Immunology Letters 214 (2019) 55–64

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Review

# ATP and adenosine: Role in the immunopathogenesis of rheumatoid arthritis

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ARTICLE INFO

*Keywords:*
Arthritis
ATP
Adenosine
Purinoreceptors
CD39
ADA

ABSTRACT

Rheumatoid arthritis (RA) is a classic inflammatory autoimmune disease. Local joint destruction and extra-articular manifestations of RA deeply compromise the life quality of the affected patients. RA immunopathogenesis depends on continuous immunogenic activation in which the purinergic system participates. The purinergic system comprises the signaling and metabolism of purines such as adenosine triphosphate (ATP) and adenosine. ATP signaling is involved in the activation and maintenance of the inflammatory state of RA through the activation of P2X7 and the production of cytokines, which orchestrate the pathogenesis of RA. The breakdown of ATP through the CD39/CD73 axis produces adenosine, which mostly inhibits the inflammatory process through activation of specific P1 receptors. Adenosine is hydrolyzed by adenosine deaminase (ADA) that interacts with other molecules playing additional roles in this disease. This review explores the release, metabolism, and the effects of binding of ATP and adenosine to their respective receptors in the context of RA, as well as their potential use as biomarkers and therapeutic targets.

## 1. Introduction

### 1.1. Onset and maintenance of rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic, inflammatory, and autoimmune disease which preferentially affects small peripheral joints such as those in the wrist or hand. Progressive articular damage leads to deformation, local deficiencies, and multiple system inflammation [1]. Its prevalence in adults around the world is about 1%, affecting mostly females and increasing with age [2,3].

The persistent inflammatory process of RA results in progressive damage of joints and may lead to pain and fatigue and systemic complications. The symptoms deeply affect the quality of life of RA patients [4] and trigger the development of depression further affecting the well-being [5]. Like other autoimmune diseases, RA pathophysiology is not fully understood and shows a diversity of clinical characteristics among patients and populations. A genetic predisposition coupled with environmental factors that trigger the activation of inflammatory and immune cells is thought to be involved in RA development [6,7].

Loss of B and T cell self-tolerance leads to self-reactivity, the initial step in the development of RA. However, evidence has shown that, much before the first symptoms of inflammation, antibodies against self-antigens are already present, indicating the onset of RA [8]. As the self-reaction expands, the release of cytokines and self-antibodies initiates an attack to the synovial joints. The recruitment of myeloid cells and the damage repair mechanisms by endothelial cells, fibroblasts, chondrocytes, and osteocytes sustain the continuous inflammatory status of RA [9,10]. Inflammation-induced neoangiogenesis further facilitates this process [11].

### 1.2. Purinergic system modulates immune responses

The purinergic system is involved in a variety of physiologic and pathologic processes [12]. By participating in the inflammatory process, this system plays a role in the physiopathology of RA [13]. At the sites of inflammation, a range of intercellular messengers and effector molecules are secreted by the various cells such as immune cells that release adenosine triphosphate (ATP) into the extracellular milieu. Elevated levels of extracellular ATP activate P2 receptors often resulting in an inflammatory response, while anti-inflammatory responses occur via adenosine binds to specific P1 receptors [14]. Two subfamilies were identified within the P2 receptor family: P2X and P2Y. P2X has 7 members (P2 X 1–7) and P2Y with 8 members (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, P2Y14). Adenosine receptors (P1) are G-protein coupled family, contain seven transmembrane domains with the N and C terminus on the extracellular side with intracellular

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https://doi.org/10.1016/j.imlet.2019.08.009
Received 3 July 2019; Received in revised form 20 August 2019; Accepted 30 August 2019
Available online 31 August 2019

and extracellular axes [15]. P1 receptors are divided into four different types: (A1, A2A, A2B, A3) [12].

The ectoenzymes play a major role in nucleotide metabolism, preventing interaction of nucleotides and nucleoside with their receptors, regulating immunity and inflammation. The expression and activity of the purinergic enzymes undergo dynamic changes that may modify or direct various pathological conditions [16]. Extracellular ATP levels are controlled by E-NTPDase (EC 3.6.1.5; CD39) which catalyzes ATP and ADP (adenosine diphosphate) hydrolysis generating AMP (adenosine monophosphate). E-5′-nucleotidase (EC 3.1.3.5; CD73), in turn, hydrolyzes AMP resulting in adenosine. Adenosine deaminase (ADA) (EC 3.5.4.4) regulates adenosine levels by deaminating this nucleoside into inosine [12].

A fine tune between the inflammatory and anti-inflammatory responses is necessary to avoid exacerbated immunosuppression or inflammation beyond control. Thus, the effects of these two key molecules, ATP and adenosine, their metabolism, and interaction with purinoreceptors, on the immune response are demonstrated in rheumatoid arthritis in this review. We searched the PubMed database using different combinations of the following the key terms: rheumatoid arthritis, pathogenesis, purine receptors, ATP, and adenosine. The most relevant papers on the proposed subject, as well as some references within the selected papers, were selected. All types of articles were included if considered suitable.

### 1.3. The purinergic system is involved in the response to RA

#### 1.3.1. ATP in early RA

The role of purinergic signaling in RA was first recognized in the 1990s after adenosine was identified as a treatment candidate for this disease [17]. Adenosine is produced from ATP during the cellular response to stress or metabolic injury and promotes an anti-inflammatory response that alleviates such damage. However, the release of ATP and UDP activates P2 receptors mobilizing calcium and activating interleukin-1 (IL-1), which stimulates the release of E2 prostaglandin (PGE2) in the synovial cells [18].

Synovial membrane inflammation is characterized by infiltration of leucocytes, such as mastocytes, dendritic cells, monocytes, and plasma cells and B, T helper 1 (Th1), and 17 (Th17) lymphocytes [6]. Endothelial damage and activation lead to changes in the endothelial permeability and increase immune cell adhesion, releasing ATP [19]. In physiological conditions, extracellular ATP is present in small amounts of approximately 10 nM, regulated by ectonucleotidases anchored in the plasma membrane [20]. However, in pathological events, extracellular ATP concentrations shift from almost undetectable to hundreds of micromolar levels [21].

When released by stressed, damaged, and necrotic cells, ATP acts as a damage-associated molecular pattern (DAMP) initiating a pro-inflammatory response DAMPs are endogenous factors specialized in recruiting and activating innate immune cells to promote healing and tissue homeostasis [22]. However, in autoimmune diseases such as RA, failure in regulatory mechanisms exacerbates inflammation [23]. DAMPs as ATP play an important role in promoting the inflammatory process during the early stages of RA and prolonging the immune activation, through positive feedback [24].

The inflammatory process of RA involves a set of immune and local cells, which interact through the release of tumor necrosis factor (TNF-α), interleukins 1β (IL-1β) and 6 (IL-6), autoantibodies, rheumatoid factor, anti-citrullinated peptide antibodies and signaling molecules (such as ATP) that contribute to the maintenance and aggravation of the lesion [25,26]. Both innate and acquired immune cells crosstalk through the interaction of nucleotides with their respective receptors.

#### 1.3.2. The role of ectonucleotidases in RA

The activity of ectonucleotidases limits the interaction of ATP with P2 receptors. A relationship between the enzymatic activity of ectonucleotidases and the activation of lymphocytes during the immune response has been demonstrated in RA patients and animal models of arthritis [27,28]. In response to elevation of ATP concentrations, there is an increase in the activity of ectonucleotidases to control the inflammatory response and favor the formation of adenosine, which has anti-inflammatory properties [13]. We have recently demonstrated, in a murine model of adjuvant-induced arthritis, that arthritis changes the activity of CD39 in immune cells resulting in a pro-inflammatory state and worse prognosis. Fortunately, these changes were reverted by the treatments proposed in the study, directing the responses to an anti-inflammatory profile [29].

Changes in the CD39/CD73 pathway may deregulate the mechanism of self-tolerance and thus contribute to the appearance of autoimmunity [30]. The increase in the activity of these enzymes results in elevated levels of adenosine, a key player in the immunosuppressive action of Tregs, which infiltrate sites of inflammation in response to high ATP concentrations. [31]. Although coexpression of CD39 and CD73 occurs in only 4% of human Tregs, adenosine production also occurs through CD39 and CD73 expressed in distinct cells [32]. CD39 expression varies among individuals especially in inflammatory sites where the expression of this enzyme is usually increased when compared to peripheral blood [33]. The expression of CD39 in Tregs is limited by single nucleotide polymorphisms (SNP) [34]. It has been shown that AA genotype of the rs10748643 SNP, a low-expressing CD39 variant, is associated with Crohn's disease [35] and is involved in the regulation of the immune system in autoimmunity [36]. A reduced response to methotrexate (MTX) in patients with rheumatoid arthritis was also shown to be related to an SNP that decreases the frequencies of CD39-expressing Tregs, the rs7071836 SNP [37].

Low CD73 expression in lymphocytes in sites of inflammation is associated with disease severity in juvenile idiopathic arthritis [38]. Also, the expression of ADA is downregulated in Tregs, suggesting that this cellular subtype not only generates adenosine but does not degrade it. Also, another feature of human Tregs is the absence of CD26 (dipeptidyl peptidase IV; DPPIV; EC3.4.14.5) expression, a molecule associated with ADA, which makes them unable to metabolize adenosine [39]. The implications of ADA activity in RA are further explored in a subsequent section.

Several mechanisms are involved in the development of an autoimmune disease such as RA, with deleterious consequences to tissues and cells damage. The components of the purinergic system are important players during the immune response to RA which shows a distinct variety of pro and anti-inflammatory actions and should be explored to understand the pathology as well as possible targets for therapeutic management.

### 1.4. ATP, P2X7 and RA

#### 1.4.1. ATP acting on P2X7 as a main regulator of RA

The vast cell/tissue damage in the joints of RA patients generates a considerable increase in purine and pyrimidine nucleotide levels in the synovial fluid [17]. Through P2 purinoreceptors, ATP regulates a number of cell responses in the joints and bones, such as chondrocytes, osteoclasts, osteoblasts, e synoviocytes [40]. Purinoreceptors are divided into two categories: G protein-coupled P2Y family (metabotropic), and ATP-specific P2X, linked to ionic channels (ionotropic). P2Y receptors activation leads to the release of $Ca^{2+}$ from the intracellular to the extracellular environment, whilst P2X receptors activation generates an influx of $Na^{+}$ and release of $K^{+}$, inducing membrane depolarization and increase of cytosolic $Ca^{2+}$ [41].

P2X7 is expressed in both inflammatory cells (monocytes, macrophages, dendritic cells) and constitutional cells such as fibroblasts [40–42]. Though all P2X receptors are expressed in immune cells, only P2X7 has a well-defined role in the inflammatory and immune responses during RA [40,43]. Moreover, single nucleotide polymorphisms in the P2X7 gene that increase the expression or activity of this receptor

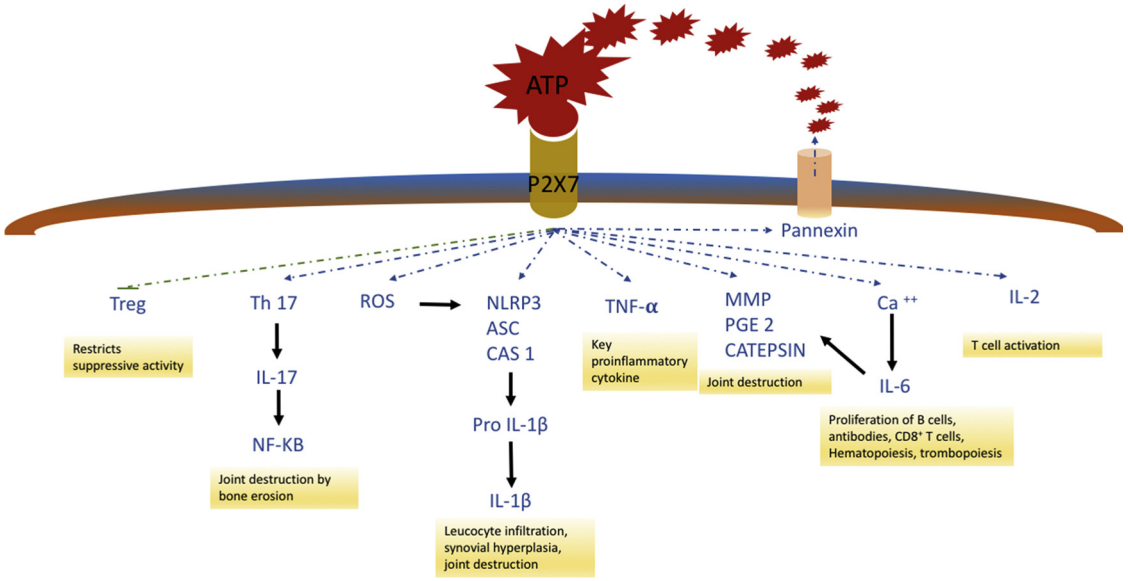


**Fig. 1.** ATP stimulation of P2X7 receptor. Upon stimulation, P2X7 signals the production and maturation of IL-1β, through the activation of inflammasomes, and the opening of pannexin channels, which causes a further release of ATP. It is involved in the production of matrix metalloproteinases (MMPs), cathepsins and prostaglandin E2 (PGE2), and stimulates the release of IL-6 through the influx of $Ca^{2+}$ and the production of IL-2. In addition, it induces ROS production, which stimulates the NLRP3 inflammasome. ATP binding to P2X7 favors the formation of Th17 cells and secretion of IL-17 as well as reducing the viability of Tregs.

are implicated in increased susceptibility to RA [44] and contribute to the pathogenesis of this disease [45]. During immune responses, ATP signaling through P2X7 elevates the levels of pro-inflammatory cytokines such as IL-1β. As a consequence, further ATP is released through pannexin channels, increasing the purinergic signaling and inflammation [46] (Fig. 1).

### *1.4.2. P2X7 in the innate response to RA*

In response to the activation of P2X7 by ATP in RA patients increased levels of IL-1β, which is primarily associated with macrophages, and promotes leukocyte infiltration and synovial hyperplasia resulting in joint destruction [40,45,46]. TNF–α released through the ATP/P2X7 interaction, is present in high concentration in the synovial fluid and serum of RA patients, performing a fundamental role in the pathogenesis of this disease. TNF-α is mainly detected in the early stages, while IL-1β is found in late stages of RA [46].

In innate and epithelial cells, the activation of P2X7-mediated inflammasome is responsible for the inadequate activation of osteoclasts by inflammatory cytokines leading to subchondral bone resorption, one of the central features of RA [47]. Also, matrix metalloproteinases (MMPs), cathepsins, and PGE2 are produced by P2X7 stimulation and linked to the degradation of the joint and pain [48,49].

In combination with TNF-α and IL-1β, IL-6 aids the production of MMPs and cathepsins, resulting in increased joint destruction [50]. Moreover, the stimulation of P2X7 by ATP has already been known to trigger the production of reactive oxygen species (ROS) by macrophages and epithelial cells. In addition to being tissue-damaging molecules, ROS mediates the proliferation and secretion of cytokines, being an essential activation factor of the NLRP3 inflammasome [51]. In RA, the sequence of events triggered by ATP binding to P2X7, which is followed by inflammasome activation, cytokine production, ROS production, tissue damage and further release of ATP, represents an activation looping.

Innate immune cells such as neutrophils are an important part of the development and progression of RA. The release of neutrophil extracellular traps (NETs) by neutrophils also causes the P2X7-dependent activation of the inflammasome, and assists in the development of autoimmune diseases [19,20,52,53]. In neutrophils, purinergic signaling rises chemotaxis and activation and generates anti-apoptotic signals, increasing cell survival and inflammation [54,55].

### *1.4.3. P2X7 in the adaptive response to RA*

The mechanisms by which pro-inflammatory cytokines are produced include the activation of the nuclear factor κB (NF-κB) pathway, mediated by toll-like receptors (TLR) and triggered by DAMPs like ATP. In addition, assembly of inflammasomes, such as NLRP3 and ASC, ends in activation of caspase-1 pathway and maturation of pro-IL-1β in IL-1 [43]. The release of IL-1β in RA via NLRP3 inflammasome is one of the most relevant events in RA, and P2X7 are some of the pathways involved in this process [40,43].

Binding of ATP to P2X7 promotes the release of IL-6 by $Ca^{2+}$ influx; IL-6 itself may generate B cell proliferation with antibody production, cytotoxic T cell proliferation, differentiation, hematopoiesis, and thrombopoiesis [56]. During cell recruitment, P2X7 is directly involved in the adaptive immune response occurring in RA, by mediating the production of interleukin 2 (IL-2) associated with T cell activation [57].

Th17 cells are involved in multiple pathological processes of RA, being recognized as a major player in the pathogenesis of RA [6]. They also upregulate the receptor activator of NF-κB (RANK) expression, which results in joint destruction and bone erosion [47]. The role of activation of P2X7 in the production of Th17 cells has been demonstrated in a study with an animal model of arthritis, which found an increase of mRNA for IL-1β, TGF-β1, IL-23, and IL-6, cytokines, necessary for the development of this cellular subtype [58]. Besides, ATP-P2X7 signaling reduces the viability of Tregs and restricts their suppressive activity as well as favoring the differentiation of IL-17-secreting Th17 cells [48].

Since Th17 cells are essential for the development of autoimmunity by the production of aforementioned pro-inflammatory cytokines, and Tregs play a key role in the maintenance immunological tolerance, a Th17/Treg balance shift by ATP/P2X7 activation may increase inflammation or initiate autoimmunity [59]. Conversely, the blockade of P2X7 aids the conversion of naïve $CD4^+$ T cells into Treg [60] and could be a strategy to control autoimmunity.

### *1.4.4. Targeting P2X7 to treat RA*

Several studies report the role of P2X7 in inflammatory diseases [61–63]. Furthermore, this receptor is highly expressed in immune cells

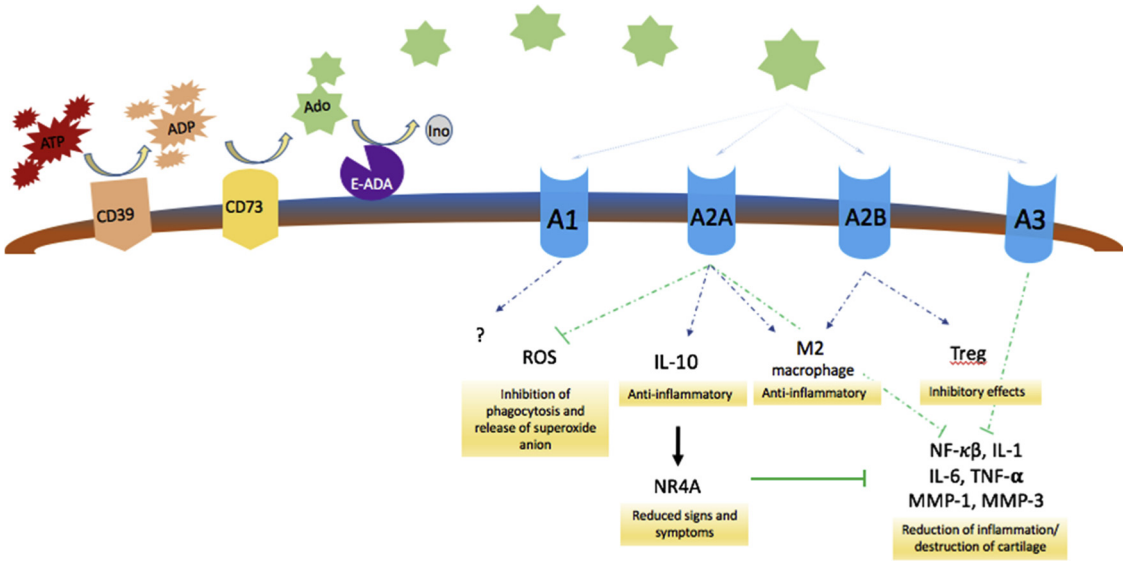


**Fig. 2.** Adenosine stimulation of P1 receptors. Adenosine generated from ATP degradation primarily elicits anti-inflammatory responses through its binding to P1 receptors, counteracting the ATP-induced inflammation.

and responds readily to the high levels of ATP released by these cells as a sign of damage or infection [64]. P2X7 triggers essential mechanisms involved in the RA-related inflammation, therefore, it has been frequently considered an anti-inflammatory therapeutic option to many inflammatory diseases including autoimmunity [55,65].

Blockade of P2X7 inhibits T cell and macrophage activation and may improve local inflammation [66]. Studies with animal models of arthritis have demonstrated a lower incidence and severity of local inflammation when P2X7 was blocked or deficient [67–69]. P2X7 antagonists have been considered as potential pharmacological targets to treat inflammatory autoimmune diseases such as RA [63,70]. However, pre-clinical and clinical trials with the P2X7antagonists have failed to inhibit disease progression [70,71], just act in local antihyperalgesic and anti-inflammatory effects [72].

P2X7 is involved in the production and release of various cytokines, enzymes, and oxidative stress factors in the inflammatory environment, thus blocking or using antagonists has promising effects on several diseases, including RA [55,70,73,74]. ATP/P2X7 signaling should be considered as a major therapeutic target, yet close attention must be paid to the implications of P2X7 blockade to the purinergic cascade and its consequences to the pathophysiology of the disease.

#### 1.4.5. A potential anti-inflammatory role for P2X7

In RA, autoantibodies participate in the inflammatory activation, formation of immune complexes and the damage of the joints [75]. The follicular auxiliary T (Tfh) cells are a subset of $CD4^+$ T cells that assist the production of antibodies by B cells [76], and exhibit an exaggerated response under conditions of autoimmunity [77]. P2X7 has a role in the proliferation not only of Th1 cells, but also Tfh cells [12,13]. Although ATP signaling in P2X7 presents in the vast majority inflammatory actions, the blockade or deficiency of this receptor as a treatment for RA has not reached the expected reduction of symptoms [71]. This may be due to the high expression of P2X7 in Tfh cells and its intrinsic action on their differentiation and function [79].

Proietti et al. [79] have shown that the lack of P2X7 in Tfh cells in Peyer patches (PPs) increases the reactions of the germinative center dependent on this type of lymphoid tissue, showing that P2X7 controls the numbers of Tfh cells. In the infectious process by *Plasmodium chabaudi* malaria, the balance between Tfh differentiation for Th1, necessary for the eradication of the disease, is dependent on the ATP binding in P2X7 [80], demonstrating that this receptor plays a role in the Tfh cell response under pathological conditions.

Deficiency or blockage of P2X7 in mice increased inflammatory arthritis induced by antibody production, due to an increased Tfh cell response, demonstrating that P2X7 plays a role in the Tfh cell response under pathological conditions [74,81]. These findings present a distinct action attributed to the blockade of P2X7 in RA, where the ATP-P2X7 binding in Tfh cells results in an anti-inflammatory response [74]. This represents one additional step in the ladder towards the use of P2X7 antagonists to treat RA, but it also presents a reasonable explanation for the different responses obtained in studies with P2X7 blockade.

### 1.5. Adenosine, P1 receptors and RA

#### 1.5.1. Distinct effects of P1 receptors in RA

A large number of messenger and effector molecules are released by immune cells and contribute to local injury in the site of inflammation. Adenosine production and binding to P1 receptors regulate a number of processes by downregulating inflammation [13]. Numerous *in vivo* studies demonstrate the anti-inflammatory potential of adenosine and, based on its well-known therapeutic power, it can be an important limiting factor for inflammation, joint destruction and consequently pain [13,82].

Several cell types produce adenosine as a result of the breakdown of extracellular ATP by CD39 and CD73 ectoenzymes. Adenosine acts on four different G protein-coupled receptors whose activation triggers distinct intracellular responses. While A1 and A3 negatively regulate the expression of cyclic AMP (cAMP) via Gαi protein, A2A and A2B lead to an increase in cAMP via Gαs protein [83]. A1 and A2A are low-affinity receptors within the nanomolar range, while A2B affinity is within micromolar ranges. A3 expression is generally low and varies among different species [84].

P1 receptors are expressed in a large number of cells, including those involved in RA pathology, lymphocytes, neutrophils, macrophages, and synovial cells, where they have different effects [82]. The effects of the binding of adenosine to different receptors are shown in Fig. 2. The anti-inflammatory effects of adenosine occur through the stimulation of A2A and A3, whose expression is under dynamic regulation by pro-inflammatory cytokines. An anti-inflammatory response can be increased as negative feedback to inflammation. A2A is upregulated by stimulatory agents such as IL-1 and TNF-α, important molecules in NF-kB activation and according to the progression of RA [69,83]. Studies indicate that the anti-inflammatory effects of adenosine come predominantly from its binding to A2A, inhibiting the release

of pro-inflammatory cytokines. The expression of A2A in most of the inflammatory cells indicates a great potential as a therapeutic target in inflammatory diseases [85]. Studies have shown an upregulation of A2A and A3 in patients with RA [86] which affects the response to treatments that increase the availability of adenosine.

*1.5.2. P1 receptors during the innate response to RA*

During the innate immune response to RA, A2A binds to adenosine on the surface of neutrophils to inhibit phagocytosis and superoxide anion production. Also, A2A is involved in the resolution of the inflammatory process, promoting apoptotic cell embolization, whereas activation of A1 and A3 promote neutrophil chemotaxis and reduces embolization [87]. Thus, these results show the opposing roles of P1 receptors in the inflammatory modulation during RA.

The effects produced by the activation of A3 in innate immune cells vary. In neutrophils, A3 mediates the inhibition of oxidative burst [88], while in macrophages it suppresses the release of TNF- α and IL-1β [89]. In summary, it is proposed that stimulation of A3 inhibits PI3K/Akt and NF-kB signaling, leading to downregulation of inflammatory mediators [90,91].

In macrophages, activation of P1 receptors has exhibited different responses. A2A and A2B stimulate the differentiation of M1 to M2 macrophages, while M1 has a pro-inflammatory character, by the release of cytokines (TNF, IL-12, INF-γ, etc.), oxidizing species mediating inflammation, M2 acts regulating this process, by engulfing inflammatory cells, resolving inflammation and promoting wound healing [33,92]; A2A mediates the production of anti-inflammatory IL-10 and stimulates expression of the nuclear receptor NR4A [85], which reduces the activation of NF-kB [88]. On the other hand, stimulation of A3 in macrophages has been shown to reduce inflammation and release of cytokines in an animal model of arthritis [89].

*1.5.3. P1 receptors during the adaptive response to RA*

Cells involved in the adaptive immune response play a major role in initiating and maintaining RA and the functions of T cells are dependent on the T cell receptor (TCR). Studies have shown that binding of adenosine to A2A results in inhibition of TCR and cause inactivation of the T cell, controlling inflammatory activation and consequent tissue damage [84,91,93]. The production and binding of adenosine to A2A drive T cells to an immunosuppressive phenotype [94]. In addition, TCR activation is linked to a rapid response to increased A2A expression, resulting in negative feedback by suppressing the production of INF-γ [95]. Considering that T cells are key cells in the maintenance of the inflammatory state in RA, the activation of A2A is beneficial for events determined by lymphocytes in the pathogenesis of RA.

Adenosine binding to A2A action activates the cAMP response element (CRE) and redirect myeloid cells, CD4+ and CD8+ T cells to produce anti-inflammatory cytokines (TGF-β, IL-10) that promote the development of Tregs and the inhibition of effector T cells (Teff) [84]. The inhibitory action of adenosine in Tregs is mediated by its binding to A2A on Teff, promoting a negative effect on the activation of NF-kB in these cells [87]. Thus, the production of cytokines and chemokines inflammatory agents important for the pathogenesis of RA is impaired [92].

However, a study with synovial fluid T cells of arthritis patients indicated that the expression of CD73 is reduced in both $Foxp3^-$ and $Foxp3^+$ Tregs and their ability to produce adenosine was impaired, contributing to local inflammation. In addition, local inflammation is enhanced by pro-inflammatory cytokines (IL-2, IL-17, and IFN-γ) released by non-Tregs cells [33]. However, as discussed above a pro-inflammatory cytokine profile may induce increased P1 receptors expression, demonstrating that synovial tissue is a potential target for pharmacological treatments with A2A agonists.

A2B signaling occurs by multiple pathways as a second signal by coupling to the G proteins Gq and Gi, promoting different effects that cannot be explained only by the elevation of cAMP [96]. Therefore, the role of A2B is not completely clear and the results of current research seem ambiguous. While some studies indicate that Th17 differentiation is stimulated by increasing IL-6 production in dendritic cells [97], others sustain that A2B, when stimulated, promotes T cell differentiation in Treg [98]. The effects of A2B binding on osteoclast differentiation and bone resorption are also controversial. Researchers [99] have observed that the A2B agonist inhibits RANKL-induced osteoclast formation in murine cells. On the other hand, one study pointed out that adenosine via A2B abolished the inhibition of osteoclasts induced by methotrexate [100]. A1 stimulation, in turn, is essential to the process of bone remodeling, through the differentiation of giant cells into osteoclasts during RA [101]. However, in a subsequent study, the expression of this receptor was low following macrophage stimulation, and no difference in A1 expression was observed after treatment with agonists or antagonists in the same study [102].

*1.5.4. P1 receptors as therapeutic targets in RA*

Adenosine production and signaling have already been indirectly explored in the treatment of RA. The anti-inflammatory effects of MTX and sulfasalazine, the most widely used medicines to treat RA, are mediated at least in part by adenosine occupancy of A2A and A3 [103]. In addition, the expression of CD39 is considered a biologic marker for methotrexate efficacy in RA patients [95,104]. Thus, as in other inflammatory diseases, the binding of adenosine to its receptors may represent a promising mechanism for the control and resolution of inflammation [97].

Upregulation and stimulation of A2A and A3 in peripheral leukocytes of RA patients inhibits the NF-kB pathway by decreasing IL-1, IL-6, and TNF-α [86]. However, the expression of A2A and A3 is normalized when anti-TNF antagonists are administrated, showing that their expression varies according to inflammatory levels during RA [105]. Furthermore, A2A and A3 agonists reduced the release of MMP-1 and MMP-3 [56,86].

Studies suggest A2A as a potential pharmacological target in RA. Treatment with A2A agonist has been shown to increase the serum levels of IL-10, an anti-inflammatory cytokine, and reduce the characteristic signs and symptoms of RA in two different animal models of arthritis [106,107]. In humans with osteoarthritis, the production of TNF-α and IL-8 by synoviocytes were inhibited by the stimulation of A2A [108].

The potential use of A3 as a therapeutic target in RA has also been described [58,82]. Treatment with agonists of this receptor reduced the formation of the inflammatory pannus, inflammation, and destruction of the cartilage. Neutralization of the anti-inflammatory response in an animal model of arthritis by the administration of an A3 agonist showed similar results [109]. In a study where RA patients were treated with A3 agonists, clinical improvement in the signs and symptoms of the pathology was observed [98]. As discussed above, RA patients show a higher expression of A3 [69] and, in contrast, A3 agonists prompt an anti-inflammatory response. Thus, the expression of this receptor also could represent a marker of disease severity and/or success of treatment for specific ligands in RA [110].

Pro-inflammatory cytokines may prevent desensitization of P1 receptors by increasing their function and potentiating the anti-inflammatory response of adenosine [66]. Thus, the success of P1 receptor agonists is determined by the increase in the expression of pro-inflammatory cytokines.

Adenosine signaling through the expression and function of its receptors, not only plays a role in the pathogenesis of RA but also has great potential as a therapeutic target. However, further studies are necessary to develop safe and effective drugs. Identifying the stages of RA with the highest expression P1 receptors is key to obtain success in the treatment with agonists. To avoid negative feedback in the expression of P1 receptors and obtain the best anti-inflammatory properties, it is necessary to identify an effective concentration of adenosine analogs agonists to attain synovial tissue-specific and systemic effects.

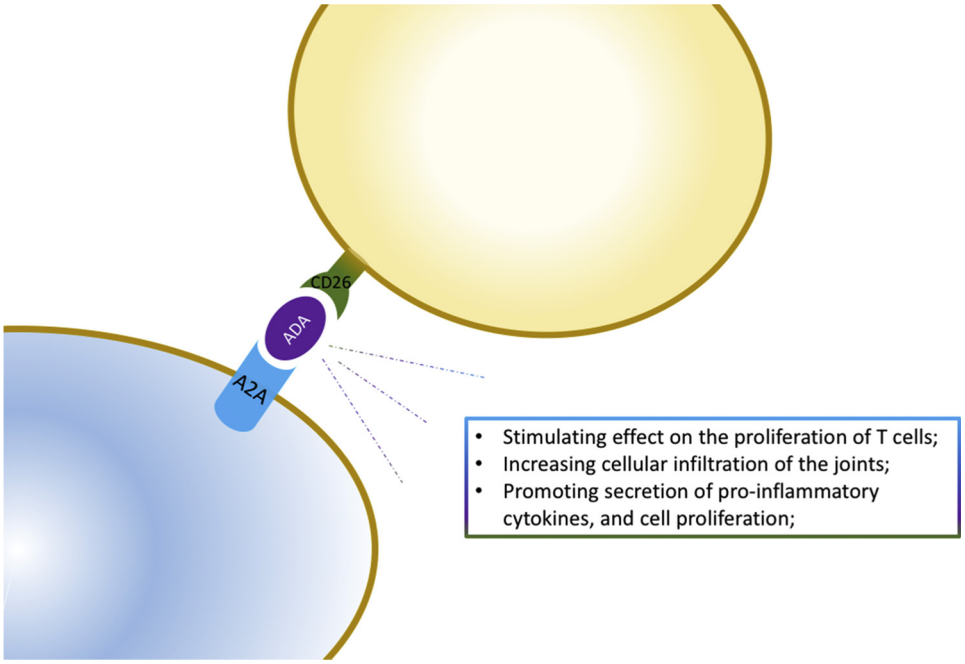


**Fig. 3.** ADA acts like a bridge between A2A and CD26 promoting cell-to-cell communication.

Also, a recently described pro-inflammatory effect of adenosine may be explored.

#### *1.5.5. The potential pro-inflammatory effect of adenosine*

As previously mentioned, adenosine coordinates the innate and adaptive immune responses to tissue injury in several different ways, such as reducing the production of pro-inflammatory cytokines. However, another important effect of adenosine on the innate immunity by increasing the macrophage production of IL-1β is yet to be discussed [111,112].

The initial activation of the inflammasome depends on TLR and DAMPs (ATP) and provides an acute production of IL-1β with resolution within hours [113]. Chronic inflammatory diseases such as RA require a sustained activation of the inflammasome, maintained by high concentrations of extracellular adenosine. Besides, adenosine is also able to re-stimulate the inflammasome [112,114].

The activity of the inflammasome is initiated by ATP and maintained by the binding of adenosine to A2A and dependent on HIF-1-alpha (Hypoxia-1-alpha inducible factor). Signaling occurs through cAMP/PKA (protein kinase A)/CREB (protein binding to the response element cAMP)/HIF-1-alpha positively regulates NLRP3 inflammasome to produce Pro-IL-1β [115,116]. This signaling is different from the NF-kB pathway, which is inhibited by the stimulation of A2A and A3 [86,117].

This response to adenosine via A2A brings a new interpretation of its effects on the inflammatory process of RA. So far, this particular response has been only described in macrophages [112], innate immune cells whose profile may vary from inflammatory to inflammatory-solving. Thus, although the actions of adenosine may seem contradictory, they play a role in an integrated response that equilibrates the pathological process of the disease.

In the adaptive response, the increase of cAMP and co-stimulation of CD28 are involved in the differentiation of T cells into Th1. The intracellular signaling is dependent on the binding PGE2 in EP2 /EP4 receptors generating PGE2/cAMP/PKA/CREB/gene transcription of IL-12, IFN-γ, and IL-2 involved in the Th1 differentiation process, while CD28 stimulates phosphoinositide 3-kinases (PI3K) that prevents the natural inhibition of T cell by cAMP/PKA [118]. This response is dependent on coupled to G-protein receptors like P1 receptors, so the author suggests that cAMP signaling in T cells could also occur by histamine or adenosine generating inflammation [118,119], however, this was not analytically tested, but should be considered due to simultaneous activation of adenosine in the signaling of PI3K and cAMP.

### *1.6. ADA as a metabolic and immunological mediator*

The immunological imbalance found in RA leads to changes in the purine metabolism, causing the release of inflammatory mediators. The bioavailability of adenosine and its action through receptors depends on its transformation into inosine through ADA, it is considered a marker of cell-mediated immunity [120].

Serum ADA activity is considered an inflammatory marker and reflects the activation of monocytes/macrophages in inflammatory diseases [121]. Serum ADA activity is elevated both in animal models of arthritis and RA patients [28,29,122]. In RA, the increase in ADA activity may be due to its release from damaged cells and increased cellular proliferation [123]. Elevated ADA activity contributes to the pathogenesis of arthritis by limiting the anti-inflammatory activities of adenosine [122], favoring the RA-related inflammatory state [124,125]. Controversial results are found in the literature regarding the meaning of serum ADA activity in RA. While some studies have suggested that serum ADA activity is a biochemical marker in the diagnosis and determination of disease activity of patients with RA [126–128], others did not find a relationship with disease activity [121,129]. This may be due to different methodological approaches with regards to parameters such as the stage at diagnosis, treatment, sample size, and different scores of disease activity.

ADA activity was found to be increased not only in the serum but also in the synovial fluid of patients with RA [130]. The reduction in adenosine levels, resultant from increased ADA activity in the serum and synovial fluid of RA patients, is indicative of a connection between the activity of this enzyme and RA-related inflammation. In addition, ADA activity may serve as a useful marker to monitor the effects of MTX since this medication acts in the metabolism of adenosine [124].

RA development is mediated by lymphocytes, antigen presentation cells, neutrophils, macrophages, and platelets, which all express cell-surface ADA [131–134]. Besides its role in adenosine metabolism, ADA interacts with A2A and CD26, which act as binding proteins, favoring

cell-to-cell communication [135,136] (Fig. 3).

Independently of the enzymatic action of ADA and CD26, their interaction stimulates the proliferation of T cells [137,138]. In fact, CD26 has been engaged in several pathologies, such as autoimmune diseases, rheumatoid arthritis, and HIV [139,140]. Moreover, by participating in the activation of T cells, CD26 influences cellular infiltration of the joints, increasing cell invasion and adhesion. Literature data report an increased CD26 density in circulating monocytes and $CD4^+$ T cells in patients with RA. Also, Th17 cells are known to express high levels of CD26, an important population committed to the pathogenesis of RA [141]. However, no conclusive data is linking the expression of CD26 to disease activity during active chronic RA [142].

There is an inverse relationship between the catalytic activity of CD26 and the density of this molecule in the cells involved in the pathogenesis of RA. On the other hand, RA patients under treatment present percentages of $CD4^+$ $CD26^+$ T cells similar to healthy individuals demonstrating a relationship between increased CD26 expression and inflammatory diseases [142,143].

Besides, ADA can function as a "bridge", forming a complex that binds CD26 to A2A [136], which results in the secretion pro-inflammatory cytokines, and cell proliferation, and activation in healthy patients. This can be transposed to autoimmune diseases as RA where the expression of CD26 [142] and P1 receptors are increased [144].

The interaction of CD26 with ADA, whose paracrine effects stimulate and activate cells involved in RA, may provide us with an alternative approach in the understanding the pathogenesis of this condition. In addition, the interaction of ADA with P1 receptors is an example of a promising field to be explored for a better knowledge of the disease. This additional function of ADA corroborates the notion that the pathogenesis of RA is strongly tied to alterations in the purinergic pathway.

## 2. Conclusion

We have collected data from recent studies and consistent historical findings describing the mechanisms by which we can draw a link between the components and mediators of the purinergic system and the immune response to RA. The actions exerted by this system may be stimulatory or inhibitory, depending on the release of nucleotides, their concentration, and their binding to the respective receptors.

We concluded that the purinergic signaling is involved in the pathogenesis, progression, severity, and maintenance of RA. Given the fundamental roles of the inflammation and immunity processes of RA, and its connection with the purinergic system addressed in this review, new therapeutic approaches focused on purinergic signaling are of great scientific interest.

## Funding information

This study was partly financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)—Financial Code 001 (PROEX number: 88882.182182/2018-01).

## Declaration of Competing Interest

The authors declare that they have no conflict of interest.

## Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) -Finance Code 001.

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