Adenosine and adenosine receptors in rheumatoid arthritis

Rheumatoid arthritis is one of the most important chronic, progressive and disabling inflammatory diseases characterized by joint destructive process associated with synovial proliferation and secretion of high levels of proinflammatory mediators including cytokines and growth factors. Early diagnosis and effective therapy are crucial in order to prevent unfavorable outcome with joint deterioration and functional disability. Treatment of rheumatoid arthritis has progressed thanks to the advent of biologic drugs targeting different specific molecules and pathways involved in the inflammation. Considerable advances could be achieved in the identification of novel inflammatory biomarkers, good predictors of outcome. Adenosine, a well-known purine nucleoside interacting with A_1, A_{2A}, A_{2B} and A_3 adenosine receptors, is a potent endogenous inhibitor of inflammatory processes involved in the pathophysiology of a variety of CNS and peripheral diseases. As a consequence, selective agonists and/or antagonists of adenosine receptors could be useful in the treatment of chronic inflammatory diseases such as rheumatoid arthritis, as they are already in other disorders in which inflammatory status is a clinical feature.

KEYWORDS: adenosine ■ adenosine receptor ■ inflammation ■ rheumatoid arthritis

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Rheumatoid arthritis: background

Rheumatoid arthritis (RA) is a chronic, progressive and disabling inflammatory disease characterized by joint destructive process associated with synovial proliferation and secretion of high levels of proinflammatory mediators including cytokines, metalloproteases and growth factors.

RA is usually characterized by symmetric inflammatory polyarthritis and affects approximately 0.5–1% of the general population worldwide [1]. Like for other rheumatic diseases, the pathophysiology of RA is not yet fully understood. One potential synthesis of the data available on pathogenesis suggests that the innate immunity activation and a favorable genetic background are the basis of the induction phase that gets the joint ready for the subsequent coming of inflammatory and immune cells [2,3]. The proinflammatory mediators released act on different cell populations including lymphocytes, neutrophils, endothelial cells, synoviocytes, osteoclasts and chondrocytes by inducing the maintenance of a Th1 inflammation with angiogenesis and chemotaxis [4]. The relative abundance of Th1 cells and cytokines suggests that the synovium resembles a Th1-like delayed-type hypersensitivity reaction. Th2 cytokines and cellular responses that normally suppress Th1 activation are nearly absent, suggesting the possibility that the lack of T-cell activation along the Th2 pathway in RA contributes to disease perpetuation. Several studies indicate that a particular type of T cell, Treg cells (thymus-derived natural regulatory T cells), and in particular the subset characterized by the production of IL-17 called Th17 cells, may play an important role in the pathogenesis of RA. The circulating Th17 and Th17/Th1 cell frequencies are different in patients with early or established RA, and active or inactive disease [5].

Among the released inflammatory mediators IL-1β, TNF-α and IL-6 are the pivotal cytokines in the physiopathology of the synovial inflammation that activate several cell types, including lymphocytes, neutrophils, endothelial cells, osteoclasts, chondrocytes and synoviocytes, and upregulate a number of pathways linked to the inflammation. Bone erosions are subsequently caused by osteoclasts, whereas cartilage dissolution results from proteolytic enzymes produced by synoviocytes in the pannus or synovial fluid neutrophils [2].

It is well reported that immunoreactivity can be identified before clinical disease and manifested by the production of rheumatoid factor (RF) and anticitrullinated peptide antibodies (ACPA) that contributes to erosiveness and severity [6–8].

Genes play a key role in susceptibility to RA and disease severity. Class II MHC genes, especially genes containing a specific five-amino

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acid sequence in the hypervariable region of HLA-DR4, are the most prominent genetic association. Newly defined genetic associations, including polymorphisms in the *PTPN22* and *PADI 4* genes, and many cytokine promoter polymorphisms, population-specific genes and other undefined genes are reported as genetic markers of diagnosis and prognosis [2,6,9].

It has been recognized that early therapeutic intervention improves clinical outcomes and reduces the accrual of joint damage and disability. The optimized use of old therapies and the availability of new drugs have dramatically enhanced the success of RA management [10]. Recently, a joint working group from the ACR and the European League Against Rheumatism (EULAR) revised the old criteria set and developed a new approach to classifying RA [11,12]. The work, which was among patients newly presenting with undifferentiated inflammatory synovitis, focused on identifying factors that best discriminated between those who were and those who were not at high risk for persistent and/or erosive disease, that is to say RA. In the new criteria set, classification as 'definite RA' is based on the confirmed presence of synovitis in at least one joint, absence of an alternative diagnosis that better explains the synovitis, and achievement of a total score of 6 or greater (of a possible 10) from the individual scores in four domains: number and site of involved joints (score range: 0–5), serologic abnormality (score range: 0–3), elevated acute-phase response (score range: 0–1) and symptom duration (two levels; range: 0–1). These new criteria focus on findings that facilitate earlier recognition of RA and outcome prediction.

Treatment of RA

Currently, optimal management of RA is needed, within 3–6 months after the onset of disease, since a narrow 'window of opportunity' is considered to be suitable to achieve remission [13]. Early prognostic assessment in order to establish the risk of aggressive disease is crucial to guide the therapeutic approach. A good early response to treatment predicts better long-term response in the following 5 years [14]. There is increasing acceptance of paradigms of adjusting therapy to achieve a predefined goal, such as remission or low-disease activity ('treat to target') with frequent monitoring and strategy adjustments, and if necessary ('tight control'), until the target is reached [15–17]. The use of a composite measure of disease activity was recommended, such as the Disease Activity Score Assessing 28 Joints (DAS-28), the Simplified Disease Activity Index (SDAI) or the Clinical Disease Activity Index (CDAI) [18,19].

In recent decades, there was an inversion of the pyramid, with earlier and more intensive and innovative approach to RA treatment [20,21]. After a long time focused on the use of conventional disease-modifying antirheumatic drugs (DMARDs) alone or in combination, the advent of 'biologic' drugs, which are able to block the cytokine pathway and B- and/or T-cell activation, has profoundly changed the therapeutic scenario and, consequently, the current strategy adopted to cure RA [10,22]. Glucocorticoids have been, and continue to be, a part of the treatment strategy throughout the years [23]. The recent EULAR recommendations for the treatment of RA identify three phases of therapy [15]. Phase one is the initiation of DMARD treatment as monotherapy, immediately after diagnosis of RA. The recommended drug is methotrexate (MTX), widely seen as an anchor drug in RA [24,25]. Phase two is the escalation of therapy by switching to a different DMARD or to a combination therapy. If this approach fails to achieve the target of clinical remission (or low disease activity) within 3–6 months, and the patients have poor prognostic factors (high disease activity, early joint damage, high levels of RF or ACPA), the new escalation of therapy is the addition of a biologic drug, TNF blockers. However, to date, approximately one-third of patients treated with anti-TNF-α agents show an inadequate response or develop side effects requiring discontinuation of therapy [26]. Phase three is in case of anti-TNF failure or lack of efficacy and/or toxicity, the recommended approach is to change the biologic treatment by switching to an alternative TNF antagonist (in combination with a synthetic DMARD) or replacing the biologic treatment with an alternative with different target therapy (B-cell-targeted therapy, IL-6, CTLA-4 modulation).

Under this point of view, it is very important to identify predictive factors related to a better or poor response or to major risk of toxicity aimed to guide the therapeutic choice and faster adjust the therapeutic intervention [17,26,27].

Adenosine & adenosine receptors

Adenosine is a purine nucleoside identified as an endogenous and ubiquitous molecule regulator of different tissues and cell functions [28]. Adenosine is generated in the extracellular space by the breakdown of ATP through a series of ectoenzymes, including apyrase

and ecto-5′-nucleotidase [29]. Adenosine is phosphorylated to AMP by adenosine kinase or degraded to inosine by adenosine deaminase [30]. Adenosine production from the hydrolysis of AMP is mediated by a cytosolic 5′-nucleotidase or by the hydrolysis of S-adenosylhomocysteine [30]. The levels of adenosine in the interstitial fluids are in the range of 20–200 nM but they dramatically increase under metabolically unfavorable conditions [31]. Adenosine effects are widespread and mediated by the interaction with different adenosine receptor (AR) subtypes, which are able to modulate cell signaling transduction (Table 1) [32]. ARs are characterized by seven transmembrane domains with the *N*- and *C*-terminus in the extracellular side, and the presence of intracellular and extracellular loops [33]. A_1AR stimulation through the interaction with Gi/Go proteins modulates different cellular effectors as adenylate cyclase (AC) and phospholypase C (PLC) [34]. The A_{2A} and A_{2B}ARs through coupling with Gs proteins activate AC and increase cyclic AMP levels [31]. A_3ARs via the interaction with Gi inhibit adenylate cyclase decreasing cyclic AMP accumulation and protein kinase A (PKA) activity. In addition, A_3ARs via coupling with Gq proteins stimulate PLC, causing an increase of calcium levels from intracellular stores, and modulate the protein kinase C (PKC) activity (Figure 1) [35,36].

The widespread distribution in different cells and tissues of the ARs could suggest their potential involvement in various pathologies and the possible use as selective pharmacological targets.

A_1 adenosine receptors

A_1ARs are widely distributed not only in the CNS, but also in peripheral tissues [37]. Adenosine, by A_1AR activation, produces inhibition of neurotransmitter release and induces neuronal hyperpolarization mediating sedative, anticonvulsant, anxiolitic and locomotor depressant effects [38]. Literature evidence has indicated the involvement of A_1ARs in controlling pain transmission, producing antinociceptive effects in various animal models [39–42]. In the cardiovascular system, A_1ARs mediate negative chronotropic, dromotropic and ionotropic effects, suggesting the potential use of A_1AR agonists as cardioprotective agents and in the treatment of arrhythmias and atrial fibrillation [43]. In the kidney, A_1ARs mediate vasoconstriction, decrease glomerular filtration rate, inhibit renin secretion and their inhibition could represent a novel strategy for the treatment of hypertension and edema [44]. The role of adenosine in regulating the respiratory system is well known and elevated levels of adenosine have been found in bronchoalveolar lavage (BAL), blood and exhaled breath condensate of patients with asthma and chronic obstructive pulmonary disease (COPD). A_1AR antagonists could also be used in asthma and in COPD since adenosine induces acute bronchoconstriction via stimulation of A_1ARs [45,46].

A_{2A} adenosine receptors

It is well known that A_{2A}ARs are found ubiquitously in the body, and their expression is highest in the immune system and the striatopallidal system in the brain [47,48]. Several studies have suggested the possible involvement of A_{2A}ARs in the pathogenesis of neuronal disorders, including Huntington's disease and Parkinson's disease. In particular, an aberrant increase of A_{2A}AR density in peripheral blood cells of Huntington's disease and Parkinson's disease patients in comparison with age-matched healthy subjects has been demonstrated [49,50]. Accordingly, A_{2A} antagonists currently constitute an attractive nondopaminergic

Table 1. Adenosine receptor subtypes, distribution and G protein coupling.

Receptor subtype	Distribution	Receptor coupling
A_1AR	Brain (cortex, hippocampus, cerebellum), spinal cord, eye, adrenal gland, atria, liver, kidney, adipose tissue, salivary glands, esophagus, colon, atrium and testis	Gi, Go
A_{2A}AR	Striatopallidal GABAergic neurons, immune cells, heart, lung and blood vessels	Gs, Golf
A_{2B}AR	Spleen, cecum, colon, bladder, lung, eye, mast cells and vasculature	Gs, Gq/11
A_3AR	Lung, liver, immune cells, kidney, brain, heart and gastrointestinal tissues	Gi, Gq/11

AR: Adenosine receptor; Gi: Inhibitory G protein; Go: Go protein; Golf: Olfactory G protein; Gq/11: G-protein q/11; Gs: Stimulatory G protein.

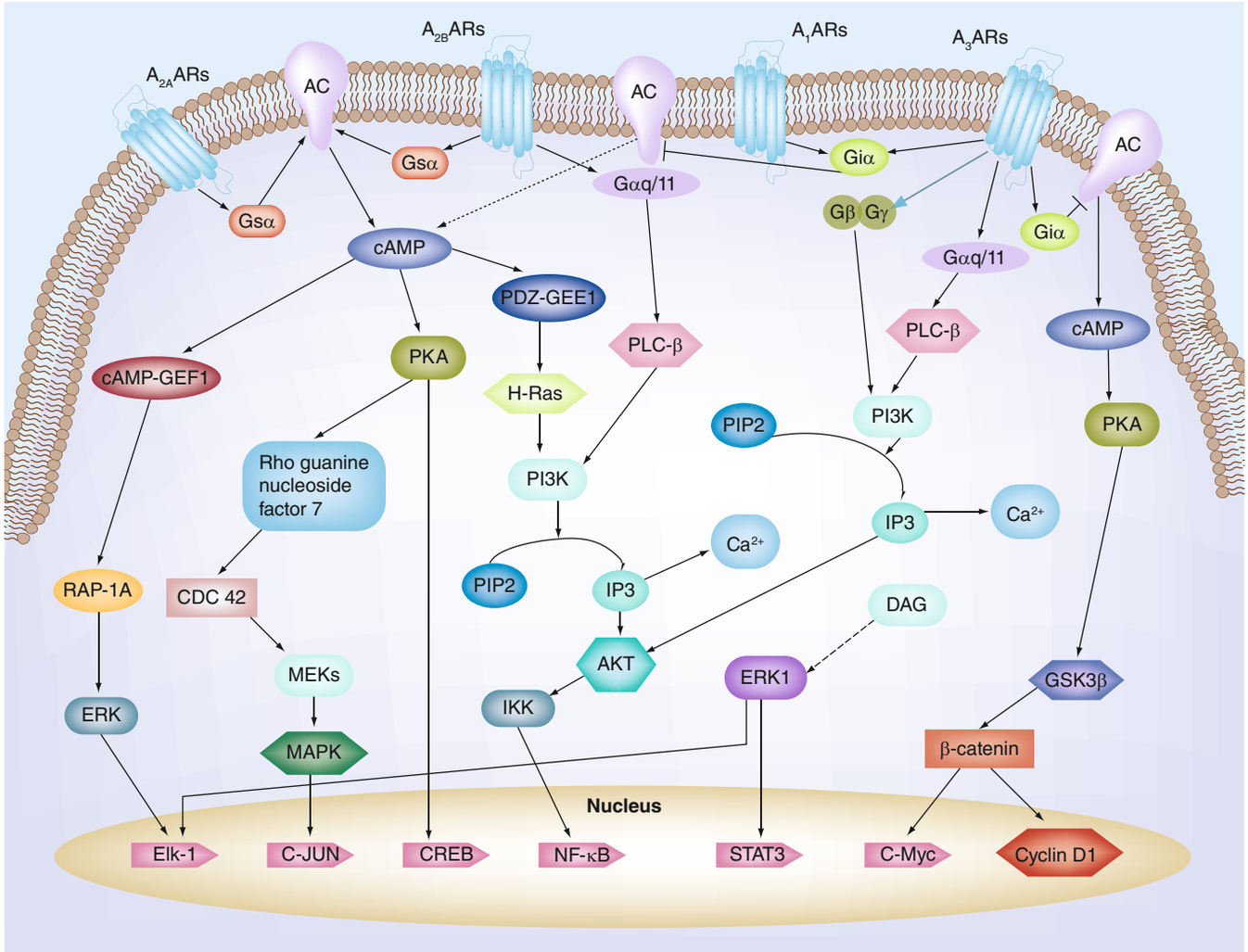

Figure 1. Principal signaling pathways activated by adenosine receptors.
AC: Adenylate cyclase; AR: Adenosine receptor; cAMP: Cyclic adenosine monophosphate; cAMP-GEF1: cAMP-regulated guanine nucleotide exchange factor 1; CDC42: Cell division control protein 42; DAG: Diacylglycerol; Elk-1: E-26-like transcription factor-1; ERK: Extracellular signal-regulated kinase; Gαq/11: G-protein q/11 α-subunit; Gβ: G-protein β-subunit; Gγ: G-protein γ-subunit; Giα: Inhibitory G-protein symbol-subunit; Gsα: Stimulatory G-protein α-subunit; GSK3β: Glycogen synthase kinase 3β; IKK: Inhibitor of NF-κB (IkB) kinase; IP3: Inositol 1,4,5-trisphosphate; MAPK: Mitogen-activated protein kinase; MEK: Mitogen-activated protein kinase kinase; NF-κB: Nuclear factor-κB; PDZ-GEF1: PDZ domain-containing guanine nucleotide exchange factor 1; PI3K: Phosphoinositide 3-kinase; PIP2: Phosphatidylinositol 4,5-bisphosphate; PKA: Protein kinase A; PLC-β: Phospholipase C-β; STAT3: Signal transducer and activator of transcription 3.

option for use in the treatment of Parkinson's disease [51]. Adenosine has important protective effects on the cardiovascular system. Activation of the A_{2A}AR subtype on coronary smooth muscle cells, endothelial cells and monocytes/macrophages results in vasodilation, neoangiogenesis and inhibition of proinflammatory cytokine production [52,53]. An upregulation of A_{2A}AR was found in peripheral circulating cells of end-stage chronic heart failure patients [54]. Literature evidence reports an important role of A_{2A}ARs in chronic airway diseases, as suggested by the genetic removal of A_{2A}AR that leads to enhanced pulmonary inflammation, mucus production and alveolar airway destruction [55].

A_{2B} adenosine receptors

A_{2B}AR is expressed in the brain, spleen, lung, colon, heart and kidney, where it is primarily localized to the vasculature [56]. A_{2B}AR expression has been detected in vascular endothelium and smooth muscle cells where it has been implicated in the regulation of vascular tone through receptor-mediated vasodilatory effects [57]. Activation of A_{2B}ARs prevent cardiac remodeling after myocardial infarction and exert protective effects from infarction in ischemic postconditioning [58]. Degranulation of mast cells and subsequent mediator release is an important component of the bronchoconstriction observed in asthma [59,60]. Importantly, investigation of ARs on mast

cells implicate A_{2B}AR signaling in degranulation and mediator release [61]. Identification of A_{2B}AR signaling as a potential pathway in the pathogenesis of asthma prompted its investigation in other chronic conditions affecting the lung, including COPD and idiopathic pulmonary fibrosis [62]. A protective role for A_{2B}AR antagonists has been proposed in the resolution of pulmonary inflammation and fibrotic processes [63,64]. In addition, it has been observed that A_{2B}ARs are downregulated in COPD patients probably due to oxidative/nitrosative stress [65]. It has also been reported that A_{2B}ARs in intestinal epithelial cells mediated Cl^- secretion through an increase in cyclic AMP levels [31].

A_3 adenosine receptors

The tissue distribution of A_3ARs has been well investigated and suggests that these receptors are primarily expressed in lung, liver and immune cells. A minor expression of A_3ARs is reported in kidney, heart, brain and gastrointestinal tissues [36]. It has been reported that A_3ARs activation in the brain may contribute to neurotransmission. [66]. A proconvulsant effect of A_3ARs has been observed in the immature brain, suggesting the possibility of facilitating seizure-induced neuronal damage [67]. A nociceptive role for A_3ARs involving both CNS and proinflammatory effects in peripheral tissues has been highlighted [68]. Moreover, prolonged A_3AR stimulation is able to transform the effects from protective to injurious, increasing the excitotoxicity [69]. Glial A_3AR activation by high adenosine levels, caused by a brain injury, may be implicated in neuroinflammatory tissue responses [70]. There is also evidence that A_3ARs enhance cellular antioxidant capacity that contribute to vasoprotection and reduce cardiac myocyte death, suggesting a strong support for an A_3-dependent cardioprotective response including the reduction in infarct size, inhibition of apoptosis and improvements in postischemic contractile function [71]. Moreover A_3ARs stimulate vascular growth acting with A_{2B}ARs to promote angiogenesis via the expression of angiogenic factors in mast cells or stimulate HIF-1α and VEGF expression [72]. Transcript levels of A_3ARs are elevated in lung biopsies of patients with asthma or COPD where their activation mediated the inhibition of eosinophil chemotaxis [73,74]. By contrast, mice treated with selective A_3 antagonists resulted in a marked attenuation of pulmonary inflammation, reduced eosinophil infiltration into the airways and decreased airway mucus production [75].

Adenosine is present at high concentrations in cancer tissues and in the interstitial fluid of several tumors, at concentrations sufficient to interact with ARs [31]. A_3ARs are present in different types of tumor cells and are involved in the tumor growth and the regulation of the cell cycle, and mediate both pro- and anti-apoptotic effects closely associated with the level of receptor activation [76–78]. A_3AR density was upregulated in colon carcinoma tissues closely correlated to the disease severity. In addition, the alteration of A_3ARs reflected a similar behavior shown in lymphocytes or neutrophils derived from colon cancer patients, suggesting that these receptors may represent an interesting biological marker [79].

ARs are present in many cell types including platelets, lymphocytes, eosinophils, neutrophils, mast cells and macrophages where they mediate pro- and anti-inflammatory effects [50,54,80]. Several authors have demonstrated that human circulating blood cells (platelets, lymphocytes and neutrophils) reproduce the same receptor alterations known to be at the basis of specific diseases mainly in the cardiovascular system and CNS [38,49,54,81,82]. As a consequence, peripheral blood cells could represent a useful and easily available model to monitor receptor changes during the course of chronic rheumatic inflammatory diseases and to assess the efficacy of specific pharmacological treatments [83–85].

Role of adenosine in the control of inflammation

Substantial lines of evidence suggest that A_{2A}ARs are able to mediate the majority of anti-inflammatory effects of endogenous adenosine [86,87]. In particular, the ability of A_{2A}AR activation to suppress cytokine and chemokine expression by immune cells is probably the dominant mechanism involved. Expression of A_{2A}ARs has been found on most inflammatory cells, where it exerts various anti-inflammatory actions [86]. In neutrophils, adenosine, acting at A_{2A}ARs, regulates the production of different cytokines including TNF-α, macrophage inflammatory protein (MIP)-1α, MIP-1β, MIP-2α and MIP-3α [88]. Studies using A_{2A}-knockout models have shown that A_{2A}AR activation inhibits IL-2 secretion by naive $CD4^+$ T cells thereby reducing their proliferation, confirming the immunosuppressive effects of A_{2A} AR stimulation [89,90]. It has also been demonstrated that A_{2A}ARs play an important role in the promotion of wound healing and angiogenesis [91]. Adenosine has been reported to reduce inflammation in several *in vivo* models, suggesting a potential value of this purine nucleoside as a therapeutic mediator of

inflammatory joint disease able to limit articular cartilage degeneration. In synoviocytes obtained from osteoarthritis patients, the activation of A_{2A}ARs inhibited p38 MAPK and NF-κB pathways, as well as the production of TNF-α and IL-8 [92]. These results indicate that A_{2A}ARs may represent a potential target in therapeutic modulation of joint inflammation. Activation of the A_{2A}ARs during reperfusion of various tissues has been found to markedly reduce ischemia-reperfusion injury. In particular, in a model of ischemia-reperfusion liver injury, A_{2A}AR stimulation with the selective agonist ATL146e was associated with decreased inflammation and profoundly protects mouse liver from injury when administered at the time of reperfusion [93]. Adenosine, acting at A_{2A}ARs, plays an important role in the pathogenesis of hepatic fibrosis in response to hepatotoxins. In particular, it has been demonstrated that A_{2A}ARs are expressed on human hepatic stellate cell lines and A_{2A}AR occupancy promotes collagen production by these cells. Furthermore, mice lacking A_{2A}ARs are protected from developing hepatic fibrosis in two different hepatic fibrosis models [94]. It is well reported that hypoxia-induced accumulation of adenosine may represent one of the most fundamental and immediate tissue-protecting mechanisms, with A_{2A}ARs triggering off signals in activated immune cells. In these regulatory mechanisms, oxygen deprivation and extracellular adenosine accumulation serve as 'reporters', while A_{2A}ARs serve as 'sensors' of excessive tissue damage [95]. The hypoxia-adenosinergic tissue-protecting mechanism is triggered by inflammatory damage to blood vessels, interruption in oxygen supply, low oxygen tension (i.e., hypoxia) and by the hypoxia-driven accumulation of extracellular adenosine acting via immunosuppressive, cAMP-elevating A_{2A} receptors [96]. Another area where A_{2A}ARs signaling has received attention as a potential therapeutic target is the GI tract. Studies have highlighted the protective effects of A_{2A} receptor activation in various animal models of colitis, and these protective effects can be ascribed to two major mechanisms: decrease of inflammatory-cell infiltration and function in the mucosa, and increased activity of Treg cells [34,89,97]. A_{2A}AR stimulation was found to attenuate gastric mucosal inflammation induced by indomethacin, blocking secondary injury due to stomach inflammation through a reduction of myeloperoxidase and proinflammatory cytokines [98]. Adenosine levels are increased in the lungs of individuals with asthma or COPD, and ARs are known to be expressed on most, if not all, inflammatory and stromal cell types involved in the pathogenesis of these diseases [99]. In addition, pharmacological treatment of allergic rats with an A_{2A}AR agonist resulted in diminished pulmonary inflammation [100]. A recent study in an ADA-deficient model demonstrated that genetic removal of A_{2A}ARs leads to enhanced pulmonary inflammation, mucus production and alveolar airway destruction [55]. Furthermore, A_{2A}ARs induced on iNKT and NK cells reduced pulmonary inflammation and injury in mice with sickle cell disease, improving baseline pulmonary function and preventing hypoxia-reoxygenation-induced exacerbation of pulmonary injury [101]. These data further confirm the involvement of A_{2A}ARs in the anti-inflammatory networks in the lung. A study performed in peripheral lung parenchyma demonstrated that affinity and/or density of adenosine receptors are altered in patients with COPD compared with control smokers with normal lung function. Moreover, there was a significant correlation between the density and affinity of adenosine receptors and the forced expiratory volume/forced vital capacity ratio, an established index of airflow obstruction. In particular A_{2A}, as well as A_3ARs, was found to be upregulated in COPD patients [80]. This alteration may represent a compensatory response mechanism and may contribute to the anti-inflammatory effects mediated by the stimulation of these receptors. Given the central role of inflammation in asthma and COPD, substantial preclinical research targeted at understanding the function of A_{2A}ARs in models of airway inflammation has been performed. In Phase II trials for COPD the use of an A_{2A}AR agonist named UK432,097 that was identified as an inhaled anti-inflammatory drug without an effect on blood pressure was reported with the aim to highlight the impressive pedigree of A_{2A}ARs as a potential anti-inflammatory agent [102]. It is well known that the anti-inflammatory effect of adenosine is also mediated by the activation of A_3ARs that are present in immune cells and involved in the physiopathologic regulation of inflammatory and immune processes. Several results from *in vitro* and *in vivo* studies suggest that the activation of the A_3ARs can be both pro- or anti-inflammatory depending on the cell type examined or on the animal species considered [103]. Binding and functional studies have shown that human neutrophils expressed A_3ARs primarily coupled to the inhibition of adenylate cyclase and calcium signaling, mediating the inhibition of oxidative burst,

representative of anti-inflammatory activity [104]. A_3ARs are also responsible for the inhibition of superoxide production and chemotaxis of mouse bone marrow neutrophils [105]. It has been reported that A_3ARs are present on human eosinophils, coupled to signaling pathways linked to cell activation and are able to protect eosinophils from apoptosis and inhibit the chemotaxis process [76]. The effects produced by A_3AR activation of macrophages seem to indicate an anti-inflammatory effect of this receptor subtype. In particular, A_3ARs suppressed TNF-α release induced by endotoxin CD14 receptor signal transduction pathway from human monocytes and murine macrophages [38]. A_3ARs directly control histamine release by antigen-stimulated mouse mast cells, because the stimulatory effect of exogenous adenosine noted in wild-type mast cells is not observed in A_3AR-knockout mast cells [106]. Literature data support a role for adenosine in dictating dendritic cell function, promoting the recruitment of immature dendritic cells to sites of inflammation and injury via A_3AR [107,108]. It has been proposed that the anti-inflammatory effect elicited by A_3AR activation could involve the inhibition of the PI3K/Akt and NF-κB signaling pathways [109,110]. The stimulation of A_3ARs decreased proliferation and exerted a cytotoxic and proapoptotic effect on malignant mesothelioma cells and on human healthy mesothelial cells exposed to asbestos through the deregulation of the Akt/NF-κB cell survival pathway [111]. The possibility that A_3ARs plays a role in the development of cancer has aroused considerable interest in recent years. In particular, A_3ARs were found to be highly expressed in tumor cells and tissues but not in normal cells or adjacent tissue. Interestingly, high A_3AR expression levels were found in peripheral blood mononuclear cells derived from tumor-bearing animals and cancer patients, reflecting receptor status in the tumors [79,112].

Adenosine pathway modulation in RA

Several studies have shown the relationship between the adenosine pathway and joint inflammation in RA *in vitro* and *in vivo* [84]. A_1, A_{2A}, A_{2B} and A_3ARs have been characterized, by using binding and functional assays, in human synoviocytes that represent key cells closely associated to articular pathologies [92]. *In vitro* stimulation of A_{2A} and A_3ARs has been shown to alter the cytokine network by decreasing inflammatory cytokine secretion by macrophages. Recently, a phosphorylated A_{2A}AR agonist was demonstrated to be a potent immunosuppressant in a model of arthritis acting by an upregulation of CD73 and A_{2A}AR expression [113]. In animal models of acute and chronic inflammation, nonselective AR antagonists reversed the anti-inflammatory effects of MTX. Furthermore, in A_{2A} and A_3ARs-deficient mice, MTX failed to suppress inflammation in the air-pouch model, thus suggesting the pivotal role of these AR subtypes in triggering an anti-inflammatory pathway in RA [91,114]. Studies on knockout animals have shown evidence that adenosine acting A_{2A} and A_3ARs mediates the anti-inflammatory effects of low-dose MTX. In adjuvant-induced arthritis in rats and in peripheral blood mononuclear cells from RA patients, MTX treatment has been shown to enhance the anti-inflammatory effects of typical A_3AR agonists via an upregulation of A_3AR expression. In RA patients, the overexpression of A_3ARs has been directly correlated with high levels of pro-inflammatory cytokines acting via upregulation of NF-κB [115–118]. Recently, it has been proposed that synovial tissue expresses ARs and there is a relationship between MTX exposure and adenosine receptor expression within the synovium [119]. Besides, among the theories about the mechanism of action of MTX, the primary anti-inflammatory action is attributable to adenosine release. MTX increases levels of adenosine, via inhibition of amino-imidazolo-carbossi-adenosine-ribonucleoside (AICAR) transformylase enzyme. The net effect of AICAR accumulation is a rise in intracellular AMP and adenosine levels [120].

Atherosclerosis is another interesting topic in which we can find correlations between RA and adenosine. RA patients have an increased mortality secondary to an increased atherosclerosis due to chronic inflammation and chronic steroid therapy. Adenosine pathway and MTX are involved in the atherogenesis. MTX, via adenosine, acting upon the A_{2A}ARs and A_3ARs produces an increased expression of important molecules of the reverse cholesterol transport system, a basic cholesterol homeostatic mechanism [121]. There are interesting data about A_{2B}ARs and the regulation of atherosclerosis in a mice model but, certainly, we are a long way from using these agents for protection of atherogenenesis [122]. The overexpression of A_3ARs in RA was directly correlated to high levels of proinflammatory cytokines acting via an upregulation of NF-κB, which is a key player in the pathogenesis of arthritis diseases [117]. In RA patients, adenosine suppressed the elevated levels of proinflammatory cytokines such as TNF-α

and IL-1β [123]. Recently it has been shown that A_{2A} and A_3ARs are upregulated in untreated RA patients and in MTX-treated RA patients. Treatment with anti-TNF-α normalized A_{2A} and A_3ARs expression and functionality [83]. It has been reported that A_3AR agonists prevented cartilage damage, osteoclast/osteophyte formation, bone destruction and markedly reduced pannus formation and lymphocyte formation [124]. The A_3ARs was also identified as a novel anti-inflammatory target that is upregulated in RA, psoriasis and Crohn's disease, if compared with healthy subjects it is associated with an altered PI3K-PKB/Akt signaling pathway and NF-κB activation [125]. The findings showing A_{2A}ARs and A_3ARs upregulation in RA patients suggest the utilization of these receptors as therapeutic targets, modulating them with specific and well-known agonists (Table 2). Clinical evidence in RA patients shows that A_3AR agonist pharmacological treatment modulates an improvement in signs and symptoms [117]. In regard to A_3ARs, there are data from animal models, healthy subjects (Phase I studies) and RA patients (Phase II studies). Upon oral treatment with the selective A_3AR agonist named CF101 the disease was ameliorated and a marked decrease in clinical manifestations was recorded. CF101 treatment reduced inflammation, pannus formation, cartilage destruction and bone resorption and lyses [126]. In a Phase I study in healthy subjects, CF101 was found to be safe and well tolerated with a linear pharmacokinetic activity [127]. In a Phase IIa study in RA patients, CF101 oral administration twice daily for 12 weeks was shown to be safe, well tolerated and able to mediate an improvement of disease signs and symptoms, suggesting the development of these type of drugs as antirheumatic agents. Interestingly, the expression level of A_3ARs at baseline was directly correlated with the high grade of efficacy, suggesting its use as a biomarker for the pharmacodynamic and therapeutic effects of this novel agent [116,117]. The anti-inflammatory effect of A_3AR was also shown in fibroblast-like synoviocytes derived from synovial fluid of RA patients [109]. In particular, the effect of a novel A_3AR agonist, CF502, with high human A_3AR affinity and selectivity is now under investigation. CF502 induces a dose-dependent inhibitory effect on the proliferation of fibroblast-like synoviocytes via deregulation of the NF-kB signaling pathway. Furthermore, CF502 markedly suppresses the clinical and pathological manifestations of adjuvant-induced arthritis in a rat experimental model. Other data have shown that the use of A_{2A}

Table 2. Adenosine receptors as an effectiveness target for treatment of rheumatoid arthritis: published experience.

Study (year)	Pharmacological target	Type of study	Summary	Ref.
Fishman *et al.* (2006)	A_3AR agonist (CI-IB-MECA-CF101)	Animal model	A_3AR stimulation exerted a potent anti-inflammatory effect manifested in the improvement of the disease clinical and histopathological score (ten rats treated for 14 days)	[118]
Ochaion *et al.* (2006)	A_3AR agonist (CI-IB-MECA-CF101)	Animal model	MTX induces increased A_3AR expression and potentiated the inhibitory effect of CF101 supporting combined use of these drugs to treat RA (ten rats treated for 25 days)	[115]
Ochaion *et al.* (2008)	A_3AR agonist (MRS3558-CF502)	Animal model	CF502 inhibited fibroblast-like synoviocyte growth and the inflammatory manifestations of arthritis, supporting the development of A_3AR agonists for the treatment of RA (30 rats treated for 14 days)	[109]
Bitto *et al.* (2011)	A_{2A}AR agonist (PDRN)	Animal model	PDRN ameliorated clinical signs of arthritis, improved histologic damage and reduced the cartilage expression of proinflammatory mediators (14 mice treated for 24 days)	[128]
Flögel *et al.* (2012)	A_{2A}AR agonist (chet-AMP)	Animal model	Phosphorylated A_{2A}AR agonists may serve as a promising new group of drugs for targeted immunotherapy of inflammation	[113]
van Troostenburg *et al.* (2004)	A_3AR agonist (CI-IB-MECA-CF101)	Phase I (healthy subjects)	CF101 was safe and well tolerated in healthy subjects (parallel group, ascending dose, double-blind and placebo-controlled trial, 15 healthy men for a single dose, 28 healthy men for a repeated dose for 7 days)	[127]
Silverman *et al.* (2008)	A_3AR agonist (CI-IB-MECA-CF101)	Phase IIa (patients)	CF101 administered to 50 patients for 12 weeks resulted in improvement in signs and symptoms of RA (parallel groups, dose finding trial)	[117]

AR: Adenosine receptor; MTX: Methotrexate; PDRN: Polydeoxyribonucleotide; RA: Rheumatoid arthritis.

and A_3AR agonists significantly reduces NF-κB levels and inhibits IL-1β, IL-6 and TNF-α release in mononuclear cells from peripheral blood samples of RA patients [85], suggesting the involvement of these ARs in the modulation of inflammatory response. It has also been found that the production of metalloproteinase (MMP) 1 and 3 was inhibited by A_{2A} or A_3AR agonists in RA patients more than in healthy controls, demonstrating the direct involvement of the adenosine receptor subtypes in the mechanism regulating joint damage in RA [85]. An inverse correlation between DAS and A_{2A}ARs and A_3ARs density was recently found, suggesting that an endogeneous activation of these ARs could attenuate the disease [85]. Thus, A_{2A} and A_3ARs upregulation in RA can be seen as a compensatory mechanism to better counteract the inflammatory status. The A_{2A}ARs modulation was investigated in an animal model where the administration of a homemade agonist significantly attenuated the development of arthritis and reduced the signs of the disease [128].

Future perspective

The role of adenosine in the modulation of chronic inflammation has been appreciated only in recent years. Interestingly, an overexpressed endogenous anti-inflammatory pathway may be a potential target therapy in RA. As a consequence, A_{2A} and A_3ARs agonists may represent a novel pharmacological treatment alone or in combination with traditional therapy, such as MTX. In the future, more preclinical and clinical studies are warranted to investigate the effect of selective A_{2A} and A_3ARs agonists in RA in order to translate these important findings into valuable benefits for RA patients. Adenosine pathway modulation may one day find its place in the therapeutic setting, especially in patients who are not fully responsive, at first as combination therapy, to obtain a more complete anti-inflammatory and, why not, atheroprotective effect.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Rheumatoid arthritis (RA) is a chronic, progressive and disabling inflammatory disease characterized by a joint destructive process associated with synovial proliferation and secretion of high levels of proinflammatory mediators including cytokines, metalloproteases and growth factors.
- It is widely accepted that RA must be treated early with effective therapy in order to prevent unfavorable outcome and disability.
- In the last 15 years, the therapeutic approach has undergone a series of innovative impulses directed towards an earlier and more aggressive treatment with a good efficacy.
- Since many patients show an inadequate response or develop side effects, researchers are always looking for new targets of therapy in the setting of RA. Exploration of adenosine mechanism revealed adenosine receptors as a potentially useful target of therapy in RA.
- Adenosine receptors are upregulated in active RA and stimulation of A_{2A} and/or A_3 adenosine receptors mediated a reduction of inflammation via nuclear factor-κB signaling pathway and a decrease of proinflammatory cytokines. Although it is very early to include this target in the therapeutics and we are along way from using these agents, experimental data with A_{2A} and A_3 adenosine receptors agonists could suggest the development of a novel treatment for RA based on adenosine receptor modulation inducing a marked anti-inflammatory effect.

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