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Modulation of myeloid cells by adenosine signaling

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Hypoxia, metabolic activity, cell death and immune responses influence the adenosine concentrations in the extracellular space. Cellular responses to hypoxia and inflammation in myeloid cells promote activation of adenosine sensing circuit, which involves increased expression of ectoenzymes that converts phospho-nucleotides such as ATP to adenosine and increased expression of G protein-coupled adenosine receptors. Adenosine sensing circuitry also involves feedforward signaling, which leads to increased expression of hypoxia-inducible factor 1-alpha (HIF1α) and feedback signaling, which leads to the suppression of inflammatory transcription factor, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation. In this review we will discuss how different subsets of myeloid cells sense adenosine accumulation and how adenosine sensing by myeloid cells influence progression of different immune-related conditions including cancer.

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Introduction

Myeloid cells are important in protection from infections, generating inflammatory responses, activation of adaptive immune responses, resolution of inflammation, tissue healing and establishment of peripheral tolerance [1,2]. Myeloid cells recognize environmental cues such as pathogen associated molecular patterns, cytokines generated by other immune cells and certain components of dead or stressed cells. In response to these immunomodulatory environmental cues myeloid cells can polarize into immunostimulatory, immunomodulatory or immunosuppressive subsets. Polarization of myeloid cells into a specific subtest dictates the set of enzymes and cell surface molecules expressed and dictates cytokines they release to their environment to shape the immune response [1,3]. Subsets of myeloid cells can also specialize in antigen processing and presentation to activate or modulate adaptive immune responses. Adaptive immune cells, particularly T cells, can further polarize into different subsets depending on cell surface signals and the cytokine milieu provided by myeloid cells [2].

Phospho-nucleotides (such as ATP and ADP) released from dead or stressed cells act as an important environmental cue both to attract myeloid cells to the site of infection or injury, and to activate myeloid cells to release inflammatory cytokines [4,5]. More specifically, phospho-nucleotides act as find me signals through P2Y2 receptors to attract monocytes and phagocytic cells [4]. In cancer setting, ATP released from tumor cells in response to chemotherapy activates purinergic P2RX7 receptors on dendritic cells (DCs) and stimulates their secretion of interleukin-1β (IL-1β). IL-1β then promotes T cell priming and polarization into tumor-fighting IFNγ producers [5]. Sustained release of immunostimulatory nucleotide signaling and chronic inflammation is counterbalanced by conversion of ATP to adenosine, which plays a more immuno-modulatory role towards tissue-healing/fibrotic reactions or immunosuppressive role in the presence of strong inflammatory stimuli [6,7•]. Several key enzymes were identified for the conversion of ATP to adenosine such as CD39, which converts ATP to ADP and AMP and CD73, which converts AMP to adenosine [8]. Another source of adenosine is nicotinamide dinucleotides (NAD^+) and NAD precursors. CD38, which is expressed by immune cells, and CD203a are other two enzymes that play a key role for the conversion of NAD^+ to AMP and AMP is dephosphorylated further to adenosine by CD73 [9]. Adenosine can also be directly released into the extracellular space through equilibrative or concentrative nucleoside transporters [3]. Accumulation of adenosine in extracellular and intracellular space is further regulated by conversion of adenosine into inosine by adenosine deaminase enzymes and transportation of adenosine and inosine through cell membrane by equilibrative or concentrative nucleoside transporters [10]. Recent evidence suggests that extracellular adenosine accumulation has profound effects on myeloid cells in different preclinical models of immune related diseases [11,12••,13,14••,15••,16–21,22•]. In this review, we will summarize recent advances in understanding the effect of adenosine signaling in different myeloid cell subsets and emphasize the importance of identification of cell intrinsic effects of adenosine signaling on myeloid cell subsets to find novel strategies of myeloid cell-targeted therapy for different immune-related diseases.

Adenosine receptor signaling

Extracellular adenosine targets G protein-coupled P1 purinergic receptors, also called adenosine receptors [23•]. There are four major adenosine receptor subtypes; A1, A2A, A2B and A3 receptors (A1R, A2AR, A2BR and A3R, respectively). Adenosine receptors regulate cellular responses by controlling cAMP accumulation [3]. Activation of A1 and A3 receptors reduce cAMP because they are coupled to Gi functional subunit, while A2A and A2B receptors are coupled to Gs subunit. Therefore, activation of adenosine A2A or A2B receptors increases cAMP accumulation. A2B and A3 receptors can also couple to Gq subunit leading to mobilization of Ca^{++} and activation of MAPK and PKC pathways [3,24].

Effect of adenosine receptor signaling on myeloid cell populations

Macrophages

Macrophages produce cytokines, clear pathogens by phagocytosis and producing antimicrobial peptides and reactive oxygen species [1]. Macrophages with M1 phenotype stimulates other immune cells by producing cytokines such as IL-12 and TNFα while macrophages with M2 phenotype suppress other immune cells and reduce inflammation by producing IL-10 and TGFβ [25]. Adenosine signaling in macrophages promotes a hybrid tissue healing phenotype characterized by reduced IL-12 and TNFα secretion while elevated production of IL-10, IL-6, Arginases and VEGF (Figure 1) [26–28]. Adenosine-prestimulated macrophages does not highly express markers such as Ym1 and CD206 [26] but express others such as arginases [29], which are associated with M2 macrophages. Expression of adenosine A2A and A2B receptors increases after activation of macrophages by inflammatory stimuli [30,31]. Activation of NF-κB and HIF1α pathway plays a major role for increased expression of adenosine A2A and A2B receptors [31–33].

A2AR-deficient macrophages produce more TNFα and less IL-10 when compared with A2AR-proficient macrophages [12••,34] A2AR signaling can block Ca^{++} mobilization by activation of phosphatases to reduce TNFα release [35]. *In vivo*, deletion of A2AR from macrophages promoted M1 polarization, characterized by reduced IL-10 and increased MHCII and IL-12 expression in tumor-associated macrophages [22•]. HIF1α is an important mediator of inflammation by causing ATP generation and increased production of pro-inflammatory IL-1β [36,37]. HIF1α has two transcriptional isoforms; HIF1αI.1 and HIF1αI.2. Although both isoforms are important for TLR-induced increases in IL-10 and VEGF, HIFαI.1 isoform is important for the suppression of immunostimulatory/ inflammatory cytokines such as TNFα, MIP-1α, IL-6, IL-12p40, and IL-1β. Stimulation of A2AR synergize with TLR signaling to upregulate HIF1αI.1 isoform in macrophages [38]. In the presence of both TLR signaling and ATP, adenosine can sustain inflammasome activation through stabilization of HIF1α and therefore, secretion of IL-1β [39]. Therefore, upregulation of HIF1α by adenosine can play an important role modulating inflammatory responses in a context dependent manner.

Adenosine A2B receptors can stimulate chronic or angiogenic inflammatory responses in macrophages in the absence or presence of inflammatory stimuli. For example, activation of adenosine receptors by NECA increase IL-6 *in vivo* and *in vitro* in peritoneal macrophages. This effect is reversed by A2BR gene ablation or A2BR blockade [40]. Along with adenosine A3 receptors A2BRs increase VEGF and IL-8 production in macrophages and promote foam cell formation under hypoxia [41]. A2BR signaling can also suppress excessive inflammation *in vitro* or *in vivo*. A2BR stimulation increases IL-10 and decreases TNFα in bone marrow-derived macrophages [12••,42,43]. A2BR-deficient peritoneal macrophages isolated from septic animals produced more IL-6 and TNFα when compared with control animals [44].

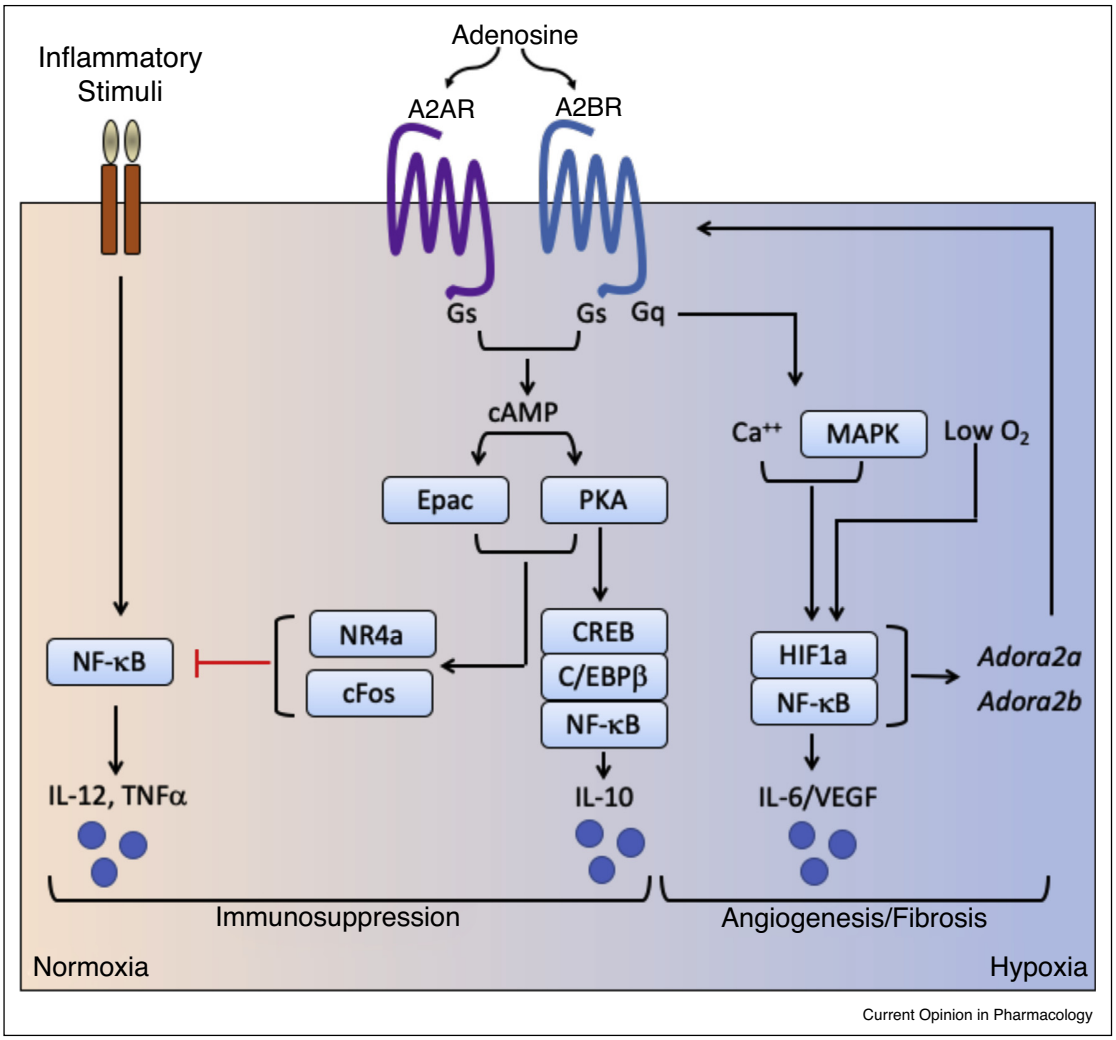
Adenosine A3 receptors are implicated in the regulation of human monocytes/macrophages. Autocrine A3 signaling mediates glucocorticoid-induced survival of a human monocyte subset with anti-inflammatory properties [45]. As mentioned previously, A3 signaling along with A2BR signaling promotes VGEF production [41].

Three major mechanisms may play important roles for adenosine-mediated immunomodulation of macrophages (Figure 1). One mechanism is the regulation of NF-κB activity as a negative feedback mechanism [33] Adenosine can inhibit NF-κB target gene expression in macrophages without changing the phosphorylation status of major components of NF-κB pathway or nuclear translocation of NF-κB [27,46,47]. Adenosine signaling strongly increase expression of NR4A nuclear orphan receptors in macrophages to regulate NF-κB activities [48]. Another mechanism is a potential feedforward mechanism involving hypoxia. Adenosine receptor expression increase in response to HIF1α activation[32], and in return adenosine signaling can promote HIF1α expression and promote angiogenic inflammation [41]. Third mechanism can be activation of MAPKs and increased expression of IL-8 and IL-6 [41].

Dendritic cells

Dendritic cells are specialized to present antigens to T cells or to produce type I interferons, cytokines that are important for protection against viral infections and neoplastic growth [1]. Depending on the cytokines produced by DCs and other myeloid cells during antigen presentation, T cells can differentiate into different functional subsets [25,49]. Cytokines such as IL-12 and TNFα promote polarization of Th1 subset, which is important to activate cytotoxic CD8+ T cell responses against

Figure 1



A2AR and A2BR signaling in myeloid cells. Activation of A2AR and A2BR causes accumulation of cAMP through Gs coupling. Two intracellular receptors of cAMP, PKA and Epac increase the expression of negative regulators of inflammation, NR4A and cFos [14]. NR4A and cFos can prevent binding of NF-κB to promoters of inflammatory cytokine genes such as IL-12 and TNFα [48,68]. PKA signaling leads to activation of CREB/C/EBPbeta pathway, which, along with NF-κB, increases the expression of anti-inflammatory IL-10 [120•]. Gq coupling of A2BR can also lead to elevation of Ca^{++} and activation of MAPKs, which along with hypoxia (low O_2) and HIF1α activation leads to increased expression of IL-6, VEGF. IL-6 and VEGF promotes angiogenesis, fibrosis and in some cased chronic inflammation [6]. Hypoxia and adenosine receptor signaling also increases expression of genes coding for A2AR and A2BR: *adora2a* and *adora2b*, respectively.

intracellular pathogens or tumors. Cytokines such as IL-6, TGFβ and IL-1β promote differentiation of Th17 subset, which promotes chronic inflammatory responses during unresolved chronic inflammatory conditions. TGFβ along with IL-10 promotes polarization of regulatory T cells (Tregs), which protects tissues from self-reactive T cells and uncontrolled inflammation [2,25]. Exposure to hypoxia promotes immunosuppressive DC phenotype characterized by decreased expression of MHCII, costimulatory molecules, inflammatory cytokines and increased expression of anti-inflammatory TGFβ. Reoxygenation of DCs restores immunostimulatory activity of DCs, leading to increased induction of CD4+ T cells with Th1 and Th17 phenotypes while reduced induction of CD4+ T cells with Treg phenotype [50]. Immature DCs express high levels of CD73. In the absence of CD73 DCs fail to cause adenosine accumulation and tolerize against hypersensitivity reactions induced by 2,4-dinitrothiocyanobenzene (DNTB) *in vivo*. CD73 deficient DCs also fails to induce T cell markers of anergy such as EGR2 and NDRG1 and suppress T cell proliferation *in vivo* and *in vitro* [13].

Adenosine signaling has been shown to play important roles in chemotaxis, maturation and immunogenicity of DCs. Chronic stimulation of human immature DCs through A2 receptors can promote several cell surface maturation and activation markers such as CD80, CD86 and MHC I [51]. However, adenosine signaling through A2 receptors also promotes anti-inflammatory IL-10 while suppressing IL-12 and TNFα release [51]. The overall functional effect of adenosine stimulation is a reduction in allostimulatory capacity in iDCs and decreased capacity to polarize T helper cells into Th1 phenotype [51]. In immature human dendritic cells adenosine can stimulate A1 and A3 receptor to increase Ca^{++} mobilization and actin polymerization, which were associated with increased chemotaxis [52]. Stimulation of A3 receptors in the presence of various inflammatory stimuli through different TLR receptors or in the presence of whole pathogens also promotes IL-12 secretion [53]. A3 signaling was also important for DC-mediated polarization of T cells into Th1 or Th17 phenotypes [53]. In mature human or mouse dendritic cells; however, adenosine signaling suppresses IL-12 production [54,55]. In mature pDCs adenosine through A2AR also reduces interleukin-6 (IL-6) and IFNα production along with IL-12 in response to CpG oligodeoxynucleotides (ODN) [52].

Polarization of DCs into suppressive phenotype is mediated mainly by A2ARs and A2BRs. DCs undergone hypoxia and reoxygenation express higher levels of A2A receptors. Activation of A2ARs in hypoxic DCs promotes immunosuppressive TGFβ expression while suppressing DC maturation and downregulating proinflammatory IL-1β, TNFα and IL-6 [56]. Regulatory T cells cause adenosine generation by dephosphorylating ATP through CD39 and CD73. Treg-generated adenosine promotes migration of dendritic cells through A2AR-cAMP-Epac pathway. Because these DCs are exposed to adenosine they are also more likely to show tolerogenic phenotype rather than immunostimulatory phenotype, which can be another important mechanism utilized by Tregs to create a tolerogenic microenvironment [57]. In three different migration settings: the emigration of epidermal and dermal dendritic cells from human skin explant culture assay, *in vivo* contact hypersensitivity assay and finally chemotaxis assay where CCL19 was used as a chemoattractant, adenosine inhibited the migration of mature dendritic cells, which have reduced A3 but elevated A2AR expression [58]. These observations suggest that adenosine may promote migration of immature dendritic cells while also promoting differentiation into a tolerogenic phenotype during maturation process in target tissues. In two different preclinical disease models (kidney injury and cancer) adenosine signaling through A2ARs has been shown to play a major role for anti-inflammatory IL-10 production in DCs, which is associated with increased tumor growth or reduced kidney injury further supporting this notion [59,60].

Hypoxia through HIF1α increases the expression of A2BR in DCs [61] Signaling through A2BRs suppresses DC maturation, which leads to impaired T cell stimulation and Th1 differentiation [55,61,62]. Adenosine generated by dephosphorylation of ATP suppresses IL-12 through A2BR and increase the expression of arginase I and II, well-known markers of tolerogenic DCs [63]. Because A2BR signaling also promotes IL-6, overall effect of A2BR signaling can be polarization of T cells into Th17 phenotype, which is associated with chronic inflammatory responses [64,65]. This suggest A2BR signaling in DCs is not solely an anti-inflammatory pathway but rather an immunomodulatory pathway, which lets certain aspects of inflammatory responses to take their course. In fact, adenosine A2B receptor stimulation polarize human and mouse DCs into a hybrid phenotype, which shares similarities both with DCs and with monocytes. Adenosine-differentiated DCs or DCs from adenosine deaminase deficient animals (therefore having elevated systemic steady-state adenosine levels) highly produce factors such as VEGF, IL-8, IL-6, IL-10, COX-2, TGFβ, and IDO, which play important roles in immunosuppression, angiogenic inflammation and tissue remodeling [66•].

Major intracellular mediator for adenosine-mediated immunosuppressive effects in DCs is cAMP accumulation, which is sensed by two intracellular receptors: PKA and Epac. In the absence of inflammatory stimuli PKA signaling alone can help DC maturation and promote chemotaxis through CXCL12/CXCR4 axis [67]. Stimulation of PKA pathway in the absence of inflammatory stimuli can reduce steady-state levels of cytokines, TNFα, IL-18, and IL-10 [67]. Epac signaling can antagonize these effects [67]. Effect of PKA stimulation alone in immature DCs translates into increased proliferation of allogeneic T cells [67]. In the presence of inflammatory stimuli adenosine/cAMP pathway targets both PKA and EPAC to polarize dendritic cells to a suppressive phenotype, characterized by ability to promote tumor growth *in vivo* and inhibiting Th1 deviation [14••]. Activation of adenosine signaling or PKA and EPAC pathways upregulate the expression of negative regulators of NF-κB such as cFos [68•] and NR4A [48] rather than directly influencing the phosphorylation status of NF-κB or MAPKs [14••].

Expression of matrix metalloproteinases are important for the migration of DCs through extracellular matrix. Hypoxia/Adenosine/A2BR/ cAMP/PKA pathway suppresses expression of MMP9 in DCs [69]. Accordingly, we have recently shown that A2BR-deficient DCs better infiltrate solid tumors to promote tumor antigen-specific CD8 T cell responses [12••]. Importance of these observations goes beyond the context of adenosine stimulation since there may be multiple mechanisms in the inflammatory milieu or in the tumor microenvironment that can

promote cAMP signaling in myeloid DCs. Therefore, these observations suggest that DC targeted therapies that will influence both PKA and EPAC activation will modulate multiple immunomodulatory mechanism that works through cAMP such as PGE2 receptors that recognize prostaglandins and G-coupled P2Y purinergic receptors, which mainly recognize ATP and some subsets of phosphorylated nucleotides such as ADP [23•].

Myeloid suppressor cells

Myeloid Suppressor Cells (MDSCs) are heterogenous population of myeloid cells, which often accumulates within solid tumors [70]. Granulocytic MDSCs (GrMDSCs) highly express Ly6G molecule on their cell surface while monocytic MDSCs (moMDSCs) highly express Ly6C, which is also a common marker for inflammatory monocytes [70]. However, unlike monocytes Ly6C+ MoMDSCs express somewhat less CD64 molecule, a marker for macropahges and monocytes. A2AR signaling is important for IL-10 production by moMDSCs *in vivo* [22•].

A2BR increases the infiltration of MDSCs in solid tumors [20]. A2BR blockade reduces CD11b+Gr1+ immune cell infiltration, and increases CD8+ T cell infiltration, which is also associated with reduced IL-10 and MCP-1 expression [71]. Accumulating GrMDSCs cells due to A2BR signaling highly express CD73, suggesting a positive feedforward signaling [72•]. A similar positive feedback loop involving A2BR is recently discovered in cancer-associated fibroblasts that trigger increased CD73 expression and immunosuppression through A2BR and A2AR [73].

Other myeloid cells

Neutrophils form extracellular net-like structures called neutrophil extracellular trap (NET) to trap extracellular pathogens and to control the infection [1]. Adenosine A1 receptors promote chemotaxis of neutrophils in a p38 MAPK-dependent manner while A2AR signaling suppresses adhesion and rolling of neutrophils causing decreased neutrophil migration [17,74,75]. They can also undergo a unique form of cell death during this process called NETosis. A2AR signaling suppresses NET formation [76]. A2AR signaling also prevents neutrophil apoptosis in response to bacterial lipopolysaccharide [56]. Mechanistically A2AR signaling targets cAMP/PKA pathway [77] and prevents oxidative burst in activated neutrophils [78], LPS-induced autophagy [56], expression of alpha 4/beta 1 integrins and binding to VCAM-1 [79] to regulate neutrophil, survival, activation and infiltration to target tissues. The role of cAMP-dependent regulation of Epac pathway downstream of A2AR in neutrophils remains elusive.

Adenosine can promote mast cell degranulation, leading to increased vascular permeability and bronchoconstriction in mice [80–82]. A3 adenosine receptors mediates the effects of adenosine in murine mast cells [80,83]. In human mast cells, A2BR signaling is important for mast cell degranulation, which is amplified by IL-4 signaling [84–86]. For the angiogenic effect in human mast cell cell line, HMC-1, cooperative action of both A2BR and A3 receptors are important [87]. Signaling through A2AR suppress human mast cell activation by C3a [88]. These results suggests that signaling through Gq signaling downstream of A2B in human mast cells and A3 in both human and rodent mast cells can be important for mast cell activation while cAMP downstream of A2AR signaling through Gs coupling can be inhibitory in human mast cells [24,88].

Effect of myeloid expression of adenosine receptors on immune-related diseases

Atherosclerosis and vascular health

Despite the evidence that A2AR signaling in myeloid cells can suppress inflammatory responses and that chronic inflammation promotes atherosclerosis, deletion of A2AR has a protective role against atherosclerosis [89]. In the absence of A2AR, foam cells undergo more apoptosis decreasing the atherosclerotic lesion size in ApoE-deficient mice, a mouse model prone to develop atherosclerosis due to increased cholesterol accumulation and foam cell formation [89]. Absence of A2AR signaling increases p38 activation to increase the foam cell apoptosis [89]. Interestingly, A2AR signaling in myeloid cells may have a protective effect on vasculature [11]. Angiotensin II promotes myeloid activation of A2AR, which prevents macrophage emigration to lymph nodes from aorta by keeping CCR7 expression low. This promotes aortic remodeling, emphasizing the importance of adenosine-mediated tissue-healing responses [11].

Global expression of A2BR was found to protect against atherosclerosis by regulating triglyceride and cholesterol synthesis in liver [90]. HIF-1α signaling has been shown to promote atherosclerosis. Adenosine can promote HIF1α accumulation under hypoxia through activation of the MAPK pathway. Adenosine increases expression of cytokines associated with increased atherosclerosis, such as VEGF, IL-8, and promotes foam cell formation by increasing HIF-1α accumulation downstream of A2BR and A3R *in vitro* [41]. Therefore, A2BR and/or A3R signaling in myeloid cells can potentially promote atherosclerosis by promoting foam cell formation, which requires further investigation.

Autoimmune diseases

Myeloid expression of adenosine receptors has been shown to play important roles in several preclinical autoimmune disease models. In a model of kidney injury representing the clinical onset of glomerulonephritis, which is induced by anti-glomerular basement membrane (GBM) antibody treatment, both acute and chronic inflammatory phase of the disease is associated with

increased infiltration of macrophages[91]. Expression of A2AR is specifically increased in infiltrating macrophages but not in CD8+ T cells. Treatment of mice with a highly selective A2AR agonist strongly abolished disease progression, decreasing the expression of inflammatory chemokines and increased expression of IL-4 and IL-10. Treatment with a selective A2AR antagonist increased the severity of the disease and the number of inflammatory infiltrates [91]. Transfer of A2AR-deficient macrophages into macrophage-depleted mice causes more severe kidney damage, which is associated with collagen deposition, in an established model of GBM-reactive serum-induced kidney injury, [19]. When A2AR-proficient macrophages are exposed to the A2AR agonist before transfer liver injury is reduced [19].

In a model of experimental autoimmune uveitis (EAU) induced by immunizing animals with immunogenic epitopes of interphotoreceptor retinoid binding protein, disease progression is associated with increased Th17 responses which was potentiated by the activation of γδ T cells [92]. A2BR agonism decreased disease severity while blockade of A2BR slowed the disease progression in EAU. A2BR agonist did not directly stimulate γδ T cells but A2BR-treated DCs or DCs isolated from A2BR agonist-treated mice caused increased activation of γδ T cells and Th17 cells [93].

Experimental autoimmune encephalomyelitis EAE model induced by immunization with myelin oligodendrocyte glycoprotein (MOG) in mice represents antidegenerative autoimmune conditions in central nervous system. A2AR blockade 11–28 days post-immunization had a neuroprotective effect in mice with EAE, which was associated with reduced macrophage/microglia infiltration in the spinal cord [94]. Further studies are needed to test if this effect is not an off-target effect, if macrophages are direct targets or alternatively if reduced macrophage infiltration and neuroinflammation is secondary to the effect of A2AR signaling on nonhematopoietic cells, which controls immune cell infiltration to the CNS [95].

Infectious diseases and sepsis

Infectious diseases and sepsis are two of the leading causes of mortality worldwide. Deletion of A2AR gene or pharmacological blockade protects mice from the lethal effects of the sepsis by cecal ligation, which is associated with increased activation (as measured by MHCII expression) of macrophages [96]. In acute septic conditions anti-inflammatory effect of A2BR signaling can theoretically provide protective effects. However, in cecal ligation and puncture (CLP) model of sepsis A2BR blockade improved survival, enhanced phagocytosis, reduced serum levels of IL-6 and MIP-2 [44]. Interestingly, A2BR-deficient macrophages isolated from septic animals produced more IL-6 and TNFα in response to LPS, suggesting early bacterial clearance is important for increased survival by A2BR blockade [44]. Interestingly, A2BR activation in nonhematopoietic cells indirectly elevates inflammation and increase mortality in the same sepsis model suggesting some of the immunological effects of A2BR signaling in immune cells can be counterbalanced by A2BR signaling in nonhematopoietic cells [97].

Adenosine signaling in myeloid cells can be exploited by infectious bacteria to evade immune system. *Leishmania amazonensis* triggers A2BR mediated cAMP accumulation and promotes polarization of tolerogenic phenotype in DCs via ERK1/2- but not PKA-dependent mechanism [18,98]. Macrophages better control *Salmonella* infection in the absence of CD73, or after inhibition of CD73 enzymatic activity, which is associated with increased proinflammatory responses and generation of anti-bacterial nitric oxide [16]. Adenosine signaling can also play a protective role during infections in the lung. Adenosine promotes neutrophil recruitment to the lungs and protects mice from lung infection after intratracheal inoculation of *Streptococcus pneumoniae* [21].

Tissue injury

Adenosine signaling in myeloid cells plays an important role in regulation of tissue healing. Myeloid expression of A2BR promotes Th2 cytokine production and increase allergic airway inflammation [99]. In a bleomycin-induced lung fibrosis model, deletion of A2BR in myeloid cells reduced lung fibrosis which is associated with reduced expression of M2 macrophage markers such as CD206 and arginase-1 [6]. Myeloid deletion of A2BR also reduced IL-6 and hyaluronan, which are associated with fibrotic reactions [6]. Adenosine signaling is an important trigger in asthmatic responses in clinic [100] Preclinical studies have shown that activation of mast cell A3R and A2BR plays a major role in mast cell activation and allergic airway hyperresponsiveness [82,87,101,102]. Unlike A2BR signaling in myeloid cells, A2AR activation can play a protective role in preclinical models of asthma and lung injury. During sensitization to model antigen myeloid stimulation of A2AR strongly prevents allergen-induced airway inflammation and permeability upon rechallenge to induce asthmatic reactions, which was associated with reduced lung infiltration of Th1 and Th17 subset of helper T cells. A2AR stimulation fails to show this effect in mice lacking A2AR expression specifically in myeloid cells [103]. Interestingly, A2AR stimulation during rechallenge is ineffective, suggesting A2AR signaling in myeloid cells during initial T cell priming not after secondary stimulation suppress T cells reactive to model antigen [103]. Targeting A2AR before LPS-induced lung injury as measured by polymorphonuclear leukocyte (PMNs) migration and vascular permeability has a protective effect. Myeloid cell A2A receptors play the major role for this protection [104].

NKT cells and NKT-DC interactions play an important role in kidney injury. When acute kidney injury is induced by clamping of kidney pedicles to produce ischemia and reperfusion, deletion of adenosine A2A receptors in DCs increased the ischemia reperfusion injury measured by plasma creatinine levels, neutrophil infiltration and tissue morphology. Adoptive transfer of DCs loaded with α-galactosylceramide (antigen that activates NKT cells) and tolerized with a highly specific A2AR agonist, reduced kidney injury in IL-10-dependent manner [60]. Inhibition of neutrophil adhesion by A2AR signaling can also potentially reduce renal ischemia/reperfusion injury [105].

Targeting adenosine A2A receptors during reperfusion also protects livers from ischemia/reperfusion injury. This protective effect is associated with reductions in both myeloid and T cell-associated inflammatory cytokines [106]. However, which immune cell subset is the main target for A2AR agonism in this setting remains to be well-defined.

Cancer

Extracellular space in solid tumors contains high concentrations of adenosine. Addition of adenosine deaminase and adenosine kinase inhibitors further increased the average adenosine concentrations up to 13 uM, which is high enough to strongly stimulate all four P1 purinergic receptors [107]. Considering there are heterogenous hypoxic and normoxic hot spots within the solid tumors, adenosine concentrations can reach even higher concentrations depending on the specific regions [108]. Adenosine generation can also be influenced by the type of therapy. For example, radiotherapy of solid tumors can increase expression of adenosine generating enzymes such as CD38 and CD73 in both mouse and human cancer cells in preclinical setting. CD73 blockade coupled with radiotherapy but not CD73 alone increased the tumor infiltration of DCs [109], suggesting therapies can be tested for changes in adenosine signature genes and selected to couple adenosine receptor blockade to obtain better therapy ourcomes.

Early evidence suggested that T cell A2ARs play the major role for suppression of anti-tumor immunity in the tumor microenvironment. Global deletion or blockade of A2AR causes rejection of immunogenic melanoma but fails to suppress growth of parental melanoma cell line. Adoptive transfer of A2AR/A2BR-silenced, antigen specific T cells suppress tumor growth as compared with A2AR/A2BR-proficient T cells [110]. However, recent evidence suggests that intrinsic A2AR and A2BR signaling in myeloid cells also play a major role to create an immunosuppressive microenvironment in solid tumors. Adenosine generated by CD39 and CD73 expressing ovarian cancer cells and cancer associated fibroblasts promotes migration of myeloid cells, which suppress T cell proliferation *ex vivo* [111]. Deletion of A2AR in myeloid cells (Macrophages, dendritic cells and granulocytes) inhibited tumor growth and polarized tumor-associated macrophages into M1 phenotype and strongly suppressed the IL-10 production by macrophages, dendritic cells and monocytic MDSCs [22•].

Upcoming studies have shown that tumor generated adenosine also targets A2BRs to suppress anti-tumor adaptive immune responses [112]. A2BR expression in hematopoietic cells suppress adaptive immune responses to promote tumor growth. A2BR blockade suppresses spontaneous metastasis of tumors. Response is associated with increased infiltration of CD11bdim DCs [112]. A2BR signaling promotes infiltration of MDSCs in mouse melanoma, causing decreased CD8+ T cell responses and increased tumor growth [20,71,72•]. Our recent study also showed that adenosine A2B receptor signaling intrinsically suppresses antigen-presenting cells and limits tumor-infiltration of DCs [12••]. In the absence of A2BR DCs better prime tumor reactive T cells and increase cytotoxic potential of pre-stimulated, adoptively transferred T cells. Adoptively transferred tumor antigen-specific T cells fail to inhibit growth of solid tumors. However, adoptive cell transfer strongly suppress tumor growth when combined with A2BR blockade [12••]. Interestingly myeloid but not CD11c-specific deletion of A2BR reduced lung colonization of melanoma tumors [12••], suggesting A2BR can target multiple myeloid cell subpopulations to promote tumor growth and metastasis.

Tissue protective role of adenosine in renal tissue translates into a tumor promoting role in clinic. Blockade of adenosine A2 receptors in renal cancer patients, who were not responsive to at least two prior therapies, caused tumor shrinkage, which is associated with T cell infiltration into the tumor tissue [15••]. Interestingly, patients, who are most-responsive to the therapy are the ones that express adenosine gene signature in pretreatment biopsies. Adenosine gene signature is defined as set of genes strongly induced by adenosine signaling in PBMCs and consists mostly of myeloid cell-associated cytokines and chemokines [15••], strongly supporting the notion that myeloid expression of adenosine receptors are important to create an immunosuppressive niche in solid tumors, which can be reversed by targeting adenosine receptors.

Future directions

Adenosine has a very limited half-life *in vivo* and *in vitro*. However, inosine, the product of adenosine deamination, is much more stable [113]. Very early studies suggested that inosine can suppress inflammatory responses in macrophages at least partially through adenosine A2A receptors [114]. Recent studies emphasized the importance of extracellular inosine as an alternative carbon source [115] or as a survival factor for T cells

Table 1

Effect of adenosine signaling on myeloid cells in preclinical models of disease

Condition	Receptor	Cell type	Condition/Disease	Model	Effect	Refs
Atherosclerosis and vascular health	A2AR	Macrophage	Atherosclerosis	ApoE$^{-/-}$ mice	Promoting	[89]
	A2AR	Macrophage	Aortic remodeling	Dissection of aorta	Protective	[11]
Autoimmunity	A2AR	Macrophage	Glomerulonephritis	Anti-GBM protein antibody injection	Protective	[19,91]
	A2BR	Dendritic cells	EAU	Immunization against IRBP	Promoting	[92,93]
	A2AR	Macrophages (?)	EAE	Immunization against MOG	Protective	[94,95]
Sepsis and Infection	A2AR	Macrophages (?)	Sepsis	Cecal ligation and puncture	Promoting	[96]
	A2BR	Macrophages	Sepsis	Cecal ligation and puncture	Promoting	[44]
	A2BR	Dendritic cells	Infection	*L. amazonensis* infection	Promoting	[18,98]
Allergy and Asthma	A2BR	Myeloid cells	Asthma/Allergy	hypopharyngeal challenges of CRA	Promoting	[99]
	A2BR	Macrophages	Asthma/Lung Injury	Bleomycin-induced lung fibrosis	Promoting	[6]
	A3, A2BR	Mast cells	Asthma/Allergy	Allergic airway hyperresponsiveness	Promoting	[82,87,101,102]
	A2AR	Myeloid cells	Asthma/Allergy	Sensitization to model antigen	Protective	[104]
Tissue Injury	A2AR	Dendritic cells	Kidney Injury	Clamping of kidney pedicles	Protective	[60]
	A2AR	Neutrophil	Kidney Injury	Ischemia reperfusion	Protective	[105]
	A2AR	Macrophages (?)	Liver injury	Ischemia reperfusion	Protective	[106]
Cancer	A2AR	Myeloid cells	Cancer	Various syngeneic tumor models	Promoting	[22]
	A2BR	MSDC	Cancer	Syngeneic melanoma	Promoting	[20,71,72]
	A2BR	Myeloid cells	Cancer	Various syngeneic tumor models	Promoting	[12,112]

ApoE, Apolipoprotein E; GBM, glomerular basal membrane; EAU, experimental autoimmune uveitis; EAE, experimental autoimmune encephalomyelitis.

through A2AR signaling [116]. Therefore, how adenosine versus inosine will shape immune responses in immune related diseases can be dissected by using tools such as immune-cell specific deletions and treatment with adenosine deaminases with improved half-lives. This will make it possible to modulate inflammatory milieu by actively regulating nucleoside balance for a desired outcome.

Adenosine signaling in myeloid cells strongly influence secretion of cytokines implicated in chronic inflammatory disorders such as inflammatory bowel disease [117] and rheumatoid arthritis [118]. One cytokine, TNFα, which has been targeted for more than 20 years for these conditions [119], is strongly suppressed by adenosine A2 receptors in myeloid cells. Therefore, new studies dissecting the roles of these cell types in these chronic inflammatory or autoimmune conditions will shed light on how adenosine through myeloid cells influence the progression of these diseases. This will lead to better therapeutic approaches.

Adenosine receptors are expressed in both hematopoietic and nonhematopoietic cells. Identification of cell-intrinsic effects of specific adenosine receptors is important to develop targeted therapies, which will prevent counteracting effects of adenosine signaling in different cell types, which may prevent the efficacy of current approaches. Identification of intracellular mediators of adenosine-regulation of immune cells are also important because current, advanced technologies such as antisense oligonucleotide silencing of target proteins or nanoparticles which will naturally accumulate in phagocytic myeloid cells can let us target previously undruggable targets in a cell-specific manner. Accumulated evidence summarized in this review suggests that patients with different immune-related conditions and cancer may benefit from novel therapies targeting adenosine signaling in myeloid cells (Table 1).

Conflict of interest statement

Caglar Cekic currently works at Omeros Corporation as a group leader II and hold stocks or stock options of Halozyme Inc. as a former employee.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

CRediT authorship contribution statement

Caglar Cekic: Conceptualization, Writing - review & editing.

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