be associated with cell migration. Indeed, this receptor controls the migration of glial cells [53] and leukocytes [55–58]. The migration of the latter cells involves either endothelial P2Y\(_{2}\) receptors whose stimulation upregulates the expression of adhesion molecules such as VCAM-1 or leukocyte P2Y\(_{2}\) receptors that trigger the release of chemokines IL-8 and MCP-1 [55, 58–60].

In agreement with an important role of ATP-activated receptors in neuroinflammation and MS, a nonsynaptic release of this nucleotide has been reported in the CNS that allows communication between axons and myelinating glia and has been shown to regulate myelination process [61]. Thus, it seems likely that the disregulation of nonsynaptic ATP release and thus alterations in P2 receptor-dependent neuron-to-glia crosstalk may disregulate myelination, and, conversely, its normalization might be beneficial in MS.

The ADP-activated P2Y\(_{12}\) is expressed by oligodendrocytes, microglia and astrocytes [62, 63], and appears to play an important role in myelination process. Indeed, there is an inverse correlation between the decrease in P2Y\(_{12}\) expression and both axonal damage and gray matter demyelination occurring in frontal cortex during the secondary progressive phase of MS [64]. In microglia, P2Y\(_{12}\) receptor plays an important role in chemotaxis [63], and as microglia present in MS lesions are negative for P2Y\(_{12}\) immunostaining [64], it is likely that at least some of the effects described above might be due to impeded microglia function that would hamper debris clearance and thus either exacerbate neurodegeneration or hamper regeneration [65]. This is in agreement with the role of glial cells in both destructive and restorative phases of MS.

The UDP-sensitive P2Y\(_{6}\) receptor is widely expressed in brain blood vessels (in vascular smooth muscle cells and endothelium) and by immune cells such as microglia and monocytes/macrophages [10]. The major role of this receptor in inflammation is associated with the production of the chemokine IL-8 that has a key role in inflammatory leukocyte recruitment [58, 60, 66]. P2Y\(_{6}\) is also involved in microglia phagocytosis [67].

The GPR17 receptor activated by both uracil nucleotides and cysteinyl leukotrienes (e.g., UDP glucose and LTD4) is expressed in neurons and a subset of parenchymal quiescent oligodendrocyte precursor cells. This receptor appears to act as a “sensor” that is activated upon brain injury and participate either in neurodegeneration or remodeling/repair response. It was shown that following brain injury, stimulation of GPR17 increases an infract size most likely by sensitizing CNS cells to ATP-induced death [68, 69].
later stages, however, GPR17 expressed in parenchymal oligodendrocyte progenitors induces differentiation of these cells into mature oligodendrocytes and, thus, promotes remyelinating process [70–72].

The role of P2 receptors in neuroinflammation is summarized in Table 1.

### Role of extracellular adenosine and P1 receptors in MS

Extracellular adenosine generally exerts potent anti-inflammatory/immunosuppressive responses and therefore plays an essential role in neuroprotection [73]. The neuroprotective effects of this molecule in the CNS include: preconditioning, increase in the level of oxygen supply/demand ratio, control of cytokine production, and immunosuppression. Importantly, adenosine also promotes neurorepair by stimulating cell proliferation and angiogenesis [74, 75]. Sitkovsky and Ohta have proposed that inflammation is always associated with mild hypoxia and that the latter increases the production of extracellular adenosine as the “stop” signal for inflammation (Fig. 1; [76]). This is accomplished by: (1) increase in the metabolism of extracellular ATP, (2) increase in the production of

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**Table 1** Role of P2 and P1 receptors in neuroinflammation and experimental encephalomyelitis

| Receptor | Natural agonist(s) | Expression in the CNS | Demonstrated and/or potential role in the CNS | Role in experimental encephalomyelitis (EAE) |
|----------|--------------------|-----------------------|-----------------------------------------------|---------------------------------------------|
| P2X7     | ATP (mM)           | Microglia, astrocytes, oligodendroglia Schwann cells, leukocytes and ↑immunoreactivity in neurons and astrocytes of MS patients | Indicates IL-1β maturation and release | P2X7 stimulation causes lesions resembling MS plaques |
| P2Y2     | ATP and UTP        | Neurons, microglia, astrocytes, and leukocytes | Controls migration of glial cells and leukocytes | ND |
| P2Y6     | UDP                | Microglia, leukocytes, and blood vessel cells | Controls leukocyte recruitment via IL-8 production | ND |
| P2Y11    | ATP and ADP        | Leukocytes | Involved in neutrophil chemotaxis and apoptosis | ND |
| P2Y12    | ADP                | Oligodendrocytes, microglia, and astrocytes | Involved in myelination | ND |
| GPR17    | UDP, UDP glucose, and cysteinyl leukotrienes | Neurons, parenchymal, oligodendrocyte, and precursor cells | Involved in neurodegeneration and remodeling/repair processes following brain injury | ND |
| A1       | Adenosine          | Neurons, microglia, astrocytes, leukocytes (except for T lymphocytes), and ↓expression in MS patients | Protects against neuroinflammation and demyelination in patients with MS and allergic EAE | A1 knockout mice display increased neuroinflammation and demyelination |
| A2A and A2B | Adenosine | Neurons, microglia (except for A2B), astrocytes, and leukocyte | A2A knockout mice display an increased production of proinflammatory cytokines | ND |
| A3       | Adenosine          | Neurons, microglia, astrocytes, and leukocytes | Induces the release of IL-6 and CCL2 from astrocytes | ND |

*ND* not determined

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intracellular adenosine and its transport outside the cells, and (3) decrease in adenosine kinase activity that phosphorylates adenosine to AMP in physiological conditions [77]. The (1) is possible due to, for example, increase in the expression of the ectonucleotidases NTPDase1 and ecto-5′-nucleotidase via induction of transcription factors Sp1 and hypoxia-inducible factor-1 (HIF-1), respectively (see “Role of ectoenzymes metabolizing extracellular nucleotides and adenosine in MS”; [78, 79]). All these mechanisms can rapidly raise the concentration of extracellular adenosine from basal nanomolar to ~10–50 μM [80]. Adenosine acts through the activation of four types of P1 receptors, namely A1, A2A, A2B, and A3. A1 and A2A receptors require lower concentrations of adenosine for activation than A2B and A3. All P1 receptor subtypes are expressed by neurons and glial cells except for microglia that do not express A2B [75, 80].

Among P1 receptors, the A1 receptor appears to have the most profound neuroprotective role in the CNS. For example, together with A3, this receptor is implicated in brain ischemic preconditioning [75]. Moreover, A1 stimulation protects against neuroinflammation and demyelination in patients with MS and allergic encephalitis [81]. In agreement, A1 receptor knockout mice develop severe demyelination and oligodendrocyte cytotoxicity due to increased production of IL-1β and metalloproteinase-12 by macrophages. This finding is in line with the decreased expression of A1 receptors in peripheral blood mononuclear cells, microglia and macrophages from MS patients [82, 83]. The neuronal A1 receptors also contribute to neuroprotection by inhibiting the release of excitatory neurotransmitters and attenuating the propagation of their signaling [84]. In addition to neuroprotection, A1 receptors are involved in tissue repair via stimulation of neuronal growth factor release from astrocytes [85]. The activation of A2A and A2B receptors leads to increase in intracellular cAMP that has a general inhibitory effect on immune cells (Fig. 2). While prevalent evidence indicates that the activation of A2A initiates potent anti-

![Fig. 1 The role of adenosine and P1 receptors in inflammation](image1)

![Fig. 2 The role of P1 receptors in the regulation of T lymphocyte functions](image2)
inflammatory responses, the role of this receptor in the CNS remains controversial as its activation is detrimental in cerebral ischemia but beneficial in lipopolysaccharide (LPS)-induced meningitis [80]. The results obtained from the model of liver injury show that A2A deletion in mice exacerbates inflammation through increased production of TNF-α, IL-12, and IFN-γ. Similar effects were observed in animals treated with the A2A antagonist ZM241385 that markedly increased severity of liver damage compared with untreated mice [76]. The protective role of A2A receptor in liver injury was mostly due to dendritic cells as the stimulation of A2A in these cells inhibits the release of proinflammatory TNF-α, IL-12, and chemokine CXCL10 but increases the release of anti-inflammatory IL-10 and CCL17 [75]. In glial cells, the activation of A2A decreases the induction of iNOS by LPS, IFN-γ, TNF-α and IL-1β but increases COX-2 expression [75]. A2A stimulation also exerts potent immunosuppressive effects on T lymphocytes by antagonizing T cell receptor (TCR) signaling that activates the release of cytokines (e.g. IL-2) and granules, upregulates the expression of CD25, CD69 and Fas ligand, and increases the cytotoxicity and proliferation of T cells [86].

As mentioned above, A2B receptor requires higher concentration of adenosine for activation than A2A suggesting that the former receptor may be more important in pathological conditions where it would potentiate the responses triggered by A2A activation. In agreement, the functional responses of A2B in astrocytes were increased by prior stimulation with TNF-α [87]. The activation of A2B receptors in T cells results in the inhibition of IL-2 production [86] whereas in astrocytes, A2B, together with A1 receptors, induce the release of IL-6 and CCL2 [88, 89]. The activation of A3 receptor was also shown to decrease LPS-induced TNF-α production by microglia and NK activation [86, 90].

Interestingly, in some cells, adenosine can exert opposite biological effects depending on the activated P1 receptors. For example, A1 stimulates astrocyte proliferation whereas A2A inhibits this process [80]. Therefore, given that either neurons or glial cells express all four P1 receptor subtypes, the final effect of extracellular adenosine will depend on P1 subtype expression level, adenosine concentration and environmental conditions (e.g., cytokine production). The role of P1 receptors in neuroinflammation is summarized in Table 1.

Role of ectoenzymes metabolizing extracellular nucleotides and adenosine in MS

The activation of P2 and P1 receptors is regulated by ectoenzymes that metabolize the ligands of these receptors [14, 15]. For example, the sequential hydrolysis of extracellular ATP to adenosine by the enzymes ectonucleotidases either terminates P2 receptor activation or promotes P1 activation. While ATP and ADP (as well as UTP and UDP) are hydrolyzed by NTPDases, AMP is dephosphorylated to adenosine by ecto-5′-nucleotidase (CD73). Some cells also possess the enzymes ectokinases, e.g., adenosine kinase, that have the capacity to re-synthesize nucleotides from their degradation products, e.g. adenosine, and thus inactivate P1 activation but promote P2 activation [15].

NTPDase1 (CD39) is a dominant ectonucleotidase originally identified as the activation marker of B lymphocytes [14]. In the CNS, this enzyme is expressed in synaptic membranes [91] and by microglia [7]. Moreover, also infiltrating leukocytes express CD39, e.g., Foxp3+ Treg cells (CD39 is used as a specific marker of this T cell subset; [92]), activated T cells, NK cells (where its expression increases upon activation), monocytes/macrophages and neutrophils [12, 93]. By contributing to the production of extracellular adenosine, NTPDase1 downregulates antigen recognition and cytotoxic T cell activation and thus has an important immunosuppressive role [94]. In agreement, patients with relapsing/remitting MS have significantly reduced numbers of CD39+ Tregs [92]. Moreover, CD39+ Tregs from MS patients display reduced capacity to suppress IL-17 production by Th17 cells [95]. Other studies demonstrate a key role of this enzyme in cytokine production. For example, endogenous NTPDase1 tightly regulates P2Y2-dependent IL-8 release by human neutrophils [96]. Endogenous NTPDase1 also controls the release of IL-1β by mouse macrophages and protects these cells from ATP-induced death [97]. Exogenous NTPDase1, i.e., apyrase, was demonstrated to abolish IL-8 release by human primary monocytes and IL-1α by human endothelial cells [60, 66, 98]. The studies performed with apyrase to augment the endogenous NTPDase1 activity may reflect an in vivo situation where hypoxia associated with ongoing inflammation upregulates the expression of this enzyme via induction of transcription factor Sp1 [79]. In RAW macrophages, in turn, the increase in NTPDase1 expression can be induced by agents driving cAMP response via cAMP response element-binding [99]. In keeping with these results, patients with relapsing–remitting MS have an increased activity of CD39 in lymphocytes [100, 101]. This increase may represent a protective mechanism that will decrease the activation of proinflammatory P2 receptors and at the same time facilitate the activation of anti-inflammatory P1 receptors.

The expression of ecto-5′-nucleotidase in the CNS was detected in astrocytes, oligodendrocytes and microglia. Moreover, this enzyme is also present in endothelium and leukocytes [7]. As for NTPDase1, the expression of ecto-5′-nucleotidase can be rapidly augmented by hypoxia via HIF-1 [78]. Interestingly, MS patients treated with IFN-β-1a and IFN-β-1b exhibited elevated expression of ecto-5′-nucleotidase in serum, astrocytes and blood–brain barrier
endothelium that suggests that these therapies target the increase in extracellular adenosine [102, 103].

Another ectoenzyme involved in MS is adenosine deaminase (ADA). This enzyme terminates P1 receptor activation by degrading adenosine to inosine and its activity was detected in thymus, lymphoid tissue and T lymphocytes [15, 104]. In agreement with the expression pattern, adenosine deaminase controls the growth, proliferation, differentiation and transendothelial migration of lymphocytes. In keeping with the role of this enzyme in MS, lymphocytes from patients with relapsing-remitting and secondary progressive MS exhibit a markedly reduced adenosine deaminase activity. The dampened expression of ADA in MS lymphocytes may facilitate the infiltration of these cells in the CNS [86, 100, 105].

Summary

As reviewed above, mounting evidence allows to link the actions of extracellular nucleotides and adenosine with the etiology of MS. The multifaceted role of these molecules in immune responses including the release of cytokines and excitatory neurotransmitters, cell trafficking, cell proliferation and death, myelination, immunosuppression, etc., opens a variety of new avenues for treatments against this debilitating disease. Moreover, we are currently testing the hypothesis whether extracellular nucleotides or adenosine measured in cerebrospinal fluid could serve as the early biomarkers of MS or the markers of the transition from remitting–relapsing MS to treatment-resistant secondary progressive MS ([106] and work in progress).

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