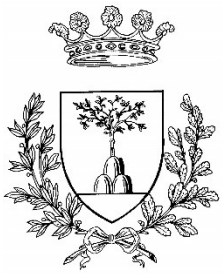


Università degli studi di Ferrara

DOTTORATO DI RICERCA IN

"Medicina Molecolare e Farmacologia"

CICLO XXX

COORDINATORE Prof. Francesco Di Virgilio

Pharmacological characterization of adenosine receptors in chronic inflammatory rheumatic diseases

Settore Scientifico Disciplinare BIO/14

Dottorando

Dott. Ravani Annalisa

Tutore

Prof. Varani Katia

Anni 2014-2017

TABLE OF CONTENTS

INTRODUCTION

Adenosine

The endogenous nucleoside adenosine, is composed of a molecule of adenine attached to a ribose sugar molecule (ribofuranose) via a β-N9-glycosidic bond (Figure 1).

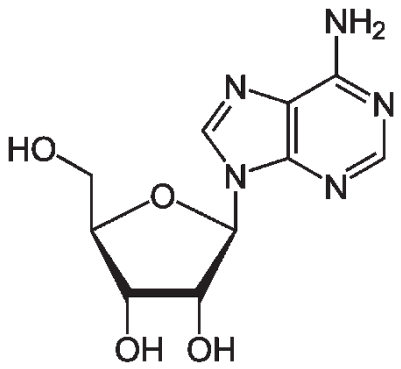


Figure 1. Chemical structure of adenosine

The purine nucleoside adenosine is a ubiquitous molecule whose importance for human health cannot be overstated. Indeed, it is the backbone of ATP and regulates the functions of every tissue and organ (Borea *et al.*, 2014), mainly, but not solely, through the activation of a family of four GPCRs, A_1, A_{2A}, A_{2B}, and A_3. Interestingly, the A_1 and A_3 subtypes have an inhibitory effect on adenylyl cyclase (AC) activity, while A_{2A} and A_{2B} stimulate it, with a consequent modulation of cyclic AMP levels (Fredholm, 2010). Although the affinity of adenosine for these receptors may vary, depending on the type of test used to evaluate it (Lane *et al*., 2011), adenosine seems to present a higher affinity for A_1, A_{2A}, and A_3 than for the A_{2B} subtype (Fredholm *et al.*, 2011a). Adenosine can be produced intracellularly, through the hydrolysis of AMP or S-adenosyl-homo- cysteine (SAH) by an intracellular 50-nucleotidase or SAH hydrolase, respectively. However, ATP dephosphorylation, orchestrated by the ectonucleoside triphosphate diphosphohydrolase CD39 and the 50-nucleotidase CD73, is the main mechanism behind high extracellular adenosine levels (Gessi *et al.*, 2011a; Antonioli *et al.*, 2016) . The bioavailability of adenosine depends upon its transformation to inosine through adenosine deaminase (ADA), of which there are intracellular and extracellular forms, and/or intracellular transport via nucleoside transporters. Once inside the cell, adenosine is phosphorylated to AMP or degraded to inosine by adenosine kinase (ADK) and ADA, respectively.

The formation of adenosine is strictly dependent on the metabolic state of a cell. Normally, the extracellular concentration of adenosine spans the low nanomolar range, but its levels

rise during conditions involving increased metabolic demand and/or lack of oxygen; for example, pathological states, such as epilepsy, ischemia, pain, inflammation, and cancer. The physiological actions of adenosine all tend to redress an imbalance between energy demand and availability, earning it the reputation of a 'retaliatory metabolite' (Fredholm *et al.*, 2011b). Adenosine-mediated tissue protection and, as a consequence, preservation of organ function, involves four main mechanisms, namely increasing and/or rebalancing the oxygen supply: demand ratio, preconditioning, anti-inflammatory effects, and stimulation of angiogenesis (Linden, 2005).

Adenosine Receptors

A_1ARs

A_1ARs are widely distributed not only in the central nervous system (CNS) but also in peripheral tissues (Baraldi *et al.*, 2008). Adenosine by the A_1AR activation produces inhibition of neurotransmitter release and induces neuronal hyperpolarization mediating sedative, anticonvulsant, ansiolitic and locomotor depressant effects (Gessi, Merighi, Varani, *et al.*, 2011a). Literature evidence indicated the involvement of A_1ARs in controlling pain transmission producing anti-nociceptive effects in various animal models (Romagnoli *et al.*, 2008; Kiesman *et al.*, 2009; Schmidt *et al.*, 2009; Sowa *et al.*, 2010b). 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 for the treatment of arrhythmias and atrial fibrillation (Elzein and Zablocki, 2008). 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 (Vallon and Osswald, 2009). 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 be also used in asthma and in COPD since adenosine induces acute bronchoconstriction via stimulation of A_1ARs (Caruso *et al.*, 2009; Wilson *et al.*, 2009).

A_{2A}ARs

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 (Fredholm *et al.*, 2001; Jenner *et al.*, 2009). Several studies have suggested the possible involvement of

A_{2A}ARs in the pathogenesis of neuronal disorders, including Huntington's disease (HD) and Parkinson's disease (PD). In particular it was demonstrated an aberrant increase of A_{2A}AR density in peripheral blood cells of Huntington's disease and PD patients in comparison with age-matched healthy subjects (Varani *et al.*, 2007; 2010c). Accordingly, A_{2A} antagonists currently constitute an attractive non-dopaminergic option for use in the treatment of PD (Dalpiaz *et al.*, 2012). Furthermore, it is well established that the selective block of adenosine A_{2A} receptors is protective in different *in vivo* models of cerebral ischemia (Maraula *et al.*, 2013).

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 pro-inflammatory cytokines production (Belardinelli *et al.*, 1998; Gessi *et al.*, 2000). An up-regulation of A_{2A}AR was found in peripheral circulating cells of end-stage chronic heart failure patients (Varani *et al.*, 2003b). 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 (Mohsenin *et al.*, 2007).

A_{2B}ARs

A_{2B}AR is expressed in the brain, spleen, lung, colon, heart and kidney, where it is primarily localized to the vasculature (Grenz *et al.*, 2008). 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 (Feoktistov and Biaggioni, 2011). Activation of A_{2B}ARs prevent cardiac remodeling after myocardial infarction and exert protective effects from infarction in ischemic post-conditioning (Methner *et al.*, 2010). Degranulation of mast cells and subsequent mediator release is an important component of the bronchoconstriction observed in asthma (Bradding *et al.*, 2006; Ryzhov *et al.*, 2008). Importantly, investigation of ARs on mast cells implicate A_{2B}AR signaling in degranulation and mediator release (Sun *et al.*, 2006). Identification of A_{2B}AR signaling as potential pathway in the pathogenesis of asthma prompted its investigation in other chronic conditions affecting the lung, including COPD and idiopathic pulmonary fibrosis (Zhou *et al.*, 2009). It has been proposed a protective role for A_{2B}AR antagonists in the resolution of pulmonary infiammation and fibrotic processes (Zaynagetdinov *et al.*, 2010; Zhou *et al.*, 2010). In addition, it has been observed that A_{2B}ARs are down-regulated in COPD patients most likely due to oxidative/nitrosative

stress (Varani *et al.*, 2010a). It has been also reported that A_{2B}ARs in intestinal epithelial cells mediated Cl^- secretion through an increase in cyclic AMP levels (Gessi *et al.*, 2011).

A_3ARs

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 (Fredholm *et al.*, 2000). The role of A_3ARs in several pathophysiological conditions is often controversial even if they may contribute to neurotransmission (Boison, 2007). A pro-convulsant effect of A_3ARs has been observed in the immature brain suggesting the possibility to facilitate seizure-induced neuronal damage (Boison, 2008). A nociceptive role for A_3ARs involving both CNS and pro-inflammatory effects in peripheral tissues has been highlighted (Yoon *et al.*, 2006). Moreover, prolonged A_3AR stimulation is able to transform the effects from protective to injurious increasing the excitotoxicity (Chen *et al.*, 2007). Glial A_3AR activation by high adenosine levels, caused by a brain injury, may be implicated in neuroinflammatory tissue responses (Hammarberg *et al.*, 2004). There is also evidence that A_3ARs enhance cellular anti-oxidant 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 post-ischemic contractile function (Zhai *et al.*, 2011). 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 vascular endothelium growth factor (VEGF) expression (Merighi *et al.*, 2007). Transcript levels for the A_3ARs are elevated in lung biopsies of patients with asthma or COPD where mediated the inhibition of eosinophil chemotaxis (Reeves *et al.*, 2000; Kay, Phipps and Robinson, 2004). In 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 (Young *et al.*, 2004).

Adenosine is present at high concentrations in cancer tissues and in the interstitial fluid of several tumors, at concentrations sufficient to interact with ARs (Gessi *et al.*, 2011a). A_3ARs are present in different types of tumor cells and are involved in the tumor growth, in the regulation of cell cycle and mediate both pro and anti-apoptotic effects closely associated with the level of receptor activation (Gao *et al.*, 2001; Gessi *et al.*, 2001, 2007). 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 (Borea *et al.*, 2009).

ARs are present in many cell types including platelets, lymphocytes, eosinophils, neutrophils, mast-cells and macrophages where they mediate pro- and anti-inflammatory effects (Varani *et al.*, 2003a; Varani *et al.*, 2006, 2010d). Several authors have demonstrated that human circulating blood cells (platelets, lymphocytes, neutrophils) reproduce the same receptor alterations known to be at the basis of specific diseases mainly in the cardiovascular system and CNS (Varani *et al.*, 2003b; Capecchi *et al.*, 2005; Varani *et al.*, 2007; Gessi *et al.*, 2011). As a consequence, peripheral blood cells could represent an 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 (Varani *et al.*, 2009, 2010b, 2011b)

Adenosine in disease states

Adenosine and epilepsy

There is a huge body of evidence showing that adenosine is an inhibitory modulator of brain activity, and its anticonvulsant effects, mediated by both receptor-dependent and -independent pathways, have been demonstrated in several experimental models of epilepsy (Boison, 2016). The ability of adenosine to prevent or ameliorate seizures induced by pentylenetetrazole, pilocarpine, NMDA, bicuculline, organophosphate treatment, and electrical stimulation has been attributed essentially to A_1AR activation, which inhibits presynaptic excitatory neurotransmitter release and hyperpolarises the postsynaptic cell membrane (Masino *et al.*, 2014). Indeed, upregulation of the A_1AR has been reported as a consequence of spontaneous seizures triggered by electrical stimulation (Hargus *et al.*, 2012). Furthermore, both upregulation of the protective A_1AR and downregulation of the proconvulsant A_{2A}AR subtype have been shown in the cerebral cortex after hyperthermia-induced seizures, suggesting the existence of a specific neuroprotective mechanism (León-Navarro *et al.*, 2015). The same study also showed a concomitant reduction of the adenosine-generating 50-nucleotidase, suggesting that this is the means by which the A_{2A} receptor effects of adenosine are attenuated, enabling adenosine to fulfil its protective role in epilepsy. Interestingly, activation of the A_1 receptor subtype has been linked to the antiepileptic effects of a ketogenic diet, a low-carbohydrate, high-fat diet protocol prescribed to treat epilepsy (Masino *et al.*, 2011, 2012). Furthermore, ketogenic diet has been shown to increase adenosine levels and reduce DNA methylation (Lusardi *et al.*,

2015). This follows, because adenosine exerts receptor-independent effects in DNA methylation homeostasis (Williams-Karnesky *et al.*, 2013). This process, triggered by DNA methyltransferases and mediated by S-adenosylmethionine (SAM)-dependent transmethylation of DNA, results in the production of adenosine, whose removal via ADK increases the transmethylation pathway potentially implicated in epileptogenesis (Kobow and Blümcke, 2012). Indeed, ADK is overexpressed in epileptogenic brain areas, where it induces seizures. This would seem to suggest the use of ADK inhibitors in epilepsy therapy, but, unfortunately, the chronic systemic use of these agents leads to liver toxicity, as well as cognitive and sedative adverse effects (Boison, 2013). Therefore, it is vital to find alternative strategies for increasing adenosine levels in epilepsy. To this end, gene therapy directed to ADK through an antisense oligonucleotide is being explored as a means of conserving adenosine by reducing ADK expression (Theofilas *et al.*, 2011; Boison and Aronica, 2015). Promisingly, adenosine has also been delivered directly to the brain ventricles of epileptic rats, thereby reducing DNA methylation and slowing disease progression (Williams-Karnesky *et al.*, 2013). Although there is still much work to be done, there is every indication that agents able to increase adenosine availability may have a place in the future treatment of epilepsy.

Adenosine and ischemia

Adenosine appears to have a role as an endogenous mediator of neuroprotection in the homeostatic response to changes occurring during ischemia and stroke (Pedata *et al.*, 2016). ARs are important targets for therapeutic implementation in the treatment of stroke because extracellular adenosine concentrations increase dramatically soon after ischemia (Melani *et al.*, 2014). Indeed, by activating A_1ARs, this nucleoside hinders Ca^{2+} influx, thereby inducing presynaptic inhibition and a reduction in the release of excitatory neurotransmitters. In addition, it increases the conductance of K^+ and Cl^- ions, mediating a fall in neuronal excitability and having a key role in ischemic preconditioning (IP) (Boison and Shen, 2010; Ciruela *et al.*, 2012). By these means, adenosine is able to reduce cellular metabolism and energy consumption in ischemia within a few hours. Moreover, during a later phase of ischemia, (i.e., in the hours and days after the insult), adenosine also exerts beneficial peripheral effects via activation of the A_{2A}AR. The ability of A_{2A}AR activation to inhibit platelet aggregation, mediate vasodilation, restrict leukocyte infiltration, and curb the inflammatory response is crucial for attenuating neuroinflammation after ischemia (Boison and Shen, 2010). By suppressing neuroinflammation, A_{2A}ARs may also produce positive effects on neurogenesis, and those located in the CNS are also known to provide a

degree of neuroprotection against brain ischemia through the increase of neurotrophic factors, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which act to restore brain activities (Gomes *et al.*, 2013). Furthermore, adenosine may limit hypoxia-triggered inflammation and vascular damage in ischemia via stimulation of A_{2B} and A_3ARs slowing neutrophil infiltration, promoting angiogenesis, and inhibiting migration of the microglia and monocytes in ischemic areas (Pedata *et al.*, 2014). Brain IP, a process by which repeated short, sublethal insults protect the tissue against subsequent ischemic damage, is an appealing therapeutic approach against stroke. However, the molecular mechanisms underlying IP, which include NMDA glutamate receptors, nitric oxide synthase, cytokines, oxidative stress, and inhibition of immune cells, have only been partially defined. Nevertheless, adenosine is known to be an important mediator of this phenomenon, acting by triggering A_1 receptors, which are able to reduce glutamatergic excitotoxicity induced by overstimulation of NMDA receptors (Constantino *et al.*, 2015). The protective effect of IP has also been observed in other tissues, particularly the heart, in which it has been most widely investigated, and where it has been found to act by stimulating A_1, A_{2B}, and A_3ARs. The molecular mechanism of cardioprotection induced by A_1ARs has been attributed to the regulation of mitochondrial ATP-dependent K^+ channels through activa-tion of protein kinase C (PKC). This prevents the destruction of the mitochondrion and consequent cell death in myocytes (Iliodromitis *et al.*, 2014). The protective effects of A_{2B}AR have been attributed to its ability to stabilise the circadian transcription factor period (Per)2, thereby flicking a HIF-1-dependent metabolic switch essential for the adaptation of myocytes to ischemia by promoting more oxygen-efficient utilisation of carbohydrates (Eckle *et al.*, 2012; Eltzschig *et al.*, 2013). Moreover, A_{2B}AR signalling has recently been ascribed different cardioprotective functions through its actions in several different tissues. Indeed, its effects in IP are exerted through vascular endothelial cells and cardiac myocytes, while its actions on inflammatory cells are critical for attenuating reperfusion injury after ischemia (Seo *et al.*, 2015). However, another critical cardioprotective effect of adenosine during IP has been observed in cardiac mast cells, in which both A_{2B} and A_3AR subtypes trigger a signalling cascade of PKC-ε and aldehyde dehydrogenase type 2. This prevents renin release from mast cells and, therefore, the dysfunctional consequences of the local renin-angiotensin system activation responsible for reperfusion arrhythmias (Koda *et al.*, 2010). However, it is difficult to exploit IP as a therapy because it is not usually possible to anticipate an ischemic event before it occurs. It is also unfeasible to maintain IP indefinitely as a prophylactic treatment. Nevertheless, there is significant evidence that A_3AR activation exerts a cardioprotective

effect, both before ischemia and during reperfusion (Gessi *et al.*, 2008; Wan *et al.*, 2011; Du *et al.*, 2012; McIntosh and Lasley, 2012; Cheong *et al.*, 2013). Postischemic protection may occur through the inhibition of either neutrophil-induced reperfusion injury or apoptotic cell death in myocytes (Ge *et al.*, 2010; Hussain *et al.*, 2014). The cardioprotective effect of one A_3AR agonist, IB-MECA, was recently attributed to A_3AR desensitisation, and the indirect activation of A_{2A}ARs on bone marrow-derived cells. This suggests that blockade of reperfusion injury by A_{2A}AR agonists added at reperfusion is a promising strategy (Tian *et al.*, 2015). Indeed, the beneficial role of A_{2A}ARs has been well described. Treatment with A_{2A}AR agonists has been reported to reduce tissue injury during reperfusion in different organs, including the liver, kidney, lung, heart, skin, and spinal cord, by reducing the neutrophil accumulation, proinflammatory cytokine/oxygen free radical release, endothelial cell activation, microvascular occlusion, and platelet aggregation that can exacerbate tissue injury during reperfusion of previously ischemic tissues (Patel *et al.*, 2009; Johnston-Cox and Ravid, 2011; Chhabra *et al.*, 2012). Therefore, adenosine or compounds modulating adenosine signalling represent a promising therapeutic means of both protecting the myocardium and enhancing its recovery after reperfusion (Fordyce *et al.*, 2015). In fact, encouraging results, in the form of a lower rate of microvascular obstruction, have been already obtained in a randomised, placebo-controlled trial entitled 'Intracoronary Nitroprusside Versus Adenosine in Acute Myocardial Infarction' (REOPEN-AMI) (Niccoli *et al.*, 2013; Nazir *et al.*, 2014; Niccoli *et al.*, 2014). In light of this, a clinical trial to assess the cardioprotective effects of adenosine administration, in terms of reducing infarct size in patients with acute myocardial infarction (MI), is currently underway. An attenuation of ischemic reperfusion injury has been reported in the lung, in which treatment with A_1, A_{2A}, or A_3AR agonists significantly improved organ function, reducing neutrophil infiltration, oedema, and the production of tumour necrosis factor alpha (TNF-α) (Gazoni *et al.*, 2010). These findings were corroborated by various recent studies showing that activation of A_1, A_{2A}, A_{2B}, or A_3ARs improved lung function and decreased inflammation, oedema, and neutrophil chemotaxis after ischemia and reperfusion (LaPar *et al.*, 2011; Fernandez *et al.*, 2013; Hoegl *et al.*, 2015). Likewise, beneficial effects have been attributed to the activation of A_{2A} and A_{2B}ARs in mouse models of kidney and hepatic reperfusion injury, through mechanisms involving the inhibition of natural killer T cell activation and nuclear factor kB (NF-κB), respectively (Grenz *et al.*, 2011; Li *et al.*, 2012; Zimmerman *et al.*, 2013). Interestingly, A_{2A}AR activation has also been found to trigger an increase in ATP production in hepatocytes and sinusoidal endothelial cells, thereby redressing the metabolic alteration

induced by ischemic reperfusion injury and increasing the availability of enzymes necessary for energy production (Mandili *et al.*, 2015). Furthermore, inhibitors of nucleoside transporters have been ascribed a protective function in a model of renal ischemia, enrolling A_{2B}ARs in vascular endothelia and thereby increasing adenosine signalling (Grenz *et al.*, 2012a). More recently, an increase in VEGF induced by A_{2B}AR activation has also been associated with the reversal of renal dysfunction (Patel and Thaker, 2014). As a whole, these results present a compelling case for adenosine agonists as potential therapeutic agents in strategies for the prevention of reperfusion injury after organ transplant, a planned insult to the body that allows for an anticipatory care and/or immediate after-care strategy that is not feasible with injuries of sudden onset, such as stroke or MI.

Adenosine and neurodegenerative diseases

There is a well-established allosteric relationship between striatal A_{2A}ARs and dopamine D_2 receptors, supported by evidence that A_{2A}ARs activation is responsible for the decreased affinity of agonist binding to D_2 receptors (Ferre *et al.*, 1991). This represented the first proof of concept for the therapeutic utility of A_{2A} receptor antagonists in PD (Armentero *et al.*, 2011; Preti *et al.*, 2015; Ferré *et al.*, 2016). Interestingly, the well-known epidemiological consideration that caffeine protects against PD has been confirmed in an animal model of PD and attributed to A_{2A}ARs antagonism (Ross *et al.*, 2000; Ascherio *et al.*, 2001, 2004; Xu *et al.*, 2016). A_{2A}ARs and D_2 receptor heteroceptor complexes have been detected in cellular models and in the striatum (Borroto-Escuela *et al.*, 2010, 2011, 2013; Varani *et al.*, 2010c; Trifilieff *et al.*, 2011; Bonaventura *et al.*, 2015). As the receptors show antagonistic interactions at bothmembrane and intracellular signalling levels, dopamine depletion may be responsible for an overactivation of A_{2A}ARs and the consequent symptoms of PD (Peterson *et al.*, 2012). Therefore, antagonists of A_{2A}ARs are beneficial for the improvement in motor function in different animal models of PD, as they attenuate the inhibition exerted via A_{2A}ARs on the effects of D_2 receptors in the gabaergic striato-pallidal neurons (Fuxe *et al.*, 2015). Recently, pharmacological data have suggested a new model that indicates A_2ARs and D_2 receptor heteromers form heterotetramers, which consist of A_{2A}ARs and D_2 receptor homodimers. This model supports the evidence that, at high concentrations, A_{2A}ARs antagonists exert the same effects as A_{2A}ARs agonists, thus reducing D_2 receptor-mediated activity in neurons; these findings could be important from a clinical point of view, when the use of A_{2A}ARs antagonists is being considered for therapy (Bonaventura *et al.*, 2015). Even though the development of A_{2A}ARs antagonists

as new drugs for PD treatment has encountered several obstacles in the clinic, istradefylline has recently been registered in Japan, as it has been found to decrease the "off time" when used in combination with levodopa (L-DOPA). However, it has not receive approval by the American Food and Drug Administration, due to the lack of significant improvement in the phenomenon of "wearing off" in comparison with L-DOPA. Other potential new drugs like preladenant and vipadenant have failed in phase III clinical studies for PD (Navarro *et al.*, 2016; Oertel and Schulz, 2016). The reasons for which these drugs failed were the lack of significant benefits of preladenant combined with L-DOPA when compared to L-DOPA alone, while vipadenant was found to have toxic effects. It has been hypothesized that administration of this class of compounds should start as early as possible to avoid a structural change in the A_{2A}ARs–D_2 receptor heteroceptor complexes that may induce dyskinesias and the onset of "off time" caused by L-DOPA (Fuxe *et al.*, 2015). Another pharmacological approach for PD treatment is suggested from the results obtained with bivalent drugs acting at A_{2A}ARs–D_2 receptor complex (Soriano *et al.*, 2009), with integrated dual acting ligands having an improved efficacy in preliminary BBB permeability tests (Jörg *et al.*, 2015). Recently, a novel protective effect of A_{2A}ARs antagonists has been attributed to the modulation of α-synuclein (αSyn) effects, as α-synuclein-induced damage to striatal neurons was clearly reduced in A_{2A}ARs KO mice and A_{2A}ARs antagonists prevented the neurotoxicity related to α-synuclein aggregation. These results provide additional evidence in support of antagonists as effective drugs for the treatment of PD and related synucleinopathies (Kachroo and Schwarzschild, 2012). The use of A_{2A}ARs antagonists has been found useful in other pathologies involving neuronal dysfunction including HD and Alzheimer's disease (AD) as well as major depression and schizophrenia, epilepsy, acute and chronic stress, restless legs syndrome (RLS) and memory fear (Canas *et al.*, 2009; Batalha *et al.*, 2013; Cunha, 2016; Krügel, 2016; Quiroz *et al.*, 2016). Different studies have reported an up-regulation of A_{2A}ARs in animal models of HD, and recently, it has been shown that A_{2A}ARs antagonists block working memory deficits at early stages of HD models (Li *et al.*, 2015). Hyperactivation of both D_1 and A_{2A}ARs has been found in HD striatum where D_1 plus A_{2A}ARs antagonists reduced PKA activity, which is involved in hippocampal-dependent cognitive dysfunction in HD, further supporting a therapy based on A_2ARs blockade. Nevertheless, evidence in favour of A_{2A}ARs activation as a strategy for HD treatment has also been reported (Gomes *et al.*, 2011; Martire *et al.*, 2013; Tyebji *et al.*, 2015). In addition, a positive action of A_{2A}ARs in the early phases of neurodegeneration has been attributed to the presynaptic facilitation of GABA transmission exerted by A_{2A}ARs agonists, involving brain-derived neurotrophic

factor–tropomyosin receptor kinase B in the hippocampus (Colino-Oliveira *et al.*, 2016). A change in the pattern of adenosine receptors with a decrease in A_1 and an increase in A_{2A}ARs has been also detected in AD. As a consequence A_{2A}ARs antagonists have been found to prevent neuronal death and the decrease in astrocytic glutamate uptake, caused by an amyloid-β (Aβ) fragment, that may be responsible for excitotoxicity in AD (Canas *et al.*, 2009; Matos *et al.*, 2012). Importantly, it has been shown that humans with AD have increased levels of A_{2A}ARs in astrocytes. Also, in young and aging mice, their genetic removal increased long-term memory and increased the levels of an immediate-early gene necessary for memory (Orr *et al.*, 2015). Furthermore, deletion of A_{2A}ARs has been shown to have a protective effect from spatial memory and hippocampal long-term depression caused by Tau pathology (Laurent *et al.*, 2016). In addition, A_{2A}ARs are up-regulated in CA3 synapses at early stages of AD and their antagonism reversed the block of long-term synaptic potentiation in a mouse model of AD amyloidosis (Viana da Silva *et al.*, 2016). Caffeine has been found to have a protective effect against cognitive impairment in both human and animal studies (Maia and de Mendonça, 2002; Smith, 2009; Santos *et al.*, 2010). Furthermore, caffeine reduced plasma and brain Aβ levels in an animal model of AD and prevented memory deficits caused by Aβ administration (Dall'Igna *et al.*, 2007; Cao *et al.*, 2009). Interestingly, the initial findings of a case–control study were the first to demonstrate that caffeine/coffee consumption is associated with a reduced risk, or delayed onset, of dementia (Cao *et al.*, 2012). Therefore, it seems that caffeine, the most popular and widely used drug in the world, by antagonizing the effects of adenosine, retains a big potential to counteract different neurodegenerative diseases (Woods *et al.*, 2016). Due to their modulation of glutamatergic and monoaminergic transmission in the striatum, A_{2A}ARs may also be involved in depression (Krügel, 2016). In behavioural animal models, A_{2A}ARs antagonists produced antidepressant effects (El Yacoubi *et al.*, 2003; Hodgson *et al.*, 2009). However, istradefylline was found to exert them independently from monoaminergic transmission (Yamada *et al.*, 2014a,b). Furthermore, caffeine or selective A_{2A}ARs antagonists reversed the performance deficits in reserpine-treated rats and prevented mood and memory dysfunctions induced by chronic stress (Batalha *et al.*, 2013; Kaster *et al.*, 2015; Minor and Hanff, 2015). In addition, in a broad-based model of depression mediated by an increase in hippocampal A_{2A}ARs, which control synaptic glutamatergic function, caffeine was able to prevent memory, but not mood, deficits (Machado *et al.*, 2017). Overall, based on the progress obtained in clarifying the molecular mechanisms underlying CNS diseases, adenosine reveals its bad side essentially through the activation of A_{2A}ARs, which may be considered as targets of therapeutic strategies and

may represent a promising future in the battle against a wide spectrum of unmet central diseases.

Adenosine and pain

Adenosine has been recognised as a potent antinociceptive agent in several different preclinical models of chronic pain and, therefore, is undergoing clinical trials for chronic regional pain syndrome, as well as perioperative and neuropathic pain (Zylka, 2011; Rauck *et al.*, 2015). Indeed, in the spinal cord and periphery, adenosine has been shown to reduce neuronal activity and, therefore, pain, through its activation of the A_1ARs. These results are consistent across several experimental pain models, including formalin-induced inflammation, carrageenan-triggered arthritis, hyperalgesia following surgical incision, neuropathic pain caused by spinal nerve ligation and chronic constriction injury, pain after spinal cord injury, and diabetic neuropathy provoked by streptozotocin (Zylka, 2011; Sawynok and Reid, 2012; Sawynok *et al.*, 2013; Sawynok, 2016). Antinociceptive effects have been demonstrated for several different A_1ARs activator types, specifically agonists in microglial cells, partial agonists and allosteric enhancers in acute and neuropathic pain models, and ADK inhibitors in chronic pain (Romagnoli *et al.*, 2010; Sowa *et al.*, 2010a; Luongo *et al.*, 2014; Vincenzi *et al.*, 2014; Imlach *et al.*, 2015; Otsuguro *et al.*, 2015). Moreover, a recent study on A_1ARs-knockout (KO) mice, previously reported as mice with an increased nociceptive response, indicated that inosine behaves as an agonist for A_1ARs, furnishing antinociceptive effects at a potency similar to adenosine itself (Nascimento *et al.*, 2015), even though inosine may also act by competing with adenosine for nucleoside transport, thereby increasing its extracellular levels. The molecular mechanisms triggered by A_1ARs that lead to antinociception include inhibition of cyclic AMP, PKA and Ca^{2+} channels, activation of K^+ currents, and interactions with phospholipase C, inositol triphosphate, diacylglycerol, and β-arrestin pathways (Chen *et al.*, 2014). Interestingly, recent studies demonstrated a role for A_1ARs in the mediation of the antinociceptive effect of acupuncture, whose analgesic effect has been replicated by direct injection of an A_1ARs agonist (Goldman *et al.*, 2010; Ren *et al.*, 2012). In particular, A_1AR activation has been shown to mimic the effect of acupuncture on miRNA profiling and protein levels (Wang *et al.*, 2014). Adenosine may also provide beneficial effects against inflammatory pain by acting through A_{2A}ARs and A_{2B}ARs located in inflammatory immune cells (Loram *et al.*, 2013), and the latest evidence suggests that A_3AR activation will be useful in the treatment of chronic neuropathic pain (Borea *et al.*, 2014; Janes *et al.*, 2016). Indeed, A_3AR

activation has been shown to inhibit neuropathic pain, induced mechanically or by chemotherapy, by enhancing the effects of available analgesics (Chen *et al.*, 2012). In particularr, A_3AR agonists were able to attenuate neuropathic pain induced by paclitaxel, by modulating glial-restricted spinal signalling pathways (Janes *et al.*, 2014). This mechanism has also been confirmed in an oxaliplatin-induced peripheral neuropathy model, in which its beneficial effects were attributed to the inhibition of an astrocyte-associated neuroinflammatory response (Janes *et al.*, 2015). A_3AR agonists have also been shown to reverse neuropathic pain via an increase in GABA inhibitory neurotransmission (Ford *et al.*, 2015). Furthermore, A_3AR stimulation has been found to reduce pain in an in vivo model of bone cancer (Varani *et al.*, 2013). This latter finding is particularly interesting given that A_3AR agonists also display anticancer activity (Borea *et al.*, 2014). It is also important to note that, unlike A_1 and A_{2A}ARs stimulation, the administration of potent, selective, and orally bioavailable A_3 agonists in humans induces no cardiac or hemodynamic adverse effects, making the A_3AR a particularly appealing therapeutic target in chronic pain of various aetiologies (Fishman *et al.*, 2012; Little *et al.*, 2015; Tosh *et al.*, 2015).

Inflammatory bone loss

Adenosine is known to have a role in the suppression of inflammatory bone resorption. Indeed, the drug methotrexate (MTX), used to inhibit bone erosion in patients with RA, exerts its anti-inflammatory effects via A_{2A}ARs (Chan and Cronstein, 2010; Mediero *et al.*, 2012; Mediero and Cronstein, 2013), activation of which inhibits osteoclast differentiation and regulates bone turnover via PKA-dependent inhibition of NF-κB nuclear translocation. This suggests a mechanism by which adenosine could target bone destruction in inflammatory diseases, such as RA (Mediero *et al.*, 2013; Mediero *et al.*, 2015). A_{2A}ARs also promote the proliferation of mouse bone marrow-derived mesenchymal stem cells, thereby having a critical role in osteoblast differentiation. By contrast, osteoblast formation is heavily regulated by A_{2B}ARs (Gharibi *et al.*, 2011; Carroll *et al.*, 2012; Takedachi *et al.*, 2012; Mediero *et al.*, 2013). Accordingly, osteoclast development in bone marrow cells from healthy controls and patients with multiple myeloma is inhibited by activation of A_{2A} and A_{2B}ARs, but with only A_{2B}ARs being implicated in human osteoblast formation (He *et al.*, 2013). The upregulation of A_{2A} and A_3ARs in joint diseases and following pulsed electromagnetic fields has also been shown to modulate chondrocyte biology and cartilage matrix, with beneficial effects on the pathology (Vincenzi *et al.*, 2013d).

Adenosine and wound healing-remodelling

Adenosine has a key role in the inflammatory response involved in wound healing and remodelling (Ferrari *et al.*, 2016). In particular, the A_{2A}AR subtype promotes several early events in these processes, including vasodilatation, angiogenesis, matrix production, and inflammation (Haskó and Cronstein, 2013). As a consequence, topical application of selective A_{2A}AR agonists accelerates the healing of dermal wounds in both normal and impaired-wound-healing animals, by a mechanism involving tissue plasminogen activator (tPA) (Arasa *et al.*, 2014; Gessi *et al.*, 2014; Montesinos *et al.*, 2015). Even though a clinical trial of an A_{2A}AR agonist for wound healing failed, a more recent clinical study reported that intramuscular and perilesional administration of an A_{2A}AR agonist improved the healing of foot ulcers in patients with diabetes (Mantell *et al.*, 2010; Squadrito *et al.*, 2014). A_{2B}ARs are also involved in wound healing and remodelling. Indeed, their expression in cardiac mesenchymal stromal cells after myocardial injury has been found to promote these processes in the myocardium, by inducing the transition of these cells into myofibroblasts (Ryzhov *et al.*, 2014). Furthermore, the A_{2B}AR is behind the increase in the production of proangiogenic factors, such as IL-6, IL-8 and VEGF, by cardiac stromal cells, revealing its role in the stimulation of angiogenesis in an injured heart (Ryzhov *et al.*, 2012, 2013). In fact, A_{2B}ARs have long been known to stimulate VEGF production and angiogenesis in various cell types, including cardiac mesenchymal stem-like cells (Ryzhov *et al.*, 2012; Merighi *et al.*, 2015b), as well as retinal and skin endothelial cells, mast cells, tumour-infiltrating hematopoietic cells, and certain types of cancer cell. Although the main transcription factor involved in this response has been identified as HIF-1, the JUN-B transcription factor has also recently been implicated (Gessi *et al.*, 2010, 2012; Gessi *et al.*, 2011b; S. Ryzhov *et al.*, 2013; Du *et al.*, 2015). Moreover, A_3AR activation also appears to be involved in tissue remodelling activity in human lung mast cells, increasing the expression of genes, such as IL8, IL6, and VEGF, all mediators of tissue remodelling and angiogenesis (Rudich *et al.*, 2015).

Adenosine and diabetes

It is well recognized that adenosine regulates insulin secretion, glucose homeostasis and lipid metabolism, by stimulation its receptors. The stimulation of A_1 and A_{2A}AR subtypes seems to promote an antidiabetic phenotype even though recently it has been shown that blockade of A_1AR activation offers protection from age-related oxidative stress and secretion of proinflammatory cytokines, thus improving insulin release and effect (Yang *et*

al., 2015). As for the A_{2B}AR, its role is still controversial with a number of studies supporting a role for its agonists as a therapy for diabetes (Johnston-Cox *et al.*, 2012, 2014; Peleli *et al.*, 2015). However, this protective effect is challenged by a series of studies reporting the beneficial effects of A_{2B}AR antagonists. It was firstly reported that A_{2B}AR antagonists behave as hypoglycaemic agents in rat models of hepatic glucose production induced by adenosine (Harada *et al.*, 2001). Furthermore, it was found that A_{2B}AR activation increases glucose production by affecting glycogenolysis and gluconeogenesis in the rat liver (Yasuda *et al.*, 2003). Following this line, A_{2B}AR antagonists were shown to counteract the reduction in insulin levels induced by a non-selective adenosine receptor agonist in pancreatic cells and plasma from rats, even though this effect is not mediated through A_{2B}AR activation (Rüsing *et al.*, 2006). A_{2B}AR antagonists were also demonstrated to reduce the levels of IL-6 and other cytokines affecting glucose and fat metabolism in a diabetic mouse model, thus improving insulin resistance (Figler *et al.*, 2011). Furthermore, A_{2B}AR blockade reduced the activation of the pro-inflammatory caspase-1 in rat retinal cells incubated in hyperglycaemic conditions (Trueblood *et al.*, 2011; Vindeirinho *et al.*, 2016). Interestingly, it has been found that high glucose levels and experimental diabetes increase the concentrations of adenosine in plasma by reducing the equilibrative NT in proximal tubule cells. This increase correlated with a marker of renal fibrosis in diabetic rats. Furthermore, the expression of profibrotic cell activation markers α-smooth muscle actin and fibronectin was increased by stimulation of A_3ARs (Kretschmar *et al.*, 2016). Therefore, before establishing a role for A_{2B}AR agonists or A_{2B}/A_3AR antagonists in diabetic therapy, when looking at the evidence it is important to accurately examine the experimental conditions associated with glucose and insulin regulation including the method used for inhibiting the receptors (pharmacological vs. genetic) and the cell types or model system used (Antonioli *et al.*, 2015; Merighi *et al.*, 2015a).

Adenosine and lung injury

An important function of the A_{2A}AR subtype has been observed in models of lung inflammation and, based on encouraging in vitro studies, several A_{2A}AR agonists have been developed for treating the asthmatic response. Unfortunately, however, these presented limited efficacy in clinical settings (Antonioli *et al.*, 2014), although scientific interest in the role of A_{2A}ARs in this context persists. Indeed, the A_{2A}AR has been

associated with lung-protective properties of propentofylline in a murine model of lipopolysaccharide (LPS)-induced acute pulmonary inflam-mation (Konrad *et al.*, 2013).

Adenosine and intestinal inflammation

Likewise, A_{2B}AR signalling has been shown to protect against inflammation in the intestine; A_{2B}-KO mice showed significantly more severe colitis of more acute onset, associated with a loss of intestinal epithelial barrier function (Aherne *et al.*, 2015). Accordingly, A_{2B}AR signalling in epithelial cells has been shown to attenuate colonic inflammation through a specific barrier-repair response, namely phosphorylation of vasodilator-stimulated phosphoprotein (Schingnitz *et al.*, 2010; Hart *et al.*, 2011). However, it has been previously reported that A_{2B}-KO mice had an attenuated colitis and that A_{2B}ARs on nonimmune cells had an important role in the induction of colitis (Kolachala *et al.*, 2008; Ingersoll *et al.*, 2012). Overall, these conflicting data suggest that A_{2B}AR signalling has opposing effects on different elements of gut inflammation. Recently, it has been found that A_{2B}AR, modulating the activity of excitatory tachykininergic nerves, participate to the enteric dysmotility associated with obesity (Antonioli *et al.*, 2017).

Adenosine and eye diseases

A_3ARs have been widely implicated in many ocular diseases, including dry eyes, glaucoma, and uveitis. A_3AR agonists have proven efficacy in glaucoma therapy, ascribed to their preventing the activation of the P2X7/NMDA receptors responsible for the increase in Ca^{2+} and apoptosis in retinal ganglion cells (Borea *et al.*, 2014). A_3AR activation is also demonstrably useful in patients with moderate-to-severe dry-eye syndrome, in which CF101 has shown good tolerance and a statistically significant improvement in a Phase II clinical trial (Avni *et al.*, 2010). Interestingly, in the same clinical trial, CF101 also demonstrated its efficacy as an agent able to lower intraocular pressure (Fishman *et al.*, 2013). This followed findings that oral treatment with CF101, initiated upon disease onset, improved clinical fundoscopy scores, and ameliorated the pathological manifestations of uveitis (Fishman *et al.*, 2011). Hence, daily oral CF101 is currently the subject of a Phase II, randomised, double-blind, placebo-controlled safety and efficacy trial in subjects with active, sight-threatening, non-infectious intermediate or posterior uveitis (NCT01905124 clinicaltrials.gov).

Adenosine and cancer

Adenosine does have a protective role in cancer, but this risks partial disturbance by its concomitant effects on the immune system. Indeed, high levels of CD39 and CD73 lead to increased adenosine concentration, which, through A_{2A} and A_{2B}AR-mediated effects on immune cells, creates an immune-tolerant tumour microenvironment (Ohta and Sitkovsky, 2014). This effect of adenosine may be considered a natural consequence of its attempting to avoid excessive inflammation during tissue injury, but suggests both inhibitors of enzyme-generating adenosine and A_{2A} and A_{2B}AR antagonists as potential anticancer targets (Antonioli *et al.*, 2013). In addition, there are in vitro and in vivo studies reporting the prosurvival and prometastatic effects of A_{2B}AR activation (Ntantie *et al.*, 2013). However, as for the specific effects of adenosine in neoplastic cells, a large amount of data points to adenosine having the role of a protective guardian in cancer. A_3AR expression is high in various tumour cells, including HL60 and K562 human leukaemia, Jurkat and U937 human lymphoma, Nb2 rat lymphoma, A375 human melanoma, PGT-beta mouse pineal gland tumour, human glioblastoma, and human prostatic and mesothelioma cells. What is more, A_3AR overexpression has also been reported in surgical colon, breast, hepatocellular, and mesothelioma cancer tissues, compared with their healthy counterparts. Given that peripheral blood cells mirror upregulation of A_3ARs in tissues, this adenosine subtype could be a novel marker for cancer (Gessi *et al.*, 2011a). Indeed, several in vitro and in vivo studies have demonstrated that A_3AR activation is responsible for inhibiting tumour cell proliferation, increasing apoptosis, and reducing tumour development and metastasis. These studies, including syngeneic, xenograft, orthotopic, and metastatic experimental animal models utilising CF-101 and Cl-IB-MECA (CF-102) in melanoma, colon, prostate, and hepatocellular carcinomas, thereby suggest A_3AR upregulation as a potential mechanism by which adenosine may reduce tumour development (Fishman *et al.*, 2009). The molecular pathway activated by A_3ARs involves deregulation of the Wnt signal, which generally actively stimulates cell cycle progression and cell proliferation during embryogenesis and tumorigenesis. In particular, downregulation of PKA and PKB/Akt leads to an increase in glycogen synthase kinase 3b (GSK-3b) activity, in turn resulting in phosphorylation and ubiquitination of b-catenin and suppression of cyclin D1 and c-myc expression. Reduced NF-κB, by inducing apoptosis, has also been implicated in the antitumor effects of A_3AR agonists, in particular IB-MECA, which provokes this effect in melanoma and hepatocellular carcinoma (Fishman *et al.*, 2012). As a consequence of the above, which opens new therapeutic perspectives against cancer, the safety and efficacy profile of A_3AR agonist CF102 has been clinically tested, further to the treatment of

hepatocellular carcinoma (Stemmer *et al.*, 2013). In light of the favourable results of this trial, more extensive Phase II liver cancer studies are ongoing (NCT02128958 clinicaltrials.gov).

Role of adenosine in the control of inflammation

By activating the A_{2A}, A_{2B}, and A_3AR subtypes, adenosine has a crucial role in the regulation of tissue homeostasis, affecting the immune system. It typically inhibits endothelial cell adhesion and superoxide anion production by neutrophils, and reduces proinflammatory cytokine release from macrophages, dendritic cells, and lymphocytes (Barletta *et al.*, 2012; Hasko and Pacher, 2012; Linden and Cekic, 2012; Vincenzi *et al.*, 2013b; Antonioli *et al.*, 2014; Perretti *et al.*, 2015). In 2001, a seminal paper by Ohta et al. reported increased inflammation, tissue damage, TNF-α/interferon (IFN)-γ levels, and mortality in A_{2A}-KO mice treated with subthreshold doses of inflammatory stimuli, thereby suggesting a role for A_{2A}ARs in inflammation (Ohta and Sitkovsky, 2001). Furthermore, several studies indicate that the A_{2B}AR subtype is selectively induced in inflamed vascular and intestinal epithelia, as well as the kidneys, heart and lung, making it a direct target in the treatment of inflammation, which is typically characterised by a hypoxic environment (Aherne *et al.*, 2011; Grenz *et al.*, 2011; Koeppen, Eckle and Eltzschig, 2011; Poth *et al.*, 2013). The A_3AR subtype may also prove useful in hypoxia, because its upregulation has been demonstrated in a variety of inflammatory conditions and immune pathologies (Borea *et al.*, 2014). Substantial line of evidence suggest that A_{2A}ARs are able to mediate the majority of anti-inflammatory effects of endogenous adenosine (Blackburn *et al.*, 2009; Ohta and Sitkovsky, 2009). In particular, the ability of A_{2A}AR activation to suppress cytokine and chemokine expression by immune cells is likely the dominant mechanism involved. Expression of A_{2A}ARs has been found on most inflammatory cells, where it exert various anti-inflammatory actions (Blackburn *et al.*, 2009). 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 (McColl *et al.*, 2006). 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 (Naganuma *et al.*, 2006; Sevigny *et al.*, 2007). It has been also demonstrated that A_{2A}ARs play an important role in the promotion of wound healing and angiogenesis (Montesinos *et al.*, 2003). 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 (Varani *et al.*, 2010d). 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 (Day *et al.*, 2004). 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 (Chan *et al.*, 2006). 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 (Sitkovsky *et al.*, 2004). 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}ARs (Sitkovsky, 2009). Another area where A_{2A}ARs signalling has received attention as potential therapeutic target is the gastrointestinal tract. Studies have highlighted the protective effects of A_{2A}AR 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 (Odashima *et al.*, 2005; Naganuma *et al.*, 2006; Haskó *et al.*, 2008). 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 (Koizumi *et al.*, 2009). 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 (Polosa and Blackburn, 2009). In addition, pharmacological treatment of allergic rats with an A_{2A}AR agonist resulted in diminished pulmonary inflammation (Fozard *et al.*, 2002). A recent study in

ADA-deficient model demonstrated that genetic removal of A_{2A}ARs leads to enhanced pulmonary inflammation, mucus production and alveolar airway destruction (Mohsenin *et al.*, 2007). 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 prevent hypoxia-reoxygenation-induced exacerbation of pulmonary injury (Wallace and Linden, 2010). 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 FEV_1/FVC ratio, an established index of airflow obstruction. In particular A_{2A}, as well as A_3ARs, was found to be upregulated in COPD patients (Varani *et al.*, 2006). 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, a substantial preclinical research activity targeted at understanding the function of A_{2A}ARs in models of airway inflammation has been performed. The use in phase II trials for COPD of an A_{2A}AR agonist named UK432,097 that was identified as inhaled anti-inflammatory drug without effects on blood pressure was reported with the aim to highlight the impressive pedigree of A_{2A}ARs as a potential anti-inflammatory agent (Trevethick *et al.*, 2008). 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 (Harish *et al.*, 2003). 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 (Gessi *et al.*, 2002). A_3ARs are also responsible for inhibition of superoxide production and chemiotaxis of mouse bone marrow neutrophils (van der Hoeven *et al.*, 2008). 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 to inhibit the chemotaxis process (Gao *et al.*, 2001). 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 (Gessi *et al.*, 2011b). 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 (Salvatore *et al.*, 2000). 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 (Panther *et al.*, 2001; Schnurr *et al.*, 2004) 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 proapoptotic effect on malignat mesothelioma cells and on human healthy mesothelial cells exposed to asbestos through the deregulation of Akt/NF-κB cell survival pathway (Varani *et al.*, 2011). 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 (Gessi *et al.*, 2008).

Several studies have shown the relationship between adenosine pathway and joint inflammation in rheumatoid arthritis (RA) *in vitro* and *in vivo* (Varani *et al.*, 2010b). 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 (Varani *et al.*, 2010d). *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 up-regulation of CD73 and A_{2A}AR expression (Flögel *et al*., 2012). In animal models of acute and chronic inflammation, non selective 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 (Montesinos *et al.*, 2003; Flogel *et al.*, 2012). Studies on knockout animals have shown evidence that adenosine acting on 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 up-regulation 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 up-regulation of NF-κB, the DNA binding site of which are present in the promoter of A_3AR gene (Montesinos *et al.*,

2000; Ochaion *et al.*, 2006; Bar-Yehuda *et al.*, 2007; Silverman *et al.*, 2008) 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 (Stamp *et al*., 2012). Besides, among the theories about 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) trasformylase enzyme. The net effect of AICAR accumulation is a rise in intracellular AMP and adenosine levels (Fishman *et al.*, 2006).

Atherosclerosis is another interesting issue about relationship between RA and adenosine. RA patient 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 (Stamp *et al.*, 2012). There are interesting data about A_{2B}ARs and the regulation of atherosclerosis in a mice model (Chan and Cronstein, 2010). The overexpression of A_3ARs in RA was directly correlated to high levels of pro-inflammatory cytokines acting via an upregulation of NF-κB which is a key player in the pathogenesis of arthritis diseases (Ochaion *et al.*, 2006). In RA patients adenosine suppressed the elevated levels of pro-inflammatory cytokines such as TNF-α and IL-1β (Coomes *et al.*, 2011). 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 (Varani *et al.*, 2009). It has been reported that A_3AR agonists prevented cartilage damage, osteoclast/osteophyte formation, bone destruction and markedly reduced pannus formation and lymphocyte formation (Koupenova *et al.*, 2012). The A_3ARs was also identified as a novel anti-inflammatory target that upregulated in RA, psoriasis and Crohn'disease if compared with healthy subjects associated with an altered PI3K-PKB/Akt signaling pathway and NF-κB activation (Forrest *et al.*, 2005). The findings showing A_{2A}ARs and A_3ARs upregulation in RA patients suggest the utilization of these receptors as therapeutic target, modulating them with specific and well-known agonists. Clinical evidences in RA patients show that A_3AR agonists pharmacological treatment modulate an improvement in signs and symptoms (Bar-Yehuda *et al.*, 2007). About 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 (Bar-Yehuda *et al.*, 2009). In a Phase I study in healthy subjects, CF 101 was found to be safe and well tolerated with a linear pharmacokinetic activity (Ochaion *et al.*, 2009). In a Phase IIa study in RA patients, CF101 oral administration twice daily for 12 weeks resulted 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 anti-rheumatic agents. Interesting, the expression level of A_3ARs at baseline was directly correlate with major responsiveness, high grading of efficacy, suggesting its use as a biomarker for the pharmacodynamic and therapeutic effects of this novel agent (Bar-Yehuda *et al.*, 2007; Silverman *et al.*, 2008). The anti-inflammatory effect of A_3AR was also proved in fibroblast-like synoviocytes derived from synovial fluid of RA patients (Ochaion *et al.*, 2008). In particular, the effect of a novel A_3AR agonist, CF502, with high human A_3AR affinity and selectivity is now under investigation. CF502 induce a dose dependent inhibitory effect on the proliferation of fibroblast-like synoviocytes via deregulation of NF-κB signaling pathway. Furthermore, CF502 markedly suppress the clinical and pathological manifestations of adjuvant induced arthritis in a rat experimental model. Other data shown that the use of A_{2A} and A_3AR agonists significantly reduces NF-κB levels and inhibits IL-1 beta, IL-6 and TNF alpha release in mononuclear cell from peripheral blood sample of RA patients (Varani *et al*., 2011b) suggesting the involvement of these ARs in the modulation of inflammatory response. It has been also 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 receptors subtypes in the mechanism regulating joint damage in RA (Varani *et al.*, 2011b). 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 (Varani *et al.*, 2011b). Thus, A_{2A} and A_3ARs up-regulation 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 an home-made agonist significantly attenuate the development of arthritis and reduced the signs of the disease (Bitto *et al.*, 2011).

Adenosine and autoimmune diseases

An enormous body of literature points towards A_{2A} and A_3AR agonists potentially having a relevant role in the treatment of RA. In particular, the gold standard therapy for RA is MTX, which is related to adenosine production, and recent evidence indicates that the capacity to generate adenosine by regulatory T cells (Tregs) is an excellent predictor of

MTX response (Chan and Cronstein, 2010, 2013; Haskó and Cronstein, 2013; Peres *et al.*, 2015). Furthermore, in an in vivo model of collagen-induced arthritis, A_{2A} receptor stimulation slowed its progression by preventing nitrosative and oxidative injury and reducing TNF-α, interleukin (IL)1-β, and IL-6 levels (Mazzon *et al.*, 2011). Interestingly, an increase in A_{2A} receptors in neutrophils and monocytes in the arthritic knee joint has been shown to mirror the upregulation of CD73 in the neutrophils, monocytes, and macrophages of the synovial fluid of mice affected by RA, leading to its pioneering exploitation as an A_{2A} agonist prodrug (Flogel *et al.*, 2012). Indeed, a CD73-dependent prodrug transformation has been shown to inhibit joint inflammation by provoking the selective activation of A_{2A} receptors on immune cells, an approach that avoids the cardiovascular adverse effects previously encountered upon administration of A_{2A} agonists. Multiple lines of evidence also point to the upregulation of A_3 receptors in RA, as well as psoriasis and Crohn's disease (Borea *et al.*, 2014). Interestingly, administration of anti-TNF-α drugs normalises the overexpression of both A_{2A} and A_3 receptors in RA. Moreover, the endogenous activation of these receptors may have a direct role in the control of RA inflammation, as suggested by the inverse correlation between their levels and the Disease Activity Score (Varani *et al.*, 2011b). Indeed, the upregulation of A_{2A} receptors by TNF-α and IL-1 is well known, and TNF-α has also been reported to increase A_{2A} receptor activity (Khoa *et al.*, 2001; Ferrante *et al.*, 2013; Perez-Aso *et al.*, 2013). Overexpression of A_3ARs in RA has been directly linked to an increase in NF-κB, a transcription factor regulating A_3 gene expression and a key player in the pathogenesis of arthritic diseases and osteoarthritis (OA) (Fishman *et al.*, 2012). Activation of A_3 receptors downregulated the NF-κB and Wnt pathways, resulting in a marked improvement in disease parameters. In this regard, the A_3AR agonist IB-MECA (CF101) has already completed Phase I clinical trials, in which it proved to be safe and well tolerated, and Phase II studies in patients with RA or OA, which confirmed that it ameliorated their signs and symptoms (Fishman *et al.*, 2012). This indicates that CF101 may represent a powerful weapon against rheumatic diseases, as do the results of another Phase II trial in patients affected by moderate-to-severe chronic plaque-type psoriasis. This multicenter, randomised, double-blind, dose-ranging, and placebo-controlled trial confirmed the safety and good tolerance of the A_3AR agonist, which brought about a progressive linear improvement in symptoms (David *et al.*, 2012). Therefore, it is no surprise that CF101 has already embarked on a Phase II/III randomised, double-blind, placebo-controlled, dose-finding study to evaluate the efficacy and safety of daily oral administration in patients with moderate-to-severe plaque psoriasis (NCT01265667 Clinicaltrials.gov).

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 pro-inflammatory mediators including cytokines, metalloproteases and growth factors.

RA is usually characterized by symmetric inflammatory polyartrhitis and affects approximately 0.5 - 1% of the general population worldwide. There is a relative lower prevalence in China (0.3%) and higher in the Pima Indians in North America (5%). Women are affected about three times more often than men (Gabriel, 2001). The pathophysiology is not yet fully understood, as for others rheumatic inflammatory disorders and systemic autoimmune diseases. One potential synthesis of the data available on pathogenesis suggests that an induction phase, initiated by innate immunity, on a favourable genetic background, get ready the joint for subsequent coming of inflammatory and immune cells (McInnes and Schett, 2011; Grenz *et al.*, 2012b). Infections, toxins and drugs have been implicated as well as an interplay among genetic and hormonal background, environmental and immunologic factors. Cigarette smoke, bacterial products, viral components, and other environmental stimuli can contribute to these responses. This process, self-limited in normal individuals, in others individuals might lead to a different outcome, due to a predetermined propensity for immune hyperreactivity or autoreactivity. The pro-inflammatory mediators released act on different cell populations including lymphocytes, neutrophils, endothelial cells, synoviocytes, osteoclasts and chondrocytes by inducing the maintenance of inflammation, angiogenesis and chemotaxis (Joseph *et al.*, 2010). 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, raising 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 (Sarkar and Fox, 2010). Among the released inflammatory mediators IL-1β, TNF-α and IL-6 are the pivotal cytokines in the physiopathology of the synovial inflammation which activate several cell types including lymphocytes, neutrophils, endothelial cells, osteoclasts, chondrocytes, synoviocytes and up-regulate 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 (McInnes and Schett, 2011).

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 (Suzuki *et al.*, 2003; Kuhn *et al.*, 2006; van der Helm-van Mil *et al.*, 2006). Genes play a key role in susceptibility to RA and disease severity. Class II MHC genes, especially genes containing a specific five-amino acid sequence in the hypervariable region of HLA-DR4, are the most prominent genetic association. Newly defined genetic associations, including polymorphism in the *PTPN22* and *PADI 4* genes, and many cytokines promoter polymorphisms, population-specific genes and other undefined genes are reported as genetic markers of diagnosis and prognosis (Begovich *et al.*, 2004; van der Helm-van Mil *et al.*, 2006; McInnes and Schett, 2011). 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 managements (Senolt *et al.*, 2009). Recently, a joint working group from the ACR and the European League Against Rheumatism (EULAR) developed and published a new approach to classifying RA (Arnett *et al.*, 1988; Aletaha *et al.*, 2010). 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 (2 levels; range 0–1). These new criteria focus on finding which facilitates earlier recognition of RA and outcome prediction.

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 (Quinn and Emery, 2003). 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 (Bakker *et al.*, 2011). There is increasing acceptance of paradigms of adjusting therapy to achieve a predefined goal, as remission or low-disease activity (“treat to target”) with frequent

monitoring and strategy adjustments, if necessary ("tight control"), until the target is reached (Smolen *et al.*, 2010; van Hulst *et al.*, 2011). 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) (Prevoo *et al.*, 1995; Anderson *et al.*, 2011).

In the past, treatment options for RA were limited to non steroidal anti-inflammatory drugs (NSAIDs), steroids and gold. A pyramidal approach to treatment was used, whereby therapy was gradually escalated and a disease modyfing anti-rheumatic drugs (DMARDs) was used relatively late in the disease course. In the last decade, there was an inversion of the pyramid, with earlier and more intensive and innovative approach to RA treatment (Sokka, no date; Bensen *et al.*, 1990). After a long time characterized by use of conventional DMARDs alone or in combination, the advent of "biologic" drugs, which are able to block cytokine pathway and B and/or T cell activation, has profoundly changed the therapeutic scenario and, consequently, the current strategy adopted to cure RA (Caporali *et al.*, 2009; Senolt *et al.*, 2009). Glucocorticoids have been, and continue to be, a part of the treatment strategy throughout the years (Gorter *et al.*, 2010). The recent EULAR recommendations for the treatment of RA identify three phases of therapy (Smolen *et al.*, 2010).

Phase one is the initiation of DMARD treatment as monotherapy, immediately after diagnosis of RA. The recommended drug is MTX, widely seen as an anchor drug in RA (Jacobs, 2012). Other DMARDs (leflunomide, sulfasalazine, injectable gold) can be used if MTX is contraindicated or not tolerated. Glucocorticoids can be added at low or medium doses (often 5-10 mg/day of prednisone).

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, shows an inadequate response or develop side effects requiring discontinuation of therapy (Furst *et al.*, 2010a).

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 (Aletaha *et al.*, 2008; Furst *et al.*, 2010a; van Hulst *et al.*, 2011).

Spondyloarthritis

Spondyloarthritis or spondyloarthropathy (SpA) refers to an interrelated group of rheumatic diseases which share some common features. Several sets of classification criteria used to classify SpA have been reviewed, which include the Assessment of SpondyloArthritis Society (ASAS) criteria, the modified New York criteria for ankylosing spondylitis (AS), the Amor criteria and the European Spondyloarthropathy Study Group (ESSG) criteria (Akgul and Ozgocmen, 2011; Taylor and Robinson, 2013). Diseases that are included in the SpA family include AS, reactive arthritis, psoriatic arthritis, enteropathic arthritis and undifferentiated SpA. Variations in the epidemiology of SpA are explained by the application of different sets of SpA classification criteria. Dean et al. estimated that there were 1.30–1.56 million and 4.63–4.98 million cases of AS in Europe and Asia respectively (Dean *et al.*, 2014). In the United States, the overall age-adjusted prevalence of definite and probable SpA by the Amor criteria was 0.9%, which corresponded to approximately 1.7 million persons, while the ageadjusted prevalence of SpA by the ESSG criteria was 1.4%, which corresponded to 2.7 million persons (Reveille and Weisman, 2013). Despite wide variations in the prevalence of SpA geographically, the reported prevalence of SpA between 0.9% and 1.4% may represent an overestimation due to recruitment biases. Some studies have reported an overall prevalence of SpA as low as 0.3% in France (Saraux *et al.*, 2005; Roux *et al.*, 2007), 0.45% in southern Sweden (Haglund *et al.*, 2011) and 0.4% in North America (Reveille, 2011). Although SpA is a class of diseases that share some similarities with RA, some distinctive features of SpA differentiate it from the latter. In RA, there is symmetrical involvement of the joints of the hands and feet accompanied by the presence of erosions and absence of new bone apposition. Auto-antibodies such as RF and anticitrullinated peptide antibody are often present in RA. On the other hand, SpA is characterised by human leukocyte antigen (HLA)-B27 association, and asymmetrical, oligoarticular peripheral arthritis that predominantly occurs in the lower extremities. Other features that link the SpA group of diseases include sacroiliitis, spondylitis, dactylitis, enthesitis and an increased susceptibility for inflammatory eye disease (Sieper *et al.*, 2006). The pathogenesis of SpA

is not fully understood. Inflammation and new bone formation, especially in the spine, are two central themes of the disease (Braun and Sieper, 2007). Several factors and mediators have been associated with the disease. For example, HLA-B27, a major histocompatibility complex (MHC) class I molecule encoded on chromosome 6p, was first described to be associated with AS in 1973 (Brewerton *et al.*, 1973). Several earlier studies have shown that more than 90% of patients with AS were HLA-B27 positive (van der Linden *et al.*, 1984) whereas reactive, psoriatic and enteropathic arthritis were less frequently associated with HLA-B27 (Brewerton *et al.*, 1973; Brown *et al.*, 1996). However, not all HLA-B27 positive persons in the general population will eventually develop AS. HLA-B27 positive individuals in the general European population had a 16-fold lower risk (1.3%) of developing AS when compared to those who were HLA-B27 positive relatives of HLA-B27 positive patients with spondylitis (21%) (van der Linden *et al.*, 1984). The multiple HLAB27 related mechanisms and theories involved in the pathogenesis of SpA have been reviewed by Colbert et al. (Colbert *et al.*, 2014) and Chatzikyriakidou et al. (Chatzikyriakidou *et al.*, 2011). Other players in the pathogenesis of SpA include inflammatory cytokines such as TNF-α (Gratacós *et al.*, 1994; Rudwaleit *et al.*, 2001), IL-1 (Monnet *et al.*, 2012), IL-6 (Gratacós *et al.*, 1994; Fiocco *et al.*, 2012), IL-7 (Rihl *et al.*, 2008), IL-17 and IL-23. The role of inflammatory cytokines and biomarkers in the pathogenesis of SpA has been reviewed by Keller et al. (Keller *et al.*, 2003), Maksymowych (Maksymowych, 2012) and Hreggvidsdottir et al. (Hreggvidsdottir *et al*., 2014), while their role in the structural remodelling in peripheral SpA by Vandooren et al. (Vandooren *et al.*, 2009). Despite new advances, novel approaches and discoveries, the treatment of SpA is largely dependent on pharmacological agents. Non-steroidal anti-inflammatory drugs (NSAIDs) remain the firstline drugs, while traditional DMARDS such as sulphasalazine and MTX and intra-articular injections of corticosteroids in local disease, are commonly and conventionally used in the treatment of SpA whereas newer approaches such as TNF-α blockers (e.g. infliximab, etanercept, adalimumab and golimumab) have been used in the treatment of SpA (reviewed by Braun & Sieper) (Braun and Sieper, 2002). Other supportive measures that play an important role in the management of SpA include patient education and exercise (Zochling *et al.*, 2006; Golder and Schachna, 2013), as well as physical therapy, rehabilitation, surgical referrals, patient associations and self-help groups (Zochling *et al.*, 2006).

AS and PsA are among the most common diseases belonging to spondyloarthritis family. Ankylosing spondylitis is a chronic inflammatory disease that affects 1% of the general population, is regarded as one of the most severe types of spondyloarthropathy, it affects

the spine and sacroiliac joints, causing debilitating pain and loss of mobility (Ghasemi-Rad *et al.*, 2015; Chen *et al.*, 2016). Psoriatic arthritis is a serious condition that causes joint destruction, disability, impaired quality of life and even increased mortality (de Andrade *et al.*, 2014; Sritheran and Leung, 2015). The first-line treatment is represented by NSAIDs in AS and by MTX in PsA. Increasing evidence suggests that many of the MTX effects are mediated by ARs activation. Indeed, it has been shown that MTX induces adenosine release in vitro, in animal models of inflammation and in patients with RA (Chan and Cronstein, 2010; Fishman and Cohen, 2016a). Furthermore, it is known that MTX selectively modulates the nuclear orphan receptor (NURR1) levels induced by inflammatory stimuli and growth factors in resident cell populations of synovial tissue (Festugato, 2015). The inhibitory effect of low-dose MTX on NURR1 activation is mediated through the A_{2A}ARs, confirming the link between the immunomodulatory actions of MTX and adenosinergic system (Ralph *et al.*, 2005). To date, a novel treatment to reduce clinical symptoms in PsA patients is represented by Apremilast, an orally available targeted inhibitor of phosphodiesterase-4 (PDE-4), the major enzyme class responsible for the hydrolysis of cAMP that modulates a wide array of inflammatory mediators implicated in PsA (Schafer, 2012).

AIM OF THE THESIS

It is notewhorty the central role of ARs in mechanisms of inflammation associated to various pathologies suggesting that their stimulation has a different effect on the release of several pro-inflammatory cytokines (Haskó *et al.*, 2008b; Chan and Cronstein, 2010). Adenosine, a purine nucleoside, is considered a potent regulator acting with four cell surface receptor subtypes A_1, A_{2A}, A_{2B} and A_3ARs, which are coupled to different G proteins . The A_1 and A_3ARs exert an inhibitory effect on cAMP production while A_{2A} and A_{2B}ARs mediate an increase of cAMP accumulation (Varani *et al.*, 2006; Fishman *et al.*, 2009; Varani *et al.*, 2010a; Gessi *et al.*, 2011b). RA, AS and PsA are chronic inflammatory rheumatic diseases that affect joints, causing debilitating pain and disability. Several papers report how in RA patients, adenosine suppresses the elevated levels of pro-inflammatory cytokines such as TNF-α and IL-1β (Forrest *et al.*, 2005; Varani *et al.*, 2010b). It has been shown that in particular A_{2A} and A_3ARs are over-expressed in patients with autoimmune inflammatory diseases, including RA, and that A_{2A} and A_3AR pharmacological treatment modulates an improvement in signs and symptoms (Silverman *et al.*, 2008; Borea *et al.*, 2009; Ochaion *et al.*, 2009). From this background it is accepted that the release of various inflammatory mediators in patients with inflammatory disorders could be closely associated with ARs, suggesting their potential role as therapeutic target and the application of novel pharmacological approaches in the treatment of RA, AS and PsA.

Considering this introduction, the aim of this thesis was to investigate the role of ARs in chronic inflammatory rheumatic diseases. In particular, we have evaluated the expression and functionality of A_{2A} and A_3ARs in RA and SpA patients.

The aim of the chapter 1 was to describe a detailed analysis on A_1, A_{2A}, A_{2B} and A_3ARs expression in patients suffering from early-RA (ERA), RA, AS and PsA, using mRNA analysis and saturation binding experiments. ARs were analyzed in age-matched healthy subjects (control group) and in patients with chronic inflammatory rheumatic diseases. Moreover, we have investigated the effect of A_{2A} and A_3AR agonists or antagonists on NF-κB activation and on the production of proinflammatory cytokines, such as TNF-α, IL-1β and IL-6. The joint degradation process that is believed to be largely mediated by proteases has been analyzed by evaluating the effect of A_{2A} and A_3AR agonists on MMP-1 and MMP-3 production.

To date conventional DMARDs such as MTX in monotherapy or in combination are currently the first line medications usually prescribed in RA, AS and PsA patients (Chan and Cronstein, 2010). More recently, the introduction of anti-TNF-α agents (e.g. adalimumab, etanercept) or the use of anti-CD20 B cell targeted therapy (rituximab, RTX) have provided a marked improvement in RA even if some patients do not respond or fail to maintain adequate response to these treatments (Furst *et al.*, 2010).

In view of the above, the aim of the chapter 2 was to evaluate in RA, AS and PsA patients the influence of different biological therapies as anti-TNF-α drugs or RTX in comparison with MTX treatment on A_{2A} and A_3AR density at various time points of treatment (from 0 to 24 months). In RA patients treated with RTX the relationship between A_{2A} and A_3AR density and the clinical parameter 28-joint Disease Activity Score (DAS 28) values, a validated and reliable composite indexes to assess RA disease activity, was also investigated.

CHAPTER 1:

ADENOSINE RECEPTORS IN RHEUMATOID ARTHRITIS AND SPONDYLOARTHRITIS PATIENTS

Introduction

In the literature, several papers have suggested the involvement of adenosine in inflammatory status by A_{2A} and A_3AR activation. In particular, it has been reported that many of the anti-inflammatory effects caused by the stimulation of A_{2A} and A_3ARs are mediated by the suppression of pro-inflammatory cytokine release (Haskó *et al.*, 2008; Gessi *et al.*, 2011b; Impellizzeri *et al.*, 2011). Furthermore, altered expression of A_{2A} and A_3ARs has been found in different inflammation-related pathologies highlighting the potential role of these receptors as therapeutic targets in inflammatory diseases (Varani *et al.*, 2006; Varani *et al.*, 2010b; Vincenzi *et al.*, 2013c). In previous studies our research group has found a significant upregulation of A_{2A} and A_3ARs in blood cells from untreated RA patients and in MTX-treated RA patients, compared with healthy subjects (Varani *et al.*, 2009). In patients' lymphocytes, it was shown that A_{2A} and A_3AR activation inhibited NF-κB pathway and diminished inflammatory cytokines such as TNF-α, IL-1β and IL-6. Moreover, A_{2A} and A_3AR agonists mediated a reduction of different MMP-1 and MMP-3 release (Varani *et al*., 2011b). Furthermore, we have demonstrated the direct involvement of A_{2A}AR in RA pathogenesis based on their expression in relation to the time of pharmacological treatments with DMARDS or different biological drugs. Using an adjuvant-induced rat model of arthritis, we previously reported that the treatment with an A_{2A}AR agonist reduced the severity of clinical signs, decreased arthritis-associated pain, and increased the serum levels of the anti-inflammatory cytokine IL-10 (Vincenzi *et al.*, 2013c). The ability of the A_{2A}AR agonist was also demonstrated in mice subjected to collagen-induced arthritis confirming its anti-inflammatory effect during chronic inflammation (Mazzon *et al.*, 2011). It is well-accepted that arthritis diseases also include spondyloarthritis, a family of chronic inflammatory diseases that share various common features such as axial arthritis and enthesitis. AS and PsA are among the most common diseases belonging to spondyloarthritis family. The role and the function of ARs during the course of chronic autoimmune rheumatic diseases are currently under study from different research groups because in the literature scarce information is available. In this study the involvement of ARs in chronic autoimmune rheumatic diseases represented by RA, AS and PsA was investigated. Of the four AR subtypes, A_{2A} and A_3ARs were overexpressed in lymphocytes obtained from patients in comparison with those of healthy subjects. A_{2A} and A_3AR agonists inhibited the activation of NF-κB, the release of typical pro-inflammatory cytokines and the metalloproteinases levels which are involved in the inflammatory responses in chronic autoimmune rheumatic diseases.

Materials and methods

Patients and healthy subjects

All patients enrolled in this study were recruited from the Rheumatology Section, Department of Medical Sciences, University of Ferrara, Italy. A total of 90 patients were included and divided in ERA patients (*n* = 30), RA patients (*n* = 30), AS patients (*n* = 16) and PsA patients (*n* = 14). RA patients with established disease for more than 12 months fulfilled the American College of Rheumatology (ACR) 1987 criteria for RA. In particular, clinical data included: (i) age, disease duration, gender, serological parameters including rheumatoid factor (RF) and anti-citrullinated cyclic peptide (ACPA); (ii) Disease Activity Score evaluated in 28 joints (DAS 28); (iii) functional status as assessed by Health Assessment Questionnaire (HAQ); (iv) pharmacological therapy as comedication with DMARDs such as MTX and leflunomide. The demographic, clinical and pharmacological details are listed in Table 1. Biological naive, adult patients with AS classified according to the Assessment of SpondyloArthritis international Society (ASAS) criteria (Rudwaleit *et al.*, 2009) and patients with PsA fulfilling the Classification of Psoriatic Arthritis (CASPAR) criteria (Taylor and Robinson, 2013) were included. Clinical data included: (i) age, disease duration, gender, human leukocyte antigen (HLA) B27 status, clinical presentation over disease history considering both the axial involvement and the presence of peripheral arthritis, dactylitis or entheseal pain; (ii) disease activity as assessed by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and DAS 28; (iii) functional status as assessed by the Bath Ankylosing Spondylitis Functional Index (BASFI) and HAQ; (iv) pharmacological therapy as specific tumor necrosis factor inhibitor (TNFi), comedication with DMARDs. The demographic, clinical and pharmacological complete details are listed in Table 1B. This study has obvious limitations mainly related to the low number of patients analyzed, especially in the spondyloarthritis (AS and PsA). This could have induced an artefactual and chance inclusion of a low number of patients with positive serology both for RF and ACPA in the RA group and HLA B27+ in AS group. Healthy subjects (*n* = 90), matched for similar age to the cohort of the examined patients, were volunteers from Ferrara University Hospital Blood Bank. The study was approved by the local Ethics Committee (Approval No. 378, February 2010) of the University Hospital of Ferrara (Italy) and informed consent was obtained from each participant in accordance with the principles outlined in the Declaration of Helsinki.

Sample collection and human lymphocyte preparation

Lymphocytes were isolated and prepared as previously described from the peripheral blood of control subjects, ERA, RA, AS and PsA patients. The isolation of blood cells started no later than 3–4 h after the samples had been taken. The blood was supplemented with 6% (by weight) Dextran T500 solution (Sigma, St. Louis, MO, USA) and erythrocytes were allowed to settle down for 60 min. Leukocytes were centrifuged for 15 min at 100× *g* and remaining erythrocytes were lysed in distilled water at 4°C. Cells were pelletted by centrifugation for 5 min at 250× *g*, suspended in Krebs-Ringer phosphate buffer and layered onto 10 mL of Fycoll-Hypaque (GE Healthcare, Little Chalfont, UK). After centrifugation, mononuclear cells were washed in 0.02 M phosphate-buffered saline at pH 7.2 containing 5 mM MgCl2 and 0.15 mM CaCl2. Finally, they were decanted into culture flask and placed in a humidified incubator (5% CO2) for 2 h at 37°C. This procedure, aimed at removing monocytes, which adhere to the culture flasks, resulted in a purified lymphocyte preparation containing at least 99% small lymphocytes identified by morphological criteria. To obtain membrane suspensions, cell fractions were centrifuged in hypotonic buffer at 20,000× *g* for 10 min. The resulting pellet was re-suspended in Tris HCl 50 mM buffer pH 7.4 containing 2 UI/mL adenosine deaminase (Sigma) and incubated for 30 min at 37°C. The suspension was then centrifuged again at 40,000× *g* for 10 min and the final pellet was used for radioligand binding experiments. The protein concentration was determined by a Bio-Rad method with bovine albumine as reference standard (Varani *et al.*, 2009).

Real-Time Quantitative Polymerase Chain Reaction Experiments

Total cytoplasmic RNA was extracted by the acid guanidinium thiocyanate phenol method. Quantitative RT-PCR assays of A_1, A_{2A}, A_{2B} and A_3AR mRNAs were carried out using gene-specific fluorescently labelled TaqMan MGB probe (minor groove binder) in a ABI Prism 7700 Sequence Detection System (Applied Biosystems, Warrington Cheshire, UK). For the RT-PCR of A_1, A_{2A}, A_{2B} and A_3ARs the Assays-on-DemandTM Gene Expression Products NM 000674, NM 000675, NM 000676 and NM 000677 were used, respectively. For the RT-PCR of the reference gene, the endogenous control human β-actin kit was used, and the probe was fluorescent-labeled with VICTM (Applied Biosystems, Monza, Italy) (Varani *et al.*, 2009).

Saturation Binding Experiments to A_1, A_{2A}, A_{2B} and A_3ARs

Saturation binding experiments to A_1ARs were carried out with the use of [^{3}H]-DPCPX ([^{3}H]-1,3-dipropyl-8-cyclopentyl-xanthine, specific activity 120 Ci/mmol, Perkin Elmer Life and Analytical Sciences, Boston, MA, USA) as radioligand (Varani *et al.*, 2009). Human lymphocyte membranes (60 μg of protein/assay) with 8 to 10 concentrations of [^{3}H]-DPCPX (0.01–20 nM) were incubated for 90 min at 25 °C. Non-specific binding was determined in the presence of 1 μM DPCPX. Saturation binding to A_{2A}ARs was performed with the use of [^{3}H]-ZM 241385 ([^{3}H]-4-(2-[7-amino-2-(2-furyl)[1,2,4]- triazolo[2,3-a][1,3,5] triazin-5-ylamino] ethyl) phenol, specific activity 27 Ci/mmol, Biotrend, Cologne, Germany), as radioligand (Varani *et al.*, 2009a). Cell membranes (60 μg of protein/assay) were incubated for 60 min at 4°C with various concentrations (0.01–20 nM) of [^{3}H]-ZM 241385. Non-specific binding was determined in the presence of 1 μM ZM 241385. Saturation binding experiments to A_{2B}ARs were performed by using [^{3}H]-MRE 2029F20 ([^{3}H]-*N*-benzo[1,3]dioxol-5-yl-2-[5-(2,6-dioxo-1,3-dipropyl- 2,3,6,7-tetrahydro-1H-purin-8-yl)-1-methyl-1H-pyrazol-3-yl-oxy]-acetamide, specific activity 123 Ci/mmol, GE Healthcare, UK) as radioligand (Varani *et al.*, 2009). Cell membranes (80 μg of protein/assay) and [^{3}H]-MRE 2029F20 (0.01–30 nM) were incubated for 60 min at 4°C and non-specific binding was determined in the presence of 1 μM MRE 2029F20. Saturation binding experiments to A_3ARs were carried out using [^{3}H]-MRE 3008F20 ([^{3}H]-5*N*-(4-methoxyphenylcarbamoyl) amio-8-propyl-2-(2- furyl) pyrazolo [4,3-e]-1,2,4-triazolo [1,5-c]pyrimidine, specific activity 67 Ci/mmol, GE Healthcare, UK) as radioligand (Varani *et al.*, 2009). The membranes (80 μg of protein/assay) with [^{3}H]-MRE 3008F20 (0.01–30 nM) were incubated at 4°C for 150 min and MRE 3008F20 1 μM was used to evaluate non-specific binding. Bound and free radioactivity were separated in a Brandel cell harvester (Brandel, Gaithersburg, MD, USA) by filtering the assay mixture through Whatman GF/B glass fiber filters (Whatman. Kent, UK). The filter-bound radioactivity was counted in a 2810 TR liquid scintillation counter (Perkin Elmer, Boston, MA, USA).

Lymphocyte Cell Culture

Isolated lymphocytes from control subjects, ERA, RA, AS and PsA patients were suspended at a density of 10^6 cells/ml in RPMI 1640 medium supplemented with 2% fetal bovine serum (Euroclone, Milan, Italy) and seeded into 24-well plates (1 ml/well). Cells were then preincubated for 15 min with 100 nM of CGS 21680 (4-[2-[[6-amino-9-(*N*-

ethyl-β-D-ribo-furanuronamidosyl)-9*H*purin- 2-yl]amino] ethyl]benzene propanoic, Sigma) or Cl-IB-MECA (N6-(3-iodo-benzyl)-2-chloroadenosine- 5′-*N*-methyluronamide, Sigma) in the absence and in the presence of selected A_{2A} or A_3AR antagonists such as SCH 442416 (2-(2-furanyl)-7-[3-(4-methoxyphenyl)propyl]-7*H*-pyrazolo[4,3-e][1,2,4] triazolo [1,5-c]pyrimidin-5-amine, Tocris Bioscience, Bristol, UK) or MRS 1334 (1,4-dihydro-2-methyl-6-phenyl-4-(phenylethynyl)-3,5-pyridinedicarboxylic acid 3-ethyl-5-[(3-nitrophenyl)methyl] ester, Tocris Bioscience) at 1 μM concentration, respectively. Lymphocytes were then activated with 5 ng/ml PMA and incubated for 24 h (Varani *et al*., 2011b). At the end of incubation, the cell suspension was collected and centrifuged at 1000× *g* for 10 min at 4°C. Then, the supernatants or cell pellets were used for enzyme-linked immunosorbent assay (ELISA) assays or nuclear extract preparation, respectively.

Measurement of cAMP Levels

In lymphocytes, the potency of the well-known A_{2A} and A_3 adenosine agonists CGS 21680 and Cl-IB-MECA (0.1 nM – 1 μM) was investigated. Cells were seeded in 96-well white half-area microplate (Perkin Elmer, Boston, MA, USA) in a stimulation buffer composed of Hank Balanced Salt Solution, 5 mM HEPES, 0.5 mM Ro 20-1724, 0.1% BSA. Forskolin (1 μM) was used to stimulate adenylyl cyclase activity after the addition of Cl-IB-MECA. cAMP levels were then quantified by using the AlphaScreen cAMP Detection Kit (Perkin Elmer) following the manufacturer's instructions. At the end of the experiments, plates were read with the Perkin Elmer EnSight Multimode Plate Reader.

NF-κB activation in human cultured lymphocytes

Nuclear extracts from human cultured lymphocytes of the examined patients were prepared by using a nuclear extract kit (Active Motif, Carlsbad, CA, USA) following the manufacturer's instructions. Activation of NF-κB was evaluated by detecting phosphorylated p65 proteins in nuclear extracts by using the TransAM NF-κB kit (Active Motif). Phosphorylated NF-κB subunits specifically bind to the immobilized oligonucleotides containing the NF-κB consensus site (5′-GGGACTTTCC- 3′). The primary antibody used to detect NF-κB recognized an epitope in the subunits that is accessible only when it is activated and bound to its DNA target. A horseradish peroxidase (HRP)-conjugated secondary antibody provided a sensitive colorimetric readout that was quantified by spectrophotometry at 450 nm wavelength with the Perkin Elmer EnSight Multimode Plate Reader (Varani *et al*., 2011b).

Pro-Inflammatory Cytokine Release in Cultured Lymphocytes

TNF-α levels were measured in human cultured lymphocytes after the treatment described above by using highly sensitive TNF-α ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Pro-inflammatory cytokine (IL-1β and IL-6) levels were determined with a quantitative sandwich ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions (Varani *et al.*, 2011b). The reaction was developed with streptavidin-horseradish peroxidase and optical density was read at 450 nm wavelength with the Perkin Elmer EnSight Multimode Plate Reader.

Measurement of Total MMP-1 and MMP-3 Release in Cultured Monocytes

To obtain human monocytes, peripheral blood mononuclear cells were seeded in petri dishes at the density of 10^6/ml. The cells were allowed to adhere to plastic tissues and non-adherent cells (lymphocytes) were removed. In cultured monocytes, MMP levels were measured after the treatment described above by using the corresponding quantitative sandwich ELISA kit (R&D Systems) according to the manufacturer's instructions (Varani *et al.*, 2011 . Briefly, the assay systems measure natural and recombinant human active and pro-MMPs (total MMPs).

Statistical Analysis

Dissociation equilibrium constants for saturation binding, affinity, or K_D values, as well as the maximum densities of specific binding sites (Bmax), were calculated for a system of one- or two-binding site populations by means of a non-linear curve fitting using GraphPad Prism software version 6.0 (GraphPad Software, Inc., San Diego, CA, USA). All data are reported as mean ± SEM of different independent experiments as indicated in the Results section or in the Figure legends. Analysis of data was performed by one-way analysis of variance (ANOVA). Differences between the groups were analyzed with Dunnett's test and were considered significant at a value of $p < 0.01$.

Results

A_{2A} and A_3ARs are up-regulated in lymphocytes from patients with chronic inflammatory rheumatic diseases

Figure 1A shows the A_1, A_{2A}, A_{2B} and A_3AR mRNA levels determined by RT-PCR in human lymphocytes from healthy subjects and patients. Interestingly, only A_{2A} and A_3AR mRNA expression in ERA, RA, AS, PsA patients was significantly increased. Figure 1B indicates the affinity (K_D, nM) of A_1, A_{2A}, A_{2B} and A_3ARs in lymphocyte membranes from ERA, RA, AS, PsA patients, compared to healthy subjects and shows that the affinity of A_{2A} and A_3ARs was lower in patients than in controls ($p < 0.01$). The receptor density (Bmax, fmol/mg protein) of ARs was examined in lymphocyte membranes from patients and healthy subjects showing that the density of A_1 and A_{2B}ARs was not significantly different in patients respect to control group (Figure 1C). Instead, A_{2A} and A_3AR density was significantly increased in patients compared with healthy subjects (Figure 2). The K_D and Bmax values of ARs in lymphocyte membranes from control group and ERA, RA, AS, PsA patients are reported in Table 2.

Increased potency of A_{2A} and A_3ARs agonists in lymphocytes from patients with chronic inflammatory rheumatic diseases

Figure 3 shows the concentration-response curves of typical A_{2A} and A_3AR agonists such as CGS 21680 and Cl-IB-MECA, in lymphocytes from healthy subjects, ERA, RA, AS and PsA patients. The EC_{50} values of CGS 21680, indicating the concentration of the agonist eliciting 50% of maximal cAMP formation, were significantly lower in patients with chronic inflammatory rheumatic diseases than in control subjects, indicating an increased potency of CGS 21680 (Figure 3A). Similarly, Cl-IB-MECA showed higher potency in ERA, RA, AS and PsA patients, suggesting that the increase in A_3ARs density was correlated with an increase of agonist potency (Figure 3B).

A_{2A} and A_3AR agonists reduces NF-κB activation in lymphocytes from the examined subjects

Higher levels of activated NF-κB p65 were found in nuclear extract from cultured lymphocytes of ERA, RA, AS and PsA patients in comparison with control subjects (Figure 4A). CGS 21680, a well-known A_{2A}AR agonist, was able to significantly inhibit NF-κB levels in cultured lymphocytes derived from the subjects investigated. This effect was

abolished by using the selective A_{2A}AR antagonist SCH 442416, suggesting the A_{2A}-mediated effect of the agonist. Similar results were obtained with the A_3AR agonist Cl-IB-MECA where its inhibitory effect on NF-κB activation was abrogated by A_3AR antagonist MRS 1334. Interestingly, the inhibitory effect mediated by A_{2A} and A_3AR agonists in patients with inflammatory rheumatic diseases was more marked than in control subjects (Figure 4A).

A_{2A} and A_3AR activation inhibits cytokines release from lymphocytes of the examined subjects

In cultured lymphocytes from patients and healthy subjects, the effect of A_{2A} and A_3AR agonists and/or antagonists on TNF-α release was investigated. A marked release of TNF-α was observed in ERA, RA, AS and PsA patients respect to healthy subjects (Figure 4B). In addition, the stimulation of A_{2A}AR with CGS 21680 (100 nM) mediated a significant inhibition of PMA-induced TNF-α release. The inhibitory effect of CGS 21680 was more evident in patients than in control subjects. Similar results were obtained through the A_3AR stimulation by using Cl-IB-MECA at the 100 nM concentration. The direct involvement of A_{2A} and A_3ARs was demonstrated by using selective antagonists such as SCH 442416 and MRS 1334 (1 μM), respectively, which were able to completely abrogate the inhibitory effect mediated by the agonists. The effect of CGS 21680 or Cl-IB-MECA on IL-1β (Figure 5A) and IL-6 (Figure 5B) release was also studied in lymphocytes. PMA (5 ng/ml) induced a marked release of these pro-inflammatory cytokines and the treatment with A_{2A} and A_3AR agonists resulted in a significant reduction of IL-1β and IL-6. In particular, the A_{2A}AR agonist mediated a significant inhibition of IL-1β and IL-6 in ERA (62% and 66%, respectively), in RA (64% and 67%, respectively), in AS (62% and 67%, respectively) or in PsA patients (63% and 68%, respectively) (Figure 5A and 5B). Similar results were obtained by using A_3AR stimulation on IL-1β and on IL-6 release. The inhibitory effect of A_{2A} or A_3AR agonists was counteracted by the A_{2A} or A_3AR antagonists SCH 442416 or MRS 1334 (1 μM), respectively.

A_{2A} and A_3AR agonists reduced MMPs activation in monocytes from the examined subjects

Monocytes were incubated with PMA (5 ng/ml) for 24 hours inducing MMP-1 and MMP-3 protein production. Incubation of monocytes with CGS 21680 or Cl-IB-MECA (100 nM) caused an inhibition of MMP-1 production (Figure 6A). The inhibitory effect was more

evident in inflammatory rheumatic disease patients respect to healthy subjects. Similar results were obtained evaluating the production of MMP-3 suggesting that these MMPs are closely associated with A_{2A} or A_3AR modulation. The direct involvement of these AR subtypes was demonstrated with selective antagonists that blocked the inhibitory effect of the A_{2A} or A_3AR agonists.

Table 1. Clinical and demographic features of the study population, including healthy subjects, patients with rheumatoid arthritis (including early forms) and spondyloarthritis.

Healthy subjects	**90**
No. female/male	59/31
Age, mean ± SEM years	54.1 ± 5.6
Early rheumatoid arthritis (ERA)	**30**
No. female/male	25/5
Age, mean ± SEM years	52.4 ± 2.5
Disease duration (months)	10.3 ± 3.1
Rheumatoid Factor	6/26 (23%)
ACPA	10/26 (38%)
DAS 28, mean ± SEM	3.2 ± 0.2
HAQ, mean ± SEM	1.2 ± 0.1
Concomitant DMARDs or TNF inhibitors	0
Rheumatoid arthritis (RA)	**30**
No. female/male	26/4
Age, mean ± SEM years	53.8 ± 2.9
Disease duration (months)	74.3 ± 8.4
Rheumatoid Factor	9/30 (30%)
ACPA	13/30 (43%)
DAS 28, mean ± SEM	3.4 ± 0.2
HAQ, mean ± SEM	1.3 ± 0.1
Concomitant DMARDs:	
Methotrexate (10–15 mg/week)	30 (100%)

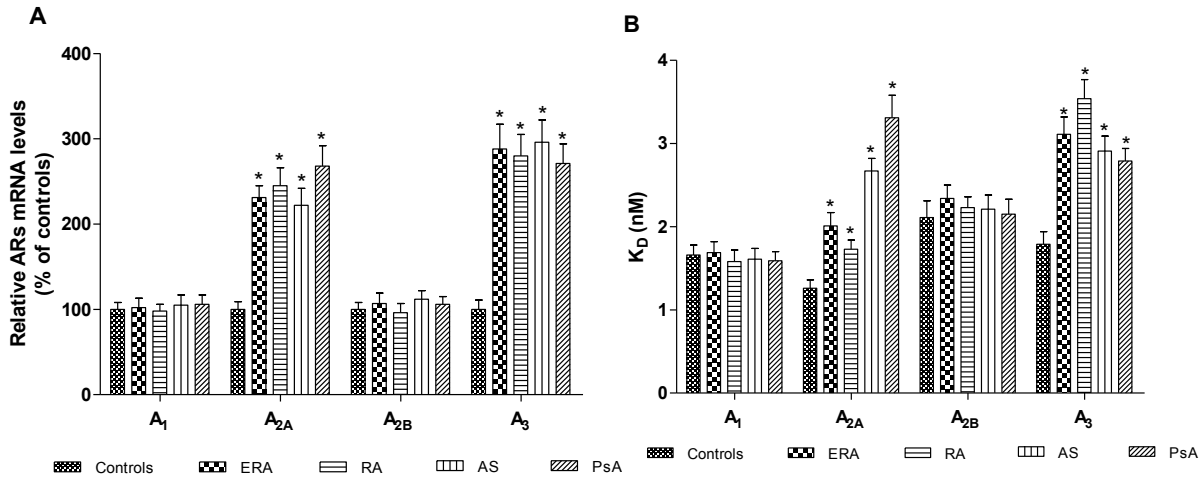
Seronegative spondyloarthritis (including AS and PsA)	**30**
No. female/male	9/21
Age, mean ± SEM years	38.4 ± 2.4
Disease duration (months)	121 ± 14
Ankylosing spondylitis (AS)	16 (53.3%)
Axial involvement (only)	13/16 (81.3%)
Entheseal involvement°	7/16 (43.8%)
Axial and peripheral involvement	3/16 (18.8%)
HLA B27 positive	7/16 (43.8%)
Psoriatic arthritis (PsA)	14 (46.7%)
Peripheral involvement (only)	10/14 (71.4%)
Axial and peripheral involvement	6/14 (42.9%)
Entheseal involvement°	7/14 (50%)
Dactylitis	2/14 (14.3%)
Clinimetric measures:	
DAS 28*, mean ± SEM	3.9 ± 0.3
HAQ*, mean ± SEM	0.8 ± 0.1
BASDAI§, mean ± SEM	5.2 ± 0.5
BASFI§, mean ± SEM	0.8 ± 0.3
Concomitant DMARDs:	
Methotrexate (10–15 mg/week)	1 (3.3%)
Leflunomide	1 (3.3%)
TNF inhibitors:	
Infliximab in AS/PsA	10/2 (55.5/25%)
Adalimumab in AS/PsA	8/4 (44.5/50%)
Etanercept in AS/PsA	0/2 (0/25%)

Table 2. Adenosine receptor binding parameters in lymphocytes from patients with chronic autoimmune rheumatic diseases in comparison with healthy subjects.

	A_1ARs - K_D (nM) Bmax (fmol/mg protein)	**A_{2A}ARs - K_D (nM) Bmax (fmol/mg protein)**	**A_{2B}ARs - K_D (nM) Bmax (fmol/mg protein)**	**A_3ARs - K_D (nM) Bmax (fmol/mg protein)**
Healthy subjects (n=90)	1.66 ± 0.12 35 ± 3	1.26 ± 0.10 55 ± 6	2.11 ± 0.20 53 ± 4	1.79 ± 0.15 136 ± 10
ERA patients (n=30)	1.69 ± 0.13 32 ± 2	2.01 ± 0.16* 167 ± 13*	2.34 ± 0.16 54 ± 3	3.11 ± 0.21* 277 ± 23*
RA patients (n=30)	1.58 ± 0.14 34 ± 3	1.73 ± 0.11* 175 ± 17*	2.23 ± 0.13 55 ± 6	3.54 ± 0.23* 298 ± 24*
AS patients (n=16)	1.61 ± 0.13 32 ± 4	2.67 ± 0.15* 185 ± 11*	2.21 ± 0.17 51 ± 4	2.91 ± 0.18* 333 ± 21*
PsA patients (n=14)	1.59 ± 0.11 33 ± 2	3.31 ± 0.27* 192 ± 11*	2.15 ± 0.18 52 ± 5	2.79 ± 0.15* 341 ± 23*

Data are expressed as the mean ± SEM. Differences were considered significant at a value of * $p < 0.01$ vs healthy controls.

Figure 1. A_{2A} and A_3ARs are up-regulated in patients' lymphocytes with chronic inflammatory rheumatic diseases. (**A**) Relative AR mRNA levels determined by RT-PCR in human lymphocytes from ERA (n = 30), RA (n = 30), AS (n = 16), PsA patients (n = 14) and control subjects (n = 90). (**B**) Affinity of A_1, A_{2A}, A_{2B}, and A_3ARs expressed as K_D values, in lymphocytes derived from ERA (n = 30), RA (n = 30), AS (n = 16) and PsA patients (n = 14) in comparison to control subjects (n = 90). (**C**) Density of A_1, A_{2A}, A_{2B}, and A_3ARs, expressed as the maximum specific binding (Bmax), in lymphocytes derived from ERA (n = 30), RA (n = 30), AS (n = 16) and PsA patients (n = 14) in comparison to control subjects (n = 90). Data are expressed as the mean ± SEM. * $p < 0.01$ vs control group.



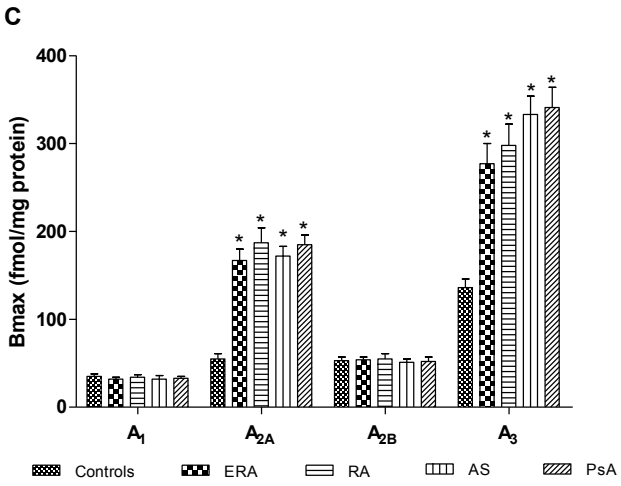


Figure 2. Saturation binding experiments in lymphocyte membranes from patients with chronic inflammatory rheumatic diseases. Saturation curves (**A**,**C**) and Scatchard plots (**B**,**D**) showing the binding of [^{3}H]-ZM 241385 to A_{2A}ARs (**A,B**) and the binding of [^{3}H]-MRE 3008F20 to A_3ARs (**C,D**) in lymphocyte membranes derived from 90 controls, 30 ERA patients, 30 RA patients, 16 AS patients and 14 PsA patients. Data are expressed as the mean ± SEM.

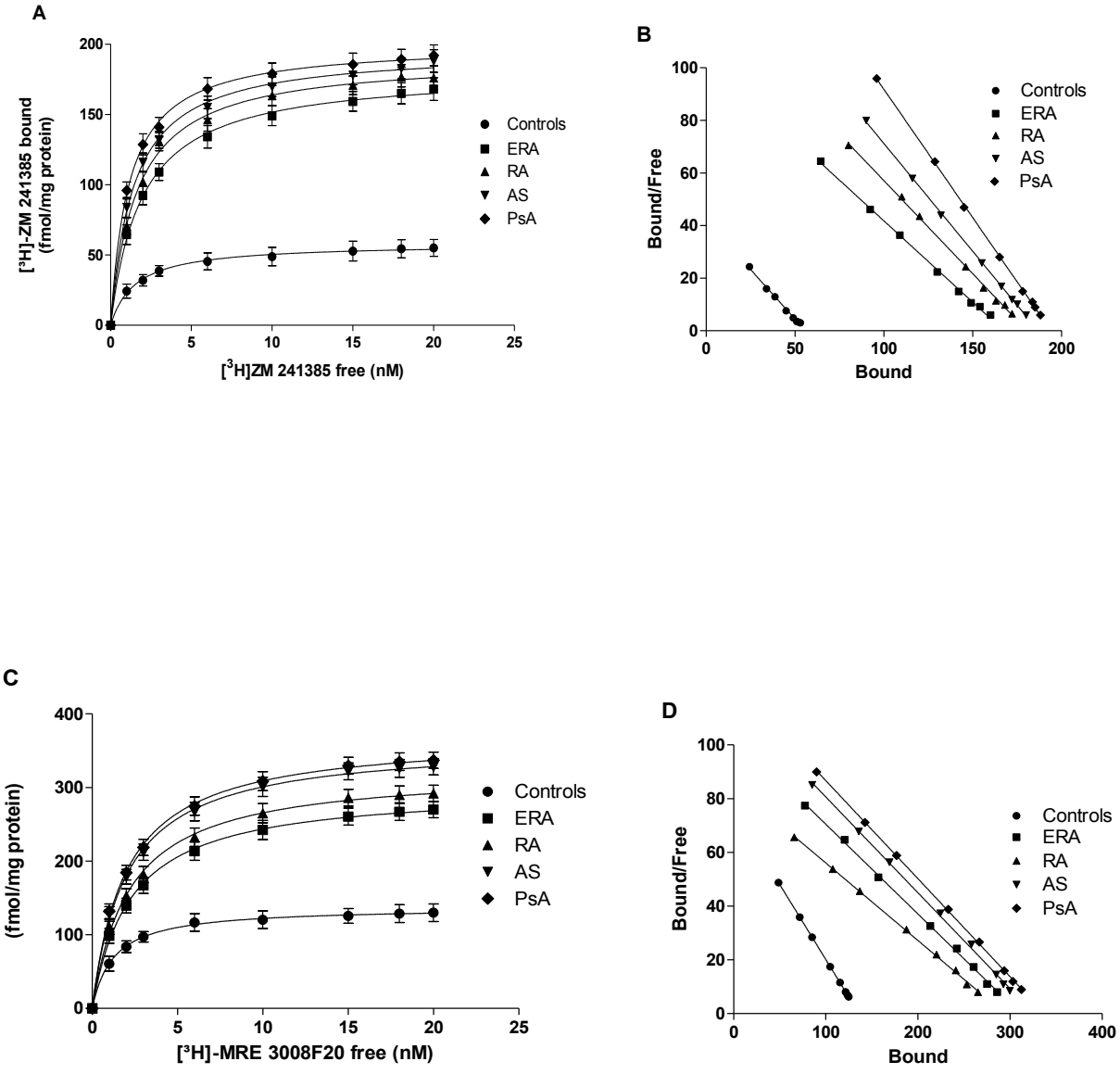
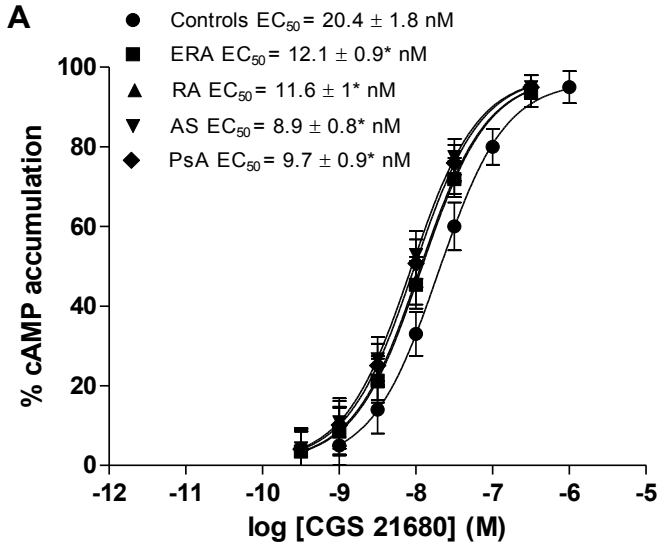


Figure 3. Increased potency of A_{2A} and A_3AR agonists in patients' lymphocytes with ERA, RA, AS and PsA diseases respect to control subjects. Concentration-response curves of CGS 21680 (**A**) or Cl-IB-MECA (**B**) on cAMP assays in lymphocytes obtained from control subjects (n = 90), ERA (n = 30), RA (n = 30), AS (n = 16) and PsA patients (n = 14). Data are expressed as the mean ± SEM. * $p < 0.01$ vs control group.



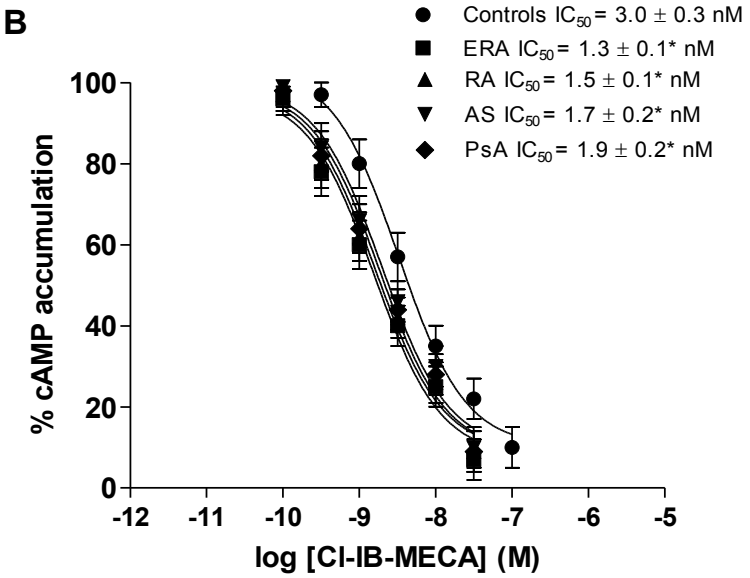


Figure 4. A_{2A} and A_3AR stimulation reduced NF-κB activation and TNF-α release. Effect of the A_{2A}AR agonist CGS 21680 (100 nM) and A_3AR agonist Cl-IB-MECA (100 nM) on NF-κB p65 subunit activation (**A**) or TNF-α release (**B**) in cultured lymphocytes from ERA (n = 30), RA (n = 30), AS (n = 16) and PsA patients (n = 14) in comparison to control subjects (n = 90). The A_{2A}AR antagonist SCH 442416 (1 μM) and the A_3AR antagonist MRS 1334 (1 μM) abrogated the effect of the agonists. Data are expressed as the mean ± SEM. * $p < 0.01$ vs untreated cells (**A**); * $p < 0.01$ vs PMA-stimulated cells (**B**).

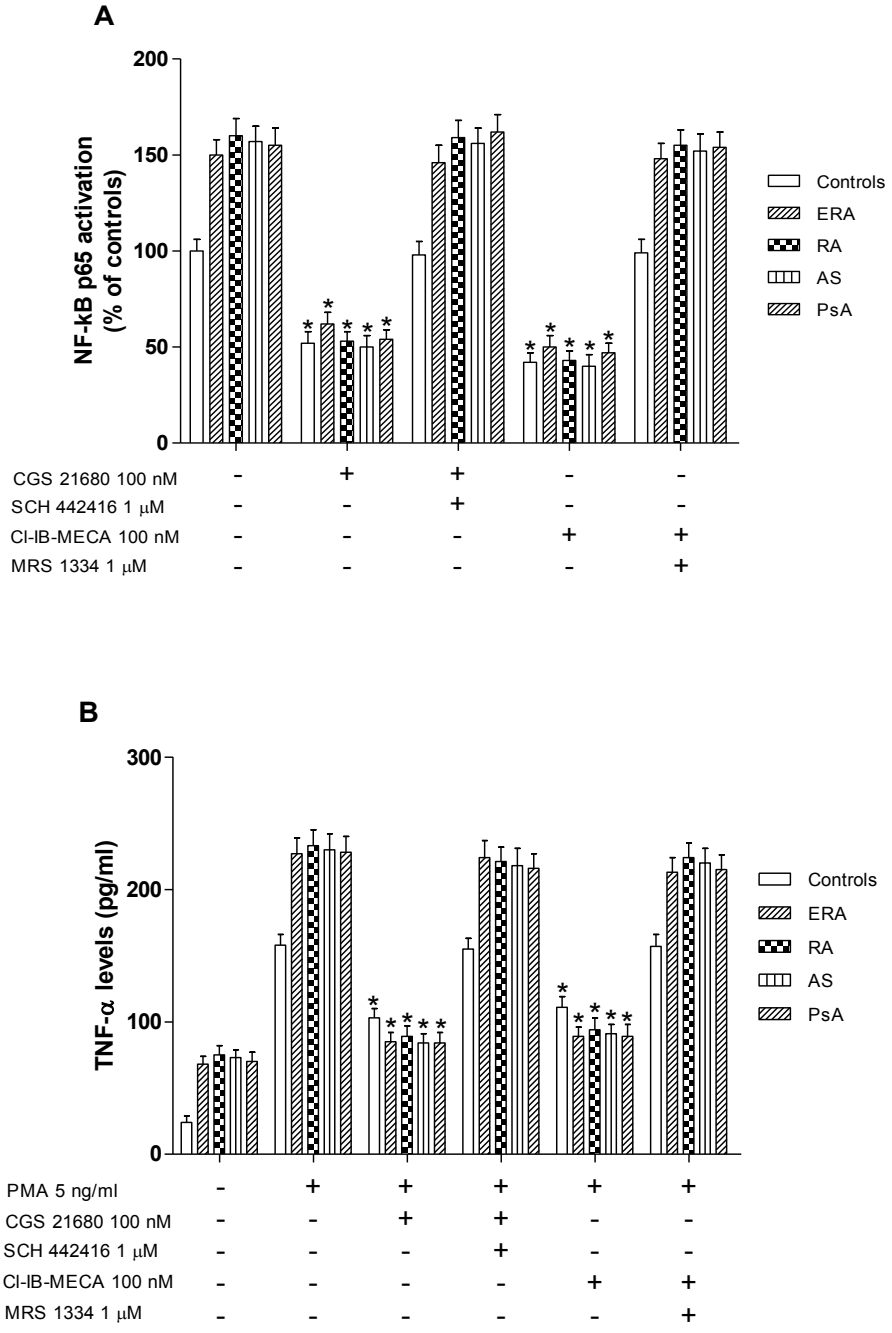


Figure 5. A_{2A} and A_3AR activation reduced IL-1β and IL-6 release. Effect of the A_{2A}AR agonist CGS 21680 (100 nM) and of the A_3AR agonist Cl-IB-MECA (100 nM) on IL-1β (**A**) or TNF-α (**B**) release in cultured lymphocytes from ERA (n = 30), RA (n = 30), AS (n = 16) and PsA patients (n = 14) in comparison to control subjects (n = 90). The A_{2A}AR antagonist SCH 442416 (1 μM) and the A_3AR antagonist MRS 1334 (1 μM) blocked the effect of the agonists. Data are expressed as a means ± SEM. * $p < 0.01$ vs PMA-stimulated cells.

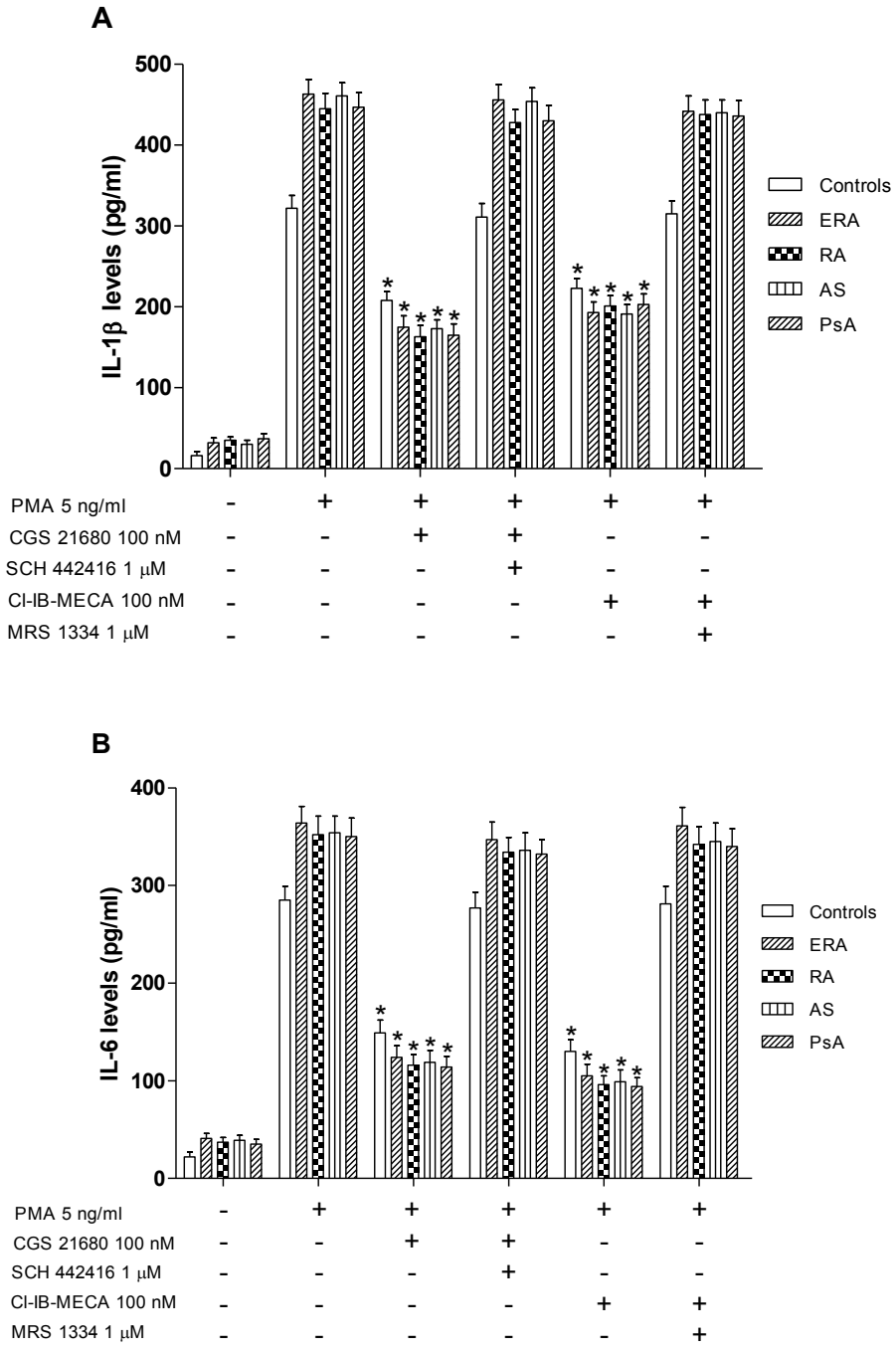
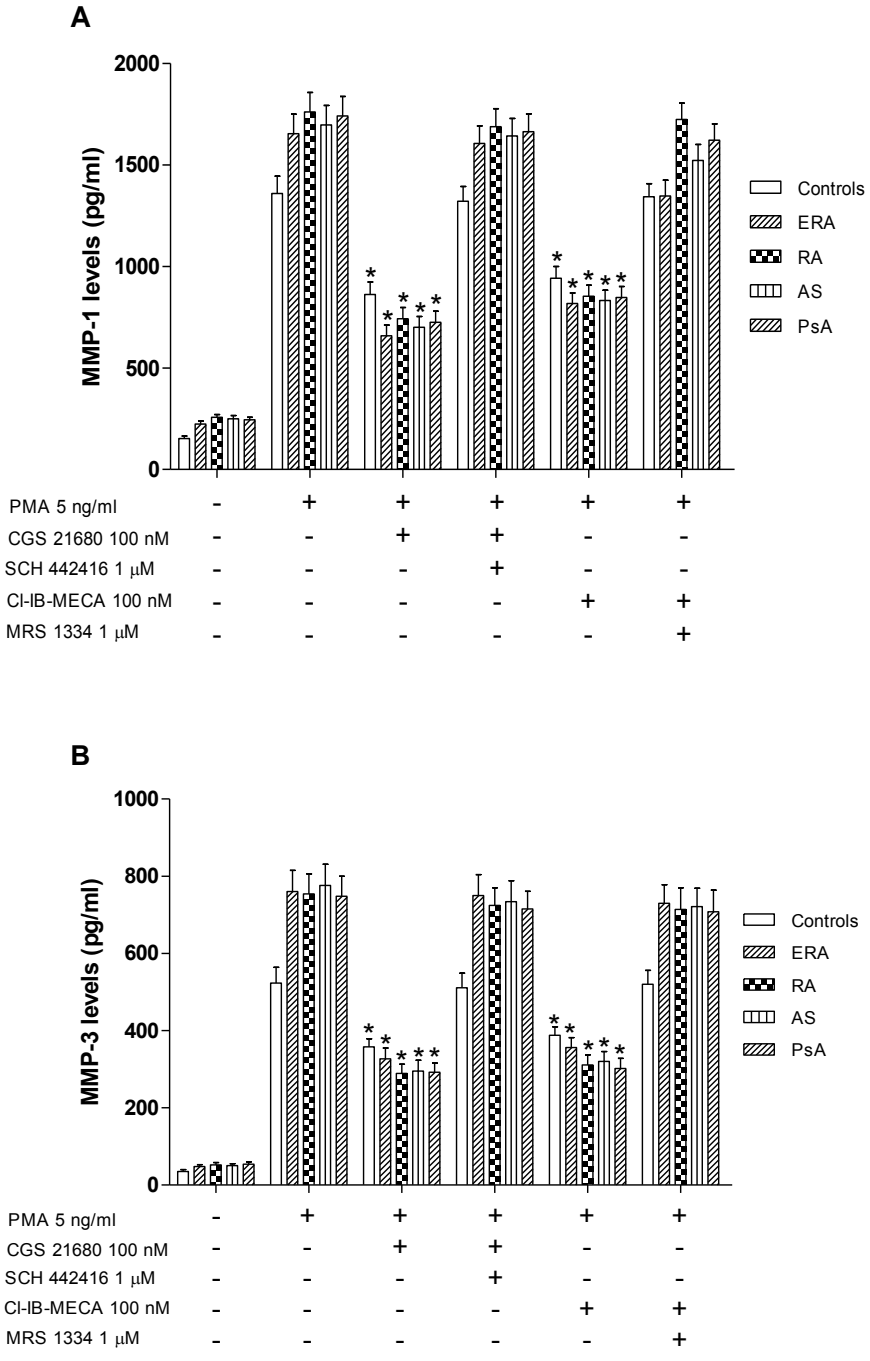


Figure 6. A_{2A} and A_3AR activation reduced MMP-1 and MMP-3 production. (**A**) Effect of the A_{2A}AR agonist CGS 21680 (100 nM) and of the A_3AR agonist Cl-IB-MECA (100 nM) on MMP-1 (**A**) or MMP-3 (**B**) production in cultured monocytes from ERA (n = 30), RA (n = 30), AS (n = 16) and PsA patients (n = 14) in comparison to control subjects (n = 90). The A_{2A}AR antagonist SCH 442416 (1 μM) and the A_3AR antagonist MRS 1334 (1 μM) blocked the effect of the agonists. Data are expressed as a means ± SEM. * $p < 0.01$ vs PMA-stimulated cells.



Discussion

It is widely accepted that adenosine based system is a key modulator of inflammatory responses affecting the release of several pro-inflammatory mediators implicated in the pathogenesis and progression of various pathologies such as rheumatic diseases (Varani *et al.*, 2010b). To shed some light on the involvement of adenosine and its receptors in chronic inflammatory rheumatic diseases, this study was primarily aimed to investigate the expression of ARs in lymphocytes obtained from RA, AS and PsA patients in comparison with healthy subjects. mRNA analysis revealed a selective increase at a transcriptional level of A_{2A} and A_3ARs in lymphocytes from arthritic patients. No significant differences were found between mRNA levels of A_1 and A_{2B}ARs in RA, AS and PsA patients respect to control subjects. The increase of A_{2A} and A_3AR mRNA expression was accompanied by an up-regulation of these receptor subtypes as confirmed by saturation binding experiments. In particular, Bmax values increased in the range from 3.0 to 3.4 fold for A_{2A}ARs and from 2.0 to 2.5 fold for A_3ARs in arthritis patients. These data confirmed our previous results obtained in RA patients were an up-regulation of A_{2A} and A_3ARs in lymphocytes was found (Varani *et al.*, 2009, 2011b; Vincenzi *et al.*, 2013a). The present study highlights, for the first time, the involvement of A_{2A} and A_3ARs in two of the most common forms of spondyloarthritis, such as AS and PsA. Spondyloarthritis are a cluster of inflammatory conditions which share clinical genetic and pathophysiological characteristics (Venken and Elewaut, 2015). From the diagnostic point of view, it is therefore clear that a cellular marker could help the clinician in the characterization of the disease, supporting the well-established clinical measures and imaging techniques. It is worth noting that an overexpression of A_3ARs was previously found in peripheral blood mononuclear cells derived from patients with RA, psoriasis and Crohn's disease compared with healthy subjects (Ochaion *et al.*, 2009). All together these data consolidate the emerging role of ARs in autoimmune inflammatory diseases (Varani, *et al.*, 2010b; Fishman *et al.*, 2012; Fishman and Cohen, 2016). The effect on cAMP production of the A_{2A}AR agonist CGS 21680 and of the A_3AR agonist Cl-IB-MECA was tested in lymphocytes from RA, AS and PsA patients in comparison to healthy subjects. The increased potency of the two compounds found in patients affected by inflammatory rheumatic diseases under examination, together with the upregulation of A_{2A} and A_3ARs, suggested the possibility to exploit these receptor subtypes as therapeutic target. To investigate the potential therapeutic of ARs modulation in arthritis diseases, the effect of specific agonists was studied in cultured lymphocytes from RA, AS and PsA patients on

several inflammatory mediators such as NF-κB, cytokines and metalloproteinases. NF-κB is a transcription factor that is recognized as a key regulator of immune development, immune responses and inflammation (Mitchell *et al.*, 2016). It is well established that NF-κB pathway is essential both in acute inflammatory responses and in chronic inflammatory diseases, including arthritis related diseases (Noort *et al.*, 2015). In a recent work, it has been reported a higher expression of NF-κB in peripheral blood leukocytes in patients with spondyloarthritis than in control group (Świerkot *et al.*, 2015). The results obtained in the present study revealed the capability of both the A_{2A}AR agonist CGS 21680 and the A_3AR agonist Cl-IB-MECA to reduce NF-κB p65 subunit activation in lymphocytes from RA, AS and PsA patients. Furthermore, the effect was more evident in patients than in healthy subjects, most likely due to the up-regulation of A_{2A} and A_3ARs. These data are consistent with those previously found in literature showing that A_{2A} and A_3AR agonists are able to inhibit NF-κB activation both in vitro and in vivo. For instance, it has been shown that A_{2A}AR activation inhibits osteoclast differentiation through the inhibition of NF-κB nuclear translocation, suggesting a mechanism by which adenosine could target bone destruction in inflammatory diseases (Mediero *et al.*, 2013). Furthermore, it has been reported that the A_3AR agonist CF502 inhibits the NF-κB signaling pathway in synoviocytes from RA patients and in adjuvant-induced arthritis rats (Ochaion *et al.*, 2008).

The advancements in understanding the molecular and cellular mechanisms of chronic autoimmune rheumatic diseases have highlighted potential therapeutic of strategies aimed to inhibit the effects of up-regulated cytokines and other pro-inflammatory mediators (Haskó *et al.*, 2008a; Cronstein and Sitkovsky, 2016). The beneficial effect of targeting pro-inflammatory cytokines is testified by the clinical efficacy of monoclonal antibodies biological drugs that works against TNF-α, IL-6 or IL-1β in RA, AS and PsA patients (Cantini *et al.*, 2016). In the present research study, we have tested the potential inhibitory effect of A_{2A} and A_3AR stimulation on the release of pro-inflammatory cytokines in lymphocytes obtained from patients with inflammatory rheumatic disorders in comparison to control subjects. The observed reduction of TNF-α, IL-6 and IL-1β achieved by using CGS 21680 or Cl-IB-MECA corroborated the anti-inflammatory effects of A_{2A} and A_3AR activation and their potential therapeutic role in chronic inflammatory rheumatic diseases. It is well-known that inflammatory cytokines such as TNF-α, IL-6 and IL-1β, expressed locally in the articular joint, stimulate the production of MMPs. Increasing evidence have highlighted that MMP activity is upregulated in arthritic cartilage and synovial fluid. For these reasons, although there are different treatment options of varying efficacy for arthritis

diseases, many alternatives are currently being explored, especially those that selectively inhibit some MMPs (Dancevic and McCulloch, 2014). The results obtained in the present work indicated that both A_{2A} and A_3AR stimulation inhibited MMP-1 and MMP-3 levels in monocytes from RA, AS and PsA patients and in control subjects. This effect was abrogated by selective antagonists for each receptor subtypes demonstrating that the inhibition of MMPs was mediated by A_{2A} and A_3AR activation. In conclusion, our findings highlight the possibility to exploit A_{2A} and A_3ARs as therapeutic target, with the aim to limit the inflammatory processes usually associated with chronic autoimmune rheumatic diseases.

CHAPTER 2:

LONGITUDINAL STUDY ON ADENOSINE RECEPTOR EXPRESSION IN CHRONIC INFLAMMATORY RHEUMATIC DISEASES

Introduction

In RA and spondyloarthritis, the inflammatory process leads to progressive cartilage degradation with synovial hyperplasia, change in underlying bone with erosions and high levels of proinflammatory mediators (Joseph *et al.*, 2010; Vincenzi *et al.*, 2013c). It is widely accepted that cytokines such as TNF-α and IL family mediate a large variety of effector functions in the context of RA pathogenesis (Varani *et al*., 2011b). Conversely, the anti-inflammatory cytokine IL-10 is relatively unique in its ability to down-regulate the production of multiple pro-inflammatory cytokines, leading to the notion that IL-10 may modulate the disease expression in RA (Vincenzi *et al.*, 2013c). Early diagnosis and therapy are crucial in order to prevent unfavourable outcome avoiding joint deterioration and functional disability (Quiñonez-Flores *et al.*, 2016). Conventional DMARDs such as MTX in monotherapy or in combination are currently the first medications usually prescribed in RA, AS and PsA patients (Cronstein and Sitkovsky, 2016). To date, the introduction of anti-TNF-α agents have provided a marked improvement in RA even if some patients do not respond or fail to maintain adequate response to these treatments (Keller *et al*., 2003).

An other interesting point was to evaluate in RA and SpA patients the influence of different biologic therapies as anti-TNF-α drugs or RTX in comparison with MTX treatment on A_{2A} and A_3ARs density at various time points of treatment (from 0 to 24 months). In RA treated patients, the relationship between A_{2A}AR density and DAS 28 values was investigated. The changes of A_{2A}AR expression after different pharmacological treatments in function of the time.

Materials and Methods

Sample Collection and Human Lymphocyte Preparation

Lymphocytes were isolated and prepared as previously described from the peripheral blood of control subjects and examined patients (Varani *et al.*, 2009). The isolation of blood cells started no later than 3 to 4 hours after the samples had been taken. The blood was supplemented with 6% (by weight) Dextran T500 solution (Sigma-Aldrich, St Louis, MO, USA) and erythrocytes were allowed to settle down for 60 min. Leukocytes were pelleted by centrifugation for 15 min at 100×g and the remaining erythrocytes were lyzed in distilled water at 4°C rapidly restoring the isotonicity by NaCl solution. Then, the cells were pelleted by centrifugation for 5 min at 250×g, suspended in Krebs-Ringer phosphate buffer and layered onto 10 ml of Fycoll-Hypaque (GE Healthcare, Little Chalfont, UK).

After centrifugation, mononuclear cells were washed in 0.02 M phosphate-buffered saline (PBS) at pH 7.2 containing 5 mM MgCl2 and 0.15 mM CaCl2. Finally, they were decanted into a culture flask and placed in a humidified incubator (5% CO2) for 2 hours at 37°C. This procedure, aimed at removing monocytes which adhere to the culture flasks, resulted in a purified lymphocyte preparation containing at least 99% small lymphocytes identified by morphological criteria. To obtain membrane suspensions, cell fractions were centrifuged in hypothonic buffer at 20000×g for 10 min. The resulting pellet was resuspended in tris HCl 50 mM buffer pH 7.4 containing 2 UI/ml adenosine deaminase (Sigma-Aldrich) and incubated for 30 min at 37°C. After the incubation the suspension was centrifuged again at 40000×g for 10 min and the final pellet was used for radioligand binding assays. The protein concentration was determined by a Bio-Rad method with bovine albumine as reference standard (Vincenzi *et al.*, 2013c).

Saturation Binding Experiments to A_{2A} and A_3ARs

Saturation binding to A_{2A}ARs was performed with the use of [^{3}H]-ZM 241385 ([3H]-4-(2-[7-amino-2-(2-furyl)[1,2,4]- triazolo[2,3-a][1,3,5] triazin-5-ylamino] ethyl) phenol, specific activity 27 Ci/mmol, Biotrend, Cologne, Germany), as radioligand. Cell membranes (60 μg of protein/assay) were incubated for 60 min at 4 °C with various concentrations (0.01–20 nM) of [^{3}H]-ZM 241385. Non-specific binding was determined in the presence of 1 μM ZM 241385. Saturation binding experiments to A_3ARs were carried out using [^{3}H]-MRE 3008F20 ([3H]-5*N*-(4-methoxyphenylcarbamoyl) amio-8-propyl-2-(2-furyl) pyrazolo [4,3-e]-1,2,4-triazolo [1,5-c]pyrimidine, specific activity 67 Ci/mmol, GE Healthcare, UK) as radioligand (Varani *et al.*, 2009). The membranes (80 μg of protein/assay) with [^{3}H]-MRE 3008F20 (0.01–30nM) were incubated at 4°C for 150 min and MRE 3008F20 1 μM was used to evaluate non-specific binding. Bound and free radioactivity were separated in a Brandel cell harvester (Brandel, Gaithersburg, MD, USA) by filtering the assay mixture through Whatman GF/B glass fiber filters (Whatman. Kent, UK). The filter-bound radioactivity was counted in a 2810 TR liquid scintillation counter (Perkin Elmer, Boston, MA, USA).

Data and Statistical Analysis

Dissociation equilibrium constants for saturation binding, affinity or K_D values, as well as the maximum densities of specific binding sites, Bmax were calculated for a system of one or two binding site populations by non-linear curve fitting using the program Ligand

purchased from Kell Biosoft (Varani *et al.*, 2010b). All data are reported as mean ± SEM. Statistical analysis of the data was performed by repeated measures analysis of variance (ANOVA) followed by Dunnett's ttest or unpaired two-sided Student's t-test for comparison of two samples. All analysis were carried out using GraphPad Prism 5.0 statistical software package and differences were considered statistically significant with a p value less than 0.05 (Graph Pad Software, San Diego, CA, USA).

Results

Patients and control subjects

All patients enrolled in this study were recruited from the Rheumatology Section, Department of Clinical and Experimental Medicine, University of Ferrara, Italy. A total of 145 patients were included and divided in RA patients (n = 67) treated with MTX (n = 26), with anti TNF-α agents (etanercept, n= 23) or with RTX (n= 18); in AS patients (n = 41) treated with DMARDs (MTX or leflunomide, n = 19), with anti TNF-α agents (infliximab, adalimumab, etanercept, n = 22) and in PsA patients (n = 37) treated with DMARDs (MTX or leflunomide, n = 21), with anti TNF-α agents (infliximab, adalimumab, etanercept, n = 16). RA patients with established disease fulfilled the American College of Rheumatology (ACR) 1987 criteria for RA (Arnett *et al.*, 1988). The demographic, clinical and pharmacological details are listed in Table 3. All patients have continued to be regularly monitored during the timeframe of the study. Clinical information about disease characteristics such as disease onset, previous and current therapy, RF and ACPA presence were reported (Table 3). DAS 28 and HAQ were completed for all patients (Prevoo *et al.*, 1995). The statistical analysis of the clinic parameters such as DAS 28 and HAQ reveals a significant difference between the groups that is considered quite common within a cohort of RA patients. Healthy controls (n= 90), matched for similar age to RA and spondyloarhtritis patients, were volunteers from Ferrara University Hospital Blood Bank.

Binding parameters of A_{2A} and A_3ARs in lymphocytes of RA patients

Affinity and density of A_{2A} and A_3ARs were evaluated in lymphocyte membranes obtained from RA patients at different time points of treatment with MTX, anti-TNF-α agents or RTX. Before the treatment start, RA patients revealed an upregulation of A_{2A}AR density and a lower affinity if compared with age matched healthy (Table 4). In MTX-treated patients, A_{2A}AR affinity and density were found to be significantly different from control subjects in the time points investigated, even if a significant reduction of K_D and Bmax

values after 24 months of treatment respect to the beginning of the study was observed. In lymphocytes from RA patients treated with anti-TNF-α drugs, a statistically significant decrease of A_{2A}AR density was obtained after 9 months of treatment. In the same patients the A_{2A}AR Bmax values were similar to control subjects after 24 months from the beginning of the treatment. Interestingly, RTX-treated patients showed a significant reduction of Bmax and K_D after 3 months of treatments reaching values similar to control subjects at the subsequent time points investigated (from 6 to 24 months). Figure 7A summarises the A_{2A}AR density values obtained in lymphocytes from RA patients treated with MTX, anti-TNF-α agents or RTX after 0, 3, 6, 9, 12 and 24 months from the treatment start. Similar results were obtained on A_3ARs K_D and Bmax during the time points investigated as shown in Figure 7B and Table 5.

Binding Parameters of A_{2A} and A_3ARs in lymphocytes of AS and PsA patients

A_{2A} and A_3ARs affinity (K_D) and density (Bmax) were also investigated in lymphocyte membranes obtained from AS and PsA patients at different time points of treatment with DMARDs (MTX or leflunomide) or anti-TNF-α agents (infliximab, adalimumab or etanercept). The examined patients revealed an upregulation of A_{2A} and A_3ARs density and a lower affinity if compared with age matched healthy controls. In DMARDs-treated patients and in anti anti-TNF-α treated patients, A_{2A}AR affinity and density were found to be significantly different from control subjects in all the time points investigated in a similar way to those observed in untreated patients (Figure 8A and 9A). Similar results were obtained on A_3ARs affinity and density as shown in Figure 8B and 9B.

The A_{2A}AR density and DAS 28 gradually reduced in RA patients treated with RTX

To date DAS 28 represents a validated composite index to assess RA disease activity. Figure 10A reports the DAS 28 values in RA patients evaluated at different time points of treatment with MTX, anti-TNFa drugs or RTX. To investigate the involvement of A_{2A} and A_3ARs in RA progression we have studied the association between these receptor subtypes and DAS 28 in function of the time of treatment. Interestingly, in RTX-treated patients the DAS 28 reduction (from 5.57 to 2.11) respect to the time of treatment was closely associated to a significant A_{2A}AR decrease of 3.5 fold after 12 months respect to RA untreated patients (Figure 10B). In particular, the timedependent relationship (from 0 to 12

months) between A_{2A}ARs and DAS 28 in lymphocytes from RA patients treated with RTX revealed a significant reduction of these parameters as reported in Figure 10B. Similar results we have obtained between A_3AR and DAS 28 in lymphocytes from RA patients treated with RTX.

Table 3. Clinical features and pharmacological treatments of the examined subjects, including patients with rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis.

RA	67
No. female/male	16/51
Age, mean ± SEM years	55.4 ± 5.4
Disease duration (months)	12 ± 3
DMARDs:	
Methotrexate (10–15 mg/week)	26 (39%)
TNF inhibitors:	
Etanercept	23 (34%)
B cells inhibitors:	
Rituximab	18 (27%)
AS	41
No. female/male	9/32
Age, mean ± SEM years	40.2±3.8
Disease duration (months)	118±8
DMARDs:	19
Methotrexate (10–15 mg/week)	12 (63%)
Leflunomide	7 (37%)
TNF inhibitors:	22
Infliximab	7 (32%)
Adalimumab	7 (32%)
Etanercept	8 (36%)
PsA	37
No. female/male	9/28
Age, mean ± SEM years	39.9±2.4
Disease duration (months)	116±7
DMARDs:	21
Methotrexate (10–15 mg/week)	13 (62%)
Leflunomide	8 (38%)
TNF inhibitors:	16
Infliximab	4 (25%)
Adalimumab	5 (31%)
Etanercept	7 (44%)

Table 4. $A_{2A}AR$ affinity (K_D, nM) and density (Bmax, fmol/mg protein) in lymphocytes from RA patients at different time points of treatment.

$A_{2A}ARs$ **K_D, nM** **Bmax, fmol/mg protein**	**RA patients** **MTX treated**	**RA patients** **anti-TNFα treated**	**RA patients** **RTX treated**
t=0	2.61±0.18*	2.64±0.22*	2.59±0.21*
	175±17*	169±15*	173±14*
t=3	2.24±0.16*	2.16±0.16*	2.06±0.16*#
	168±16	162±13	140±13*#
t=6	2.18±0.19*	2.17±0.18*	1.81±0.18*#
	170±14*	155±14*	113±12*#
t=9	2.14±0.18*	2.08±0.17*#	1.25±0.17#
	168±15*	141±11*#	75±10#
t=12	2.12±0.20*	2.02±0.16*#	1.05±0.19#
	164±13*	106±10*#	46±7#
t=24	1.95±0.15*#	1.27±0.18#	1.26±0.21#
	144±14*#	60±6#	45±7#

Healthy controls (n=90): K_D = 1.34 ± 0.85 nM; Bmax = 57 ± 46 fmol/mg protein. MTX = methotrexate; RTX = rituximab. Time (t) is expressed as months of pharmacological treatment. Data are expressed as mean ± SEM. Differences were considered significant at a value of $p<0.01$ vs healthy controls (*) or vs t=0 (#).

Table 5. A_3AR affinity (K_D, nM) and density (Bmax, fmol/mg protein) in lymphocytes from RA patients at different time points of treatment.

A_3ARs **K_D, nM** **Bmax, fmol/mg protein**	**RA patients** **MTX treated**	**RA patients** **anti-TNFα treated**	**RA patients** **RTX treated**
t=0	2.95±0.20*	2.89±0.18*	3.05±0.23*
	298±19*	297±19*	298±22*
t=3	2.53±0.21*	2.36±0.19*	2.43±0.17*$^{\#}$
	298±18*	281±21*	224±19*$^{\#}$
t=6	2.46±0.23*	2.38±0.21*	2.13±0.22*$^{\#}$
	297±21*	258±19*	189±15*$^{\#}$
t=9	2.42±0.23*	2.28±0.20*$^{\#}$	1.47±0.13$^{\#}$
	289±19*	226±18*$^{\#}$	158±30$^{\#}$
t=12	2.37±0.22*	2.21±0.21*$^{\#}$	1.34±0.11$^{\#}$
	279±20*	174±20*$^{\#}$	134±13$^{\#}$
t=24	2.20±0.18*$^{\#}$	1.49±0.12$^{\#}$	1.45±0.10$^{\#}$
	225±19*$^{\#}$	137±19$^{\#}$	135±14$^{\#}$

Healthy controls (n=90): K_D = 1.78 ± 0.13 nM; Bmax = 134 ± 15 fmol/mg protein. MTX = methotrexate; RTX = rituximab. Time (t) is expressed as months of pharmacological treatment. Data are expressed as mean ± SEM. Differences were considered significant at a value of $p<0.01$ vs healthy controls (*) or vs t=0 (#).

Figure 7. (**A**) $A_{2A}AR$ and (**B**) A_3AR Bmax values in lymphocytes from RA patients evaluated at different time points of treatment with MTX, anti-TNF-α drugs or RTX. Data are expressed as mean ± SEM. *, $p<0.01$ vs healthy controls; #, $p<0.01$ vs t = 0.

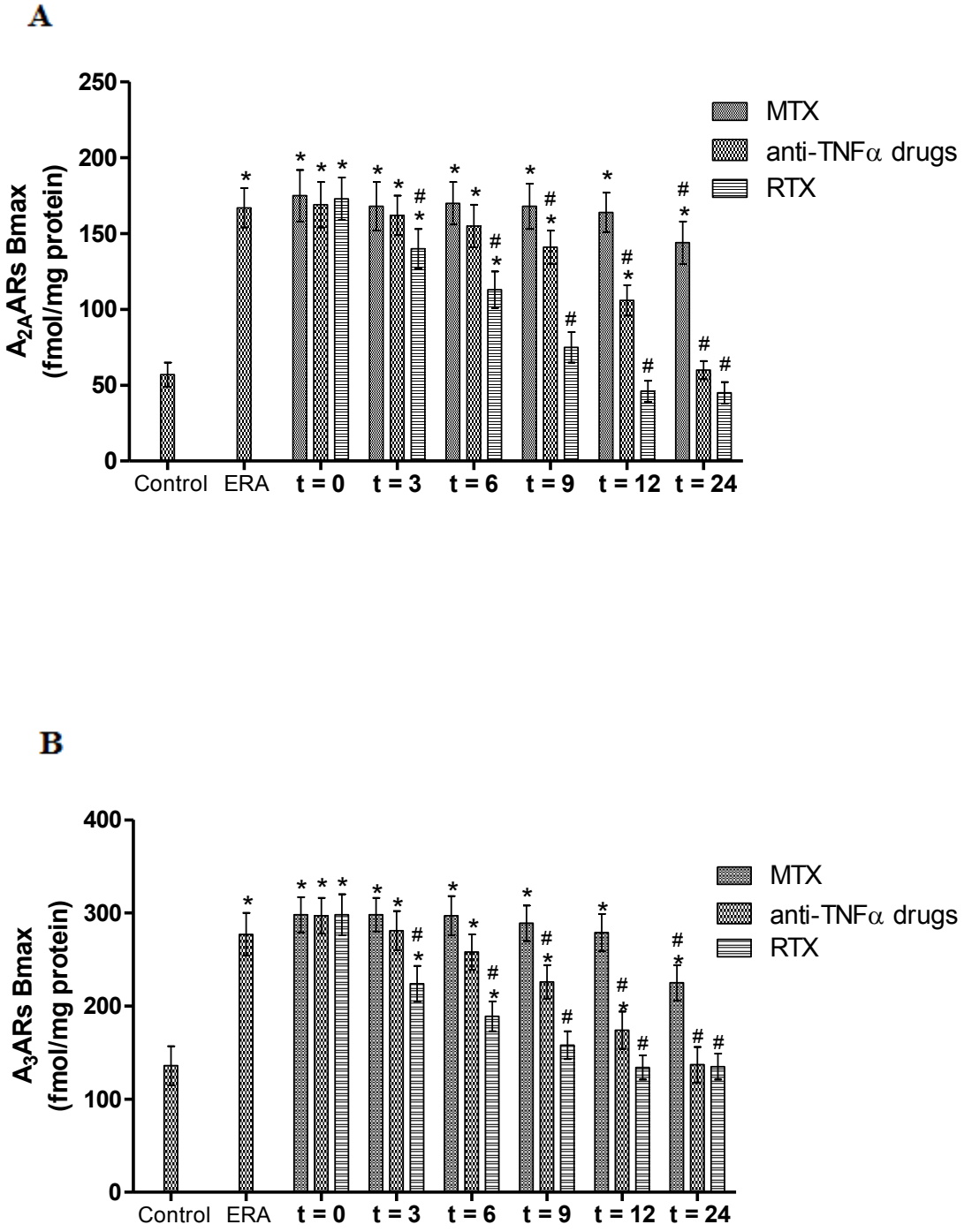


Figure 8. (**A**) $A_{2A}AR$ and (**B**) A_3AR Bmax values in lymphocytes from AS patients evaluated at different time points of treatment with DMARDs or anti-TNF-α drugs. Data are expressed as mean ± SEM. *, $p<0.01$ vs healthy controls.

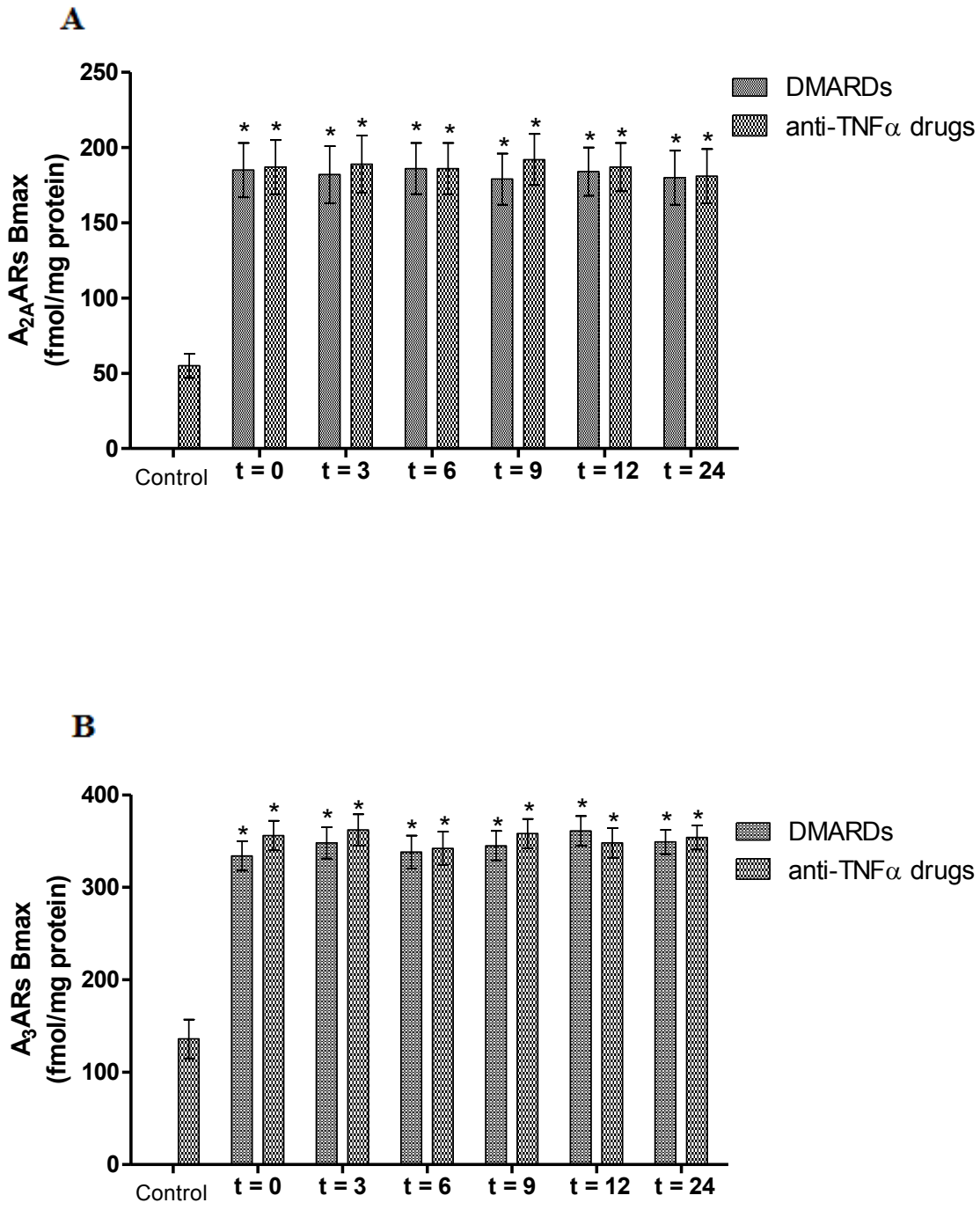


Figure 9. (**A**) $A_{2A}AR$ and (**B**) A_3AR Bmax values in lymphocytes from PsA patients evaluated at different time points of treatment with DMARDs or anti-TNF-α drugs. Data are expressed as mean ± SEM. *, $p<0.01$ vs healthy controls.

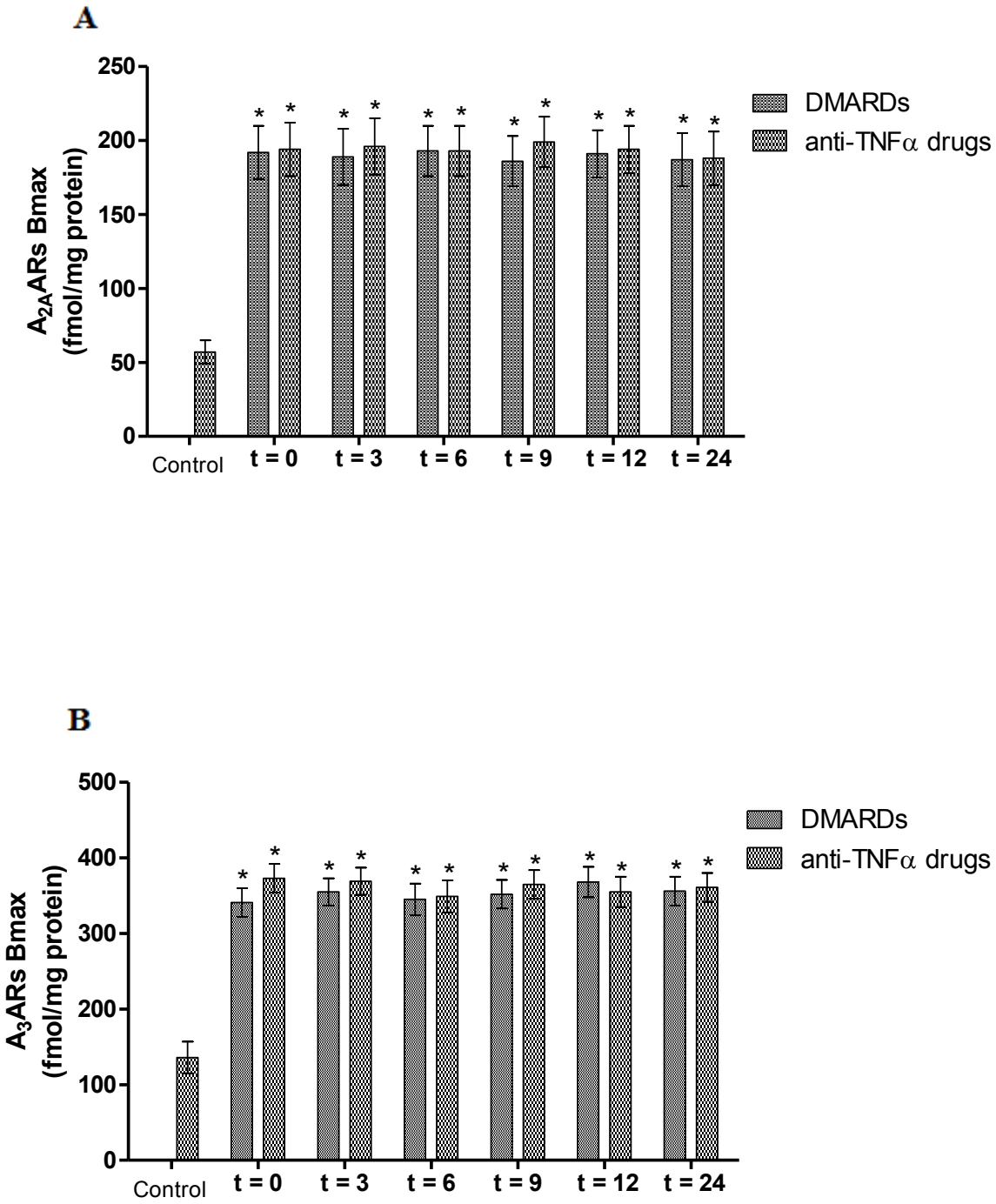


Figure 10. DAS 28 values in RA patients evaluated at different time points of treatment with MTX, anti-TNFa drugs or RTX. Data are expressed as mean ± SEM.*, $p<0.01$ vs t = 0 months.

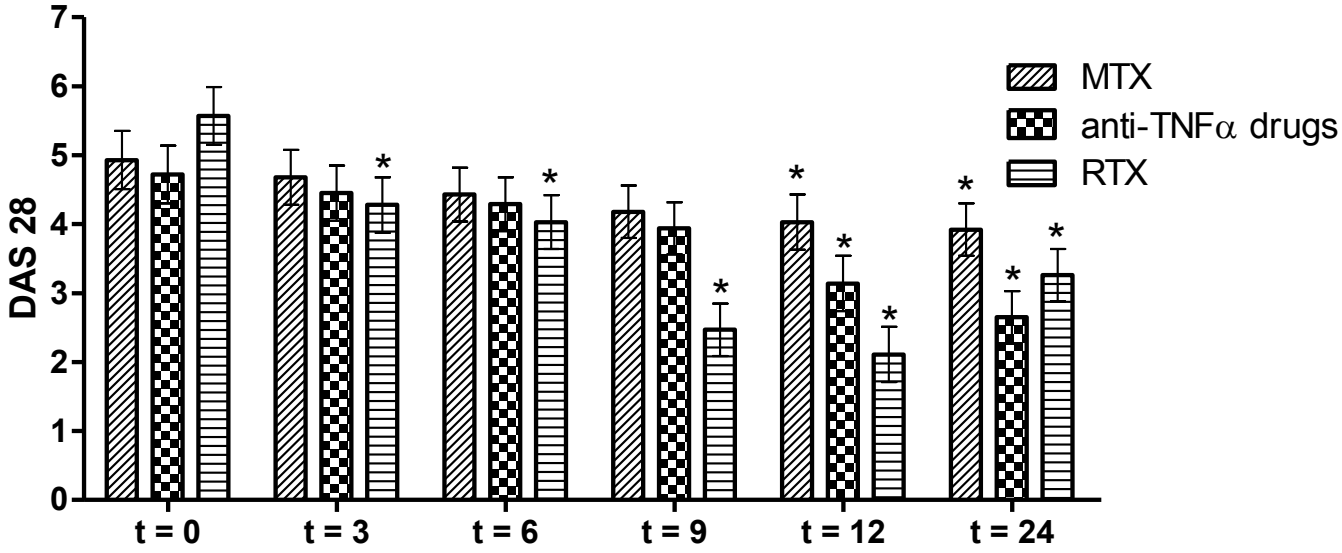
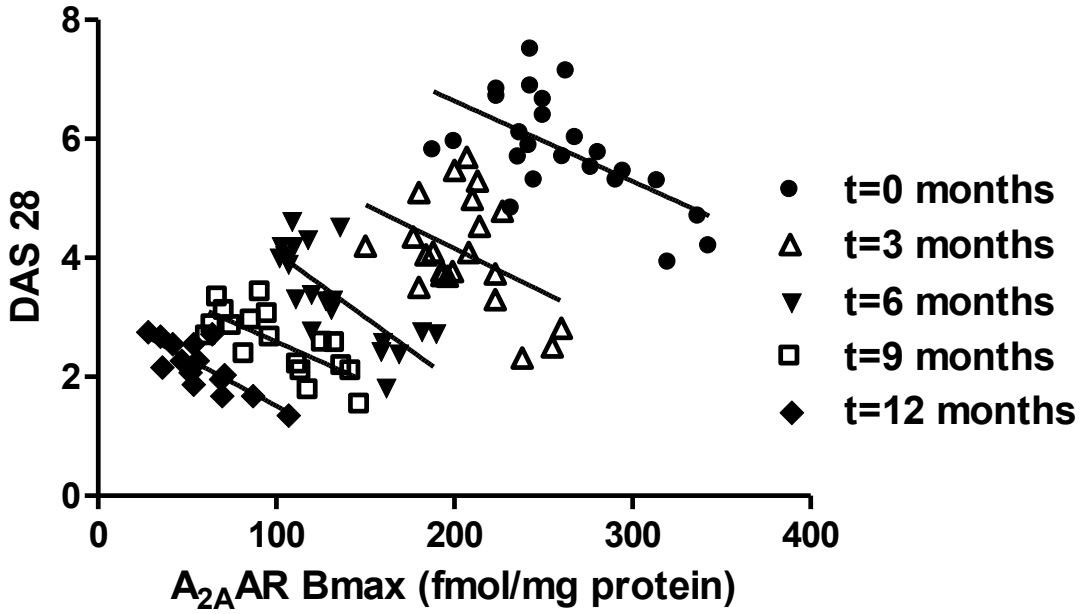


Figure 11. Time-dependent relationship between $A_{2A}AR$ Bmax and DAS 28 in lymphocytes from RTX treated RA patients.



Discussion

To date no clinical trials are present evaluating the effects of A_{2A} and A_3AR agonists in RA, AS and PsA patients since more preclinical studies are needed to better elucidate their pharmacological properties. RA treatment has progressed for the advent of biologic DMARDs even if the risks of infection, infusion-associated reactions or malignancy together to the high costs of these treatments could limit their clinical use. In this study, we have investigated the involvement of A_{2A} and A_3ARs in RA, AS and PsA by evaluating their affinity and density in lymphocytes from patients at different time points after the treatment with classic or biologic DMARDs. We have found an up-regulation of A_{2A} and A_3ARs in untreated RA patients that was gradually reduced in function of the treatment time and in different ways depending on the type of drug used. The longitudinal study (from 0 to 24 months) performed in this study, aimed to assess the time-dependent changes of A_{2A} and A_3AR expression in differentially treated RA, AS and PsA patients. In MTX-treated RA patients A_{2A}ARs were present in high levels at all the time points of treatment whilst in anti-TNF-α-treated RA patients A_{2A} and A_3AR density normalized to control values after 24 months of treatment. RA patients treated with RTX exhibited A_{2A}AR binding characteristics similar to control values after 9 and until 24 months of treatment.

In AS and PsA patients A_{2A} and A_3ARs were found to be significantly different from control subjects in all the time points investigated.

So it is tempting to speculate that the different behavior of examined drugs in RA patients on A_{2A} and A_3AR density could be correlated with the several mechanisms of action involving the inflammatory process regulated by these heterogeneous therapies. DAS 28 values progressively reduced in function of the time of treatment and in different way respect to the type of drugs used with a significant reduction after 3 months of RTX treatment. Moreover, the time-dependent relationship between A_{2A} and A_3ARs and DAS 28 in lymphocytes from RTX treated RA patients strongly suggested the involvement of A_{2A} and A_3ARs in RA progression. Interestingly, high levels of DAS 28 indicating the presence of a marked inflammatory status are accompanied by an increased A_{2A} and A_3AR density. These data suggest that A_{2A}AR expression is sensibly affected by inflammation and tend to normalize with the disease remission. Thus, A_{2A}ARs could be a useful tool to monitor RA progression following conventional or biologic therapies. On the other hand, A_{2A} and A_3ARs upregulation could be interpreted as a possible compensatory mechanism that is needed to opposite the inflammatory status.

In conclusion, we have demonstrated the involvement of A_{2A} and A_3ARs in RA pathogenesis based on the modulation of their expression in function of the time after different pharmacological treatments. As a matter of fact, A_{2A} and A_3ARs agonists, mimicking an endogenous protective system, could have less limitation and side effects than DMARDs or biologic drugs although with a comparable effectiveness. These experimental results support the role of A_{2A} and A_3AR as therapeutic target and strongly suggest the potential use of novel pharmacological approaches based on A_{2A} and A_3AR stimulation in RA treatment.

GENERAL CONCLUSIONS

Several papers report the key role of ARs in mechanisms of inflammation associated to various disorders suggesting that their stimulation has a different effect on the release of several pro-inflammatory cytokines (Haskó et al., 2008b; Chan and Cronstein, 2010).

The results of the first part of this thesis suggest that A_{2A} and A_3ARs are differentially expressed in chronic rheumatic inflammatory diseases and the affinity and/or density of these receptors are altered in patients compared with healthy control subjects. Furthermore our data highlight that A_{2A} and A_3AR agonists inhibited NF-κB activation and significantly reduced inflammatory cytokines release, such as TNF-α, IL-1β and IL-6 in patient lymphocytes. Moreover, A_{2A} and A_3AR activation mediated a reduction of MMP-1 and MMP-3. The effect of the A_{2A} and A_3AR agonists was abrogated by selective antagonists demonstrating the direct involvement of these receptor subtypes.

The second part of the study was undertaken to investigate the modulation of A_{2A} and A_3ARs in patients suffering from RA, AS and PsA after different pharmacological treatments. We evaluated A_{2A} and A_3AR density and functionality in pathologies progression using a longitudinal study in RA, AS and PsA patients before and after MTX, anti-TNFα agents or rituximab treatments. A_{2A} and A_3ARs were analyzed by saturation binding assays in lymphocytes from patients throughout the 24-month study timeframe. In lymphocytes obtained from RA patients, the A_{2A} and A_3AR up-regulation was gradually reduced in function of the treatment time.

In conclusion, these data highlight, for the first time, the involvement of A_{2A} and A_3ARs in two of the common forms of spondyloarthritis, such as AS and PsA. Interestingly, an over-expressed endogenous anti-inflammatory pathway may be a potential target therapy in RA, AS and PsA. 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. From a clinical point of view, the highest levels of A_{2A} and A_3AR density are closely associated with the lowest levels of DAS 28, suggesting that the endogenous activation of these receptors could attenuate the disease activity.

From the pharmacological point of view, it could be of crucial importance that the stimulation of the over-expressed A_{2A} and A_3AR leads to the inhibition of cellular proinflammatory and degenerative mediators.

In the future, more preclinical and clinical studies are warranted to investigate the effect of selective A_{2A} and A_3AR agonists in chronic inflammatory rheumatic diseases in order to translate these important findings into valuable benefits for patients.

Taken together, these data confirmed the involvement of ARs in chronic autoimmune rheumatic diseases highlighting the possibility to exploit A_{2A} and A_3ARs as therapeutic targets, with the aim to limit the inflammatory responses usually associated with RA, AS and PsA.

REFERENCES

Aherne, C.M., Kewley, E.M., Eltzschig, H.K., 2011. The resurgence of A2B adenosine receptor signaling. Biochim. Biophys. Acta - Biomembr. 1808, 1329–1339. https://doi.org/10.1016/j.bbamem.2010.05.016

Aherne, C.M., Saeedi, B., Collins, C.B., Masterson, J.C., McNamee, E.N., Perrenoud, L., Rapp, C.R., Curtis, V.F., Bayless, A., Fletcher, A., Glover, L.E., Evans, C.M., Jedlicka, P., Furuta, G.T., de Zoeten, E.F., Colgan, S.P., Eltzschig, H.K., 2015. Epithelial-specific A2B adenosine receptor signaling protects the colonic epithelial barrier during acute colitis. Mucosal Immunol. 8, 1324–1338. https://doi.org/10.1038/mi.2015.22

Akgul, O., Ozgocmen, S., 2011. Classification criteria for spondyloarthropathies. World J. Orthop. 2, 107–15. https://doi.org/10.5312/wjo.v2.i12.07

Aletaha, D., Funovits, J., Smolen, J.S., 2008. The importance of reporting disease activity states in rheumatoid arthritis clinical trials. Arthritis Rheum. 58, 2622–31. https://doi.org/10.1002/art.23733

Aletaha, D., Neogi, T., Silman, A.J., Funovits, J., Felson, D.T., Bingham, C.O., Birnbaum, N.S., Burmester, G.R., Bykerk, V.P., Cohen, M.D., Combe, B., Costenbader, K.H., Dougados, M., Emery, P., Ferraccioli, G., Hazes, J.M.W., Hobbs, K., Huizinga, T.W.J., Kavanaugh, A., Kay, J., Kvien, T.K., Laing, T., Mease, P., Ménard, H.A., Moreland, L.W., Naden, R.L., Pincus, T., Smolen, J.S., Stanislawska-Biernat, E., Symmons, D., Tak, P.P., Upchurch, K.S., Vencovský, J., Wolfe, F., Hawker, G., 2010. 2010 Rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 62, 2569–2581. https://doi.org/10.1002/art.27584

Anderson, J.K., Zimmerman, L., Caplan, L., Michaud, K., 2011. Measures of rheumatoid arthritis disease activity: Patient (PtGA) and Provider (PrGA) Global Assessment of Disease Activity, Disease Activity Score (DAS) and Disease Activity Score With 28-Joint Counts (DAS28), Simplified Disease Activity Index (SDAI), Cl. Arthritis Care Res. (Hoboken). 63, S14–S36. https://doi.org/10.1002/acr.20621

Antonioli, L., Blandizzi, C., Csóka, B., Pacher, P., Haskó, G., 2015. Adenosine signalling in diabetes mellitus—pathophysiology and therapeutic considerations. Nat. Rev. Endocrinol. 11, 228–241. https://doi.org/10.1038/nrendo.2015.10

Antonioli, L., Blandizzi, C., Malavasi, F., Ferrari, D., Haskó, G., 2016. Anti-CD73 immunotherapy: A viable way to reprogram the tumor microenvironment. Oncoimmunology 5, e1216292. https://doi.org/10.1080/2162402X.2016.1216292

Antonioli, L., Blandizzi, C., Pacher, P., Haskó, G., 2013. Immunity, inflammation and cancer: a leading role for adenosine. Nat. Rev. Cancer 13, 842–857. https://doi.org/10.1038/nrc3613

Antonioli, L., Csóka, B., Fornai, M., Colucci, R., Kókai, E., Blandizzi, C., Haskó, G., 2014. Adenosine and inflammation: what's new on the horizon? Drug Discov. Today 19, 1051–1068. https://doi.org/10.1016/j.drudis.2014.02.010

Antonioli, L., Pellegrini, C., Fornai, M., Tirotta, E., Gentile, D., Benvenuti, L., Giron, M.C., Caputi, V., Marsilio, I., Orso, G., Bernardini, N., Segnani, C., Ippolito, C., Csóka, B., Németh, Z.H., Haskó, G., Scarpignato, C., Blandizzi, C., Colucci, R., 2017. Colonic motor dysfunctions in a mouse model of high-fat diet-induced obesity: an involvement of A2B adenosine receptors. Purinergic Signal. https://doi.org/10.1007/s11302-017-9577-0

Arasa, J., Martos, P., Terencio, M.C., Valcuende-Cavero, F., Montesinos, M.C., 2014. Topical application of the adenosine A 2A receptor agonist CGS-21680 prevents phorbol-induced epidermal hyperplasia and inflammation in mice. Exp. Dermatol. 23, 555–560. https://doi.org/10.1111/exd.12461

Armentero, M.T., Pinna, A., Ferré, S., Lanciego, J.L., Müller, C.E., Franco, R., 2011. Past, present and future of A2A adenosine receptor antagonists in the therapy of Parkinson's disease. Pharmacol. Ther. 132, 280–299. https://doi.org/10.1016/j.pharmthera.2011.07.004

Arnett, F.C., Edworthy, S.M., Bloch, D.A., McShane, D.J., Fries, J.F., Cooper, N.S., Healey, L.A., Kaplan, S.R., Liang, M.H., Luthra, H.S., 1988. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 31, 315–24.

Ascherio, A., Weisskopf, M.G., O'Reilly, E.J., McCullough, M.L., Calle, E.E., Rodriguez, C., Thun, M.J., 2004. Coffee Consumption, Gender, and Parkinson's Disease Mortality in the Cancer Prevention Study II Cohort: The Modifying Effects of Estrogen. Am. J. Epidemiol. 160, 977–984. https://doi.org/10.1093/aje/kwh312

Ascherio, A., Zhang, S.M., Hernán, M.A., Kawachi, I., Colditz, G.A., Speizer, F.E., Willett, W.C., 2001. Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Ann. Neurol. 50, 56–63.

Avni, I., Garzozi, H.J., Barequet, I.S., Segev, F., Varssano, D., Sartani, G., Chetrit, N., Bakshi, E., Zadok, D., Tomkins, O., Litvin, G., Jacobson, K.A., Fishman, S., Harpaz, Z., Farbstein, M., Yehuda, S.B., Silverman, M.H., Kerns, W.D., Bristol, D.R., Cohn, I., Fishman, P., 2010. Treatment of dry eye syndrome with orally administered CF101: data from a phase 2 clinical trial. Ophthalmology 117, 1287–93. https://doi.org/10.1016/j.ophtha.2009.11.029

Bakker, M.F., Jacobs, J.W.G., Welsing, P.M.J., Vreugdenhil, S.A., van Booma-Frankfort, C., Linn-Rasker, S.P., Ton, E., Lafeber, F.P.J.G., Bijlsma, J.W.J., Utrecht Arthritis Cohort Study Group, 2011. Early clinical response to treatment predicts 5-year outcome in RA patients: follow-up results from the CAMERA study. Ann. Rheum. Dis. 70, 1099–103. https://doi.org/10.1136/ard.2010.137943

Bar-Yehuda, S., Rath-Wolfson, L., Del Valle, L., Ochaion, A., Cohen, S., Patoka, R., Zozulya, G., Barer, F., Atar, E., Piña-Oviedo, S., Perez-Liz, G., Castel, D., Fishman, P., 2009. Induction of an antiinflammatory effect and prevention of cartilage damage in rat knee osteoarthritis by CF101 treatment. Arthritis Rheum. 60, 3061–71. https://doi.org/10.1002/art.24817

Bar-Yehuda, S., Silverman, M.H., Kerns, W.D., Ochaion, A., Cohen, S., Fishman, P., 2007. The anti-inflammatory effect of A3 adenosine receptor agonists: a novel targeted therapy for rheumatoid arthritis. Expert Opin. Investig. Drugs 16, 1601–13. https://doi.org/10.1517/13543784.16.10.1601

Baraldi, P.G., Tabrizi, M.A., Gessi, S., Borea, P.A., 2008. Adenosine receptor antagonists: translating medicinal chemistry and pharmacology into clinical utility. Chem. Rev. 108, 238–63. https://doi.org/10.1021/cr0682195

Barletta, K.E., Ley, K., Mehrad, B., 2012. Regulation of Neutrophil Function by Adenosine. Arterioscler. Thromb. Vasc. Biol. 32, 856–864. https://doi.org/10.1161/ATVBAHA.111.226845

Batalha, V.L., Pego, J.M., Fontinha, B.M., Costenla, A.R., Valadas, J.S., Baqi, Y., Radjainia, H., Müller, C.E., Sebastião, A.M., Lopes, L. V, 2013. Adenosine A2A receptor blockade reverts hippocampal stress-induced deficits and restores corticosterone circadian oscillation. Mol. Psychiatry 18, 320–331. https://doi.org/10.1038/mp.2012.8

Begovich, A.B., Carlton, V.E.H., Honigberg, L.A., Schrodi, S.J., Chokkalingam, A.P., Alexander, H.C., Ardlie, K.G., Huang, Q., Smith, A.M., Spoerke, J.M., Conn, M.T., Chang, M., Chang, S.-Y.P., Saiki, R.K., Catanese, J.J., Leong, D.U., Garcia, V.E., McAllister, L.B., Jeffery, D.A., Lee, A.T., Batliwalla, F., Remmers, E., Criswell, L.A., Seldin, M.F., Kastner, D.L., Amos, C.I., Sninsky, J.J., Gregersen, P.K., 2004. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am. J. Hum. Genet. 75, 330–7. https://doi.org/10.1086/422827

Belardinelli, L., Shryock, J.C., Snowdy, S., Zhang, Y., Monopoli, A., Lozza, G., Ongini, E., Olsson, R.A., Dennis, D.M., 1998. The A2A adenosine receptor mediates coronary vasodilation. J. Pharmacol. Exp. Ther. 284, 1066–73.

Bensen, W.G., Bensen, W., Adachi, J.D., Tugwell, P.X., 1990. Remodelling the pyramid: the therapeutic target of rheumatoid arthritis. J. Rheumatol. 17, 987–9.

Bitto, A., Polito, F., Irrera, N., D'Ascola, A., Avenoso, A., Nastasi, G., Campo, G.M., Micali, A., Bagnato, G., Minutoli, L., Marini, H., Rinaldi, M., Squadrito, F., Altavilla, D., 2011. Polydeoxyribonucleotide reduces cytokine production and the severity of collagen-induced arthritis by stimulation of adenosine A2A receptor. Arthritis Rheum. 63, 3364–3371. https://doi.org/10.1002/art.30538

Blackburn, M.R., Vance, C.O., Morschl, E., Wilson, C.N., 2009. Adenosine receptors and inflammation. Handb. Exp. Pharmacol. 215–69. https://doi.org/10.1007/978-3-540-89615-9_8

Boison, D., 2007. Adenosine as a modulator of brain activity. Drug News Perspect. 20, 607. https://doi.org/10.1358/dnp.2007.20.10.1181353

Boison, D., 2008. The adenosine kinase hypothesis of epileptogenesis. Prog. Neurobiol. 84, 249–262. https://doi.org/10.1016/j.pneurobio.2007.12.002

Boison, D., 2013. Adenosine Kinase: Exploitation for Therapeutic Gain. Pharmacol. Rev. 65, 906–943. https://doi.org/10.1124/pr.112.006361

Boison, D., 2016. Adenosinergic signaling in epilepsy. Neuropharmacology 104, 131–139. https://doi.org/10.1016/j.neuropharm.2015.08.046

Boison, D., Aronica, E., 2015. Comorbidities in Neurology: Is adenosine the common link? Neuropharmacology 97, 18–34. https://doi.org/10.1016/j.neuropharm.2015.04.031

Boison, D., Shen, H.-Y., 2010. Adenosine kinase is a new therapeutic target to prevent ischemic neuronal death. Open Drug Discov. J. 2, 108–118.

Bonaventura, J., Navarro, G., Casadó-Anguera, V., Azdad, K., Rea, W., Moreno, E., Brugarolas, M., Mallol, J., Canela, E.I., Lluís, C., Cortés, A., Volkow, N.D., Schiffmann, S.N., Ferré, S., Casadó, V., 2015. Allosteric interactions between agonists and antagonists within the adenosine A 2A receptor-dopamine D 2 receptor heterotetramer. Proc. Natl. Acad. Sci. 112, E3609–E3618. https://doi.org/10.1073/pnas.1507704112

Borea, P.A., Gessi, S., Bar-Yehuda, S., Fishman, P., 2009. A3 Adenosine Receptor: Pharmacology and Role in Disease, in: Handbook of Experimental Pharmacology. pp. 297–327. https://doi.org/10.1007/978-3-540-89615-9_10

Borea, P.A., Varani, K., Vincenzi, F., Baraldi, P.G., Tabrizi, M.A., Merighi, S., Gessi, S., 2014. The A3 Adenosine Receptor: History and Perspectives. Pharmacol. Rev. 67, 74–102. https://doi.org/10.1124/pr.113.008540

Borroto-Escuela, D.O., Romero-Fernandez, W., Garriga, P., Ciruela, F., Narvaez, M., Tarakanov, A.O., Palkovits, M., Agnati, L.F., Fuxe, K., 2013. G Protein–Coupled Receptor Heterodimerization in the Brain, in: Methods in Enzymology. pp. 281–294. https://doi.org/10.1016/B978-0-12-391862-8.00015-6

Borroto-Escuela, D.O., Romero-Fernandez, W., Tarakanov, A.O., Gómez-Soler, M., Corrales, F., Marcellino, D., Narvaez, M., Frankowska, M., Flajolet, M., Heintz, N., Agnati, L.F., Ciruela, F., Fuxe, K., 2010. Characterization of the A2AR–D2R interface: Focus on the role of the C-terminal tail and the transmembrane helices. Biochem. Biophys. Res. Commun. 402, 801–807. https://doi.org/10.1016/j.bbrc.2010.10.122

Borroto-Escuela, D.O., Tarakanov, A.O., Guidolin, D., Ciruela, F., Agnati, L.F., Fuxe, K., 2011. Moonlighting characteristics of G protein-coupled receptors: focus on receptor heteromers and relevance for neurodegeneration. IUBMB Life 63, 463–72. https://doi.org/10.1002/iub.473

Bradding, P., Walls, A.F., Holgate, S.T., 2006. The role of the mast cell in the pathophysiology of asthma. J. Allergy Clin. Immunol. 117, 1277–84. https://doi.org/10.1016/j.jaci.2006.02.039

Braun, J., Sieper, J., 2002. Therapy of ankylosing spondylitis and other spondyloarthritides: established medical treatment, anti-TNF-alpha therapy and other novel approaches. Arthritis Res. 4, 307–21. https://doi.org/10.1186/ar592

Braun, J., Sieper, J., 2007. Ankylosing spondylitis. Lancet (London, England) 369, 1379–90. https://doi.org/10.1016/S0140-6736(07)60635-7

Brewerton, D.A., Hart, F.D., Nicholls, A., Caffrey, M., James, D.C., Sturrock, R.D., 1973. Ankylosing spondylitis and HL-A 27. Lancet (London, England) 1, 904–7.

Brown, M.A., Pile, K.D., Kennedy, L.G., Calin, A., Darke, C., Bell, J., Wordsworth, B.P., Cornélis, F., 1996. HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom. Ann. Rheum. Dis. 55, 268–70.

Canas, P.M., Porciuncula, L.O., Cunha, G.M.A., Silva, C.G., Machado, N.J., Oliveira, J.M.A., Oliveira, C.R., Cunha, R.A., 2009. Adenosine A2A Receptor Blockade Prevents Synaptotoxicity and Memory Dysfunction Caused by -Amyloid Peptides via p38 Mitogen-

Activated Protein Kinase Pathway. J. Neurosci. 29, 14741–14751. https://doi.org/10.1523/JNEUROSCI.3728-09.2009

Cantini, F., Niccoli, L., Nannini, C., Cassarà, E., Kaloudi, O., Giulio Favalli, E., Becciolini, A., Biggioggero, M., Benucci, M., Li Gobbi, F., Grossi, V., Infantino, M., Meacci, F., Manfredi, M., Guiducci, S., Bellando-Randone, S., Matucci-Cerinic, M., Foti, R., Di Gangi, M., Mosca, M., Tani, C., Palmieri, F., Goletti, D., Italian board for the TAilored BIOlogic therapy (ITABIO), 2016. Tailored first-line biologic therapy in patients with rheumatoid arthritis, spondyloarthritis, and psoriatic arthritis. Semin. Arthritis Rheum. 45, 519–32. https://doi.org/10.1016/j.semarthrit.2015.10.001

Cao, C., Cirrito, J.R., Lin, X., Wang, L., Verges, D.K., Dickson, A., Mamcarz, M., Zhang, C., Mori, T., Arendash, G.W., Holtzman, D.M., Potter, H., Potter, H., 2009. Caffeine Suppresses Amyloid-β Levels in Plasma and Brain of Alzheimer's Disease Transgenic Mice. J. Alzheimer's Dis. 17, 681–697. https://doi.org/10.3233/JAD-2009-1071

Cao, C., Loewenstein, D.A., Lin, X., Zhang, C., Wang, L., Duara, R., Wu, Y., Giannini, A., Bai, G., Cai, J., Greig, M., Schofield, E., Ashok, R., Small, B., Potter, H., Arendash, G.W., 2012. High Blood caffeine levels in MCI linked to lack of progression to dementia. J. Alzheimers. Dis. 30, 559–72. https://doi.org/10.3233/JAD-2012-111781

Capecchi, P.L., Camurri, A., Pompella, G., Mazzola, A., Maccherini, M., Diciolla, F., Lazzerini, P.E., Abbracchio, M.P., Laghi-Pasini, F., 2005. Upregulation of A2A adenosine receptor expression by TNF-alpha in PBMC of patients with CHF: a regulatory mechanism of inflammation. J. Card. Fail. 11, 67–73.

Caporali, R., Pallavicini, F.B., Filippini, M., Gorla, R., Marchesoni, A., Favalli, E.G., Sarzi-Puttini, P., Atzeni, F., Montecucco, C., 2009. Treatment of rheumatoid arthritis with anti-TNF-alpha agents: a reappraisal. Autoimmun. Rev. 8, 274–80. https://doi.org/10.1016/j.autrev.2008.11.003

Carroll, S.H., Wigner, N.A., Kulkarni, N., Johnston-Cox, H., Gerstenfeld, L.C., Ravid, K., 2012. A2B Adenosine Receptor Promotes Mesenchymal Stem Cell Differentiation to Osteoblasts and Bone Formation in Vivo. J. Biol. Chem. 287, 15718–15727. https://doi.org/10.1074/jbc.M112.344994

Caruso, M., Varani, K., Tringali, G., Polosa, R., 2009. Adenosine and adenosine receptors: their contribution to airway inflammation and therapeutic potential in asthma. Curr. Med. Chem. 16, 3875–85.

Chan, E.S.L., Cronstein, B.N., 2010. Methotrexate—how does it really work? Nat. Rev. Rheumatol. 6, 175–178. https://doi.org/10.1038/nrrheum.2010.5

Chan, E.S.L., Cronstein, B.N., 2013. Mechanisms of action of methotrexate. Bull. Hosp. Jt. Dis. 71 Suppl 1, S5-8.

Chan, E.S.L., Montesinos, M.C., Fernandez, P., Desai, A., Delano, D.L., Yee, H., Reiss, A.B., Pillinger, M.H., Chen, J.-F., Schwarzschild, M.A., Friedman, S.L., Cronstein, B.N., 2006. Adenosine A 2A receptors play a role in the pathogenesis of hepatic cirrhosis. Br. J. Pharmacol. 148, 1144–1155. https://doi.org/10.1038/sj.bjp.0706812

Chatzikyriakidou, A., Voulgari, P. V., Drosos, A.A., 2011. What is the role of HLA-B27 in spondyloarthropathies? Autoimmun. Rev. 10, 464–468. https://doi.org/10.1016/j.autrev.2011.01.011

Chen, C., Zhang, X., Xiao, L., Zhang, X., Ma, X., 2016. Comparative Effectiveness of Biologic Therapy Regimens for Ankylosing Spondylitis: A Systematic Review and a Network Meta-Analysis. Medicine (Baltimore). 95, e3060. https://doi.org/10.1097 MD.0000000000003060

Chen, J.-F., Sonsalla, P.K., Pedata, F., Melani, A., Domenici, M.R., Popoli, P., Geiger, J., Lopes, L. V, de Mendonça, A., 2007. Adenosine A2A receptors and brain injury: broad spectrum of neuroprotection, multifaceted actions and "fine tuning" modulation. Prog. Neurobiol. 83, 310–31. https://doi.org/10.1016/j.pneurobio.2007.09.002

Chen, Z., Janes, K., Chen, C., Doyle, T., Bryant, L., Tosh, D.K., Jacobson, K.A., Salvemini, D., 2012. Controlling murine and rat chronic pain through A3 adenosine receptor activation. FASEB J. 26, 1855–65. https://doi.org/10.1096/fj.11-201541

Chen, Z., Xiong, C., Pancyr, C., Stockwell, J., Walz, W., Cayabyab, F.S., 2014. Prolonged Adenosine A1 Receptor Activation in Hypoxia and Pial Vessel Disruption Focal Cortical Ischemia Facilitates Clathrin-Mediated AMPA Receptor Endocytosis and Long-Lasting Synaptic Inhibition in Rat Hippocampal CA3-CA1 Synapses: Differential Regulation of GluA2 and GluA1 Subunits by p38 MAPK and JNK. J. Neurosci. 34, 9621–9643. https://doi.org/10.1523/JNEUROSCI.3991-13.2014

Cheong, S.L., Federico, S., Venkatesan, G., Mandel, A.L., Shao, Y.-M., Moro, S., Spalluto, G., Pastorin, G., 2013. The A 3 adenosine receptor as multifaceted therapeutic target: pharmacology, medicinal chemistry, and in silico approaches. Med. Res. Rev. 33, 235–335. https://doi.org/10.1002/med.20254

Chhabra, P., Linden, J., Lobo, P., Okusa, M.D., Brayman, K.L., 2012. The immunosuppressive role of adenosine A2A receptors in ischemia reperfusion injury and islet transplantation. Curr. Diabetes Rev. 8, 419–33.

Ciruela, F., Fernández-Dueñas, V., Llorente, J., Borroto-Escuela, D., Cuffí, M.L., Carbonell, L., Sánchez, S., Agnati, L.F., Fuxe, K., Tasca, C.I., 2012. G protein-coupled receptor oligomerization and brain integration: Focus on adenosinergic transmission. Brain Res. 1476, 86–95. https://doi.org/10.1016/j.brainres.2012.04.056

Colbert, R.A., Tran, T.M., Layh-Schmitt, G., 2014. HLA-B27 misfolding and ankylosing spondylitis. Mol. Immunol. 57, 44–51. https://doi.org/10.1016/j.molimm.2013.07.013

Colino-Oliveira, M., Rombo, D.M., Dias, R.B., Ribeiro, J.A., Sebastião, A.M., 2016. BDNF-induced presynaptic facilitation of GABAergic transmission in the hippocampus of young adults is dependent of TrkB and adenosine A2A receptors. Purinergic Signal. 12, 283–294. https://doi.org/10.1007/s11302-016-9502-y

Constantino, L.C., Pamplona, F.A., Matheus, F.C., Ludka, F.K., Gomez-Soler, M., Ciruela, F., Boeck, C.R., Prediger, R.D., Tasca, C.I., 2015. Adenosine A1 receptor activation modulates N-methyl-d-aspartate (NMDA) preconditioning phenotype in the brain. Behav. Brain Res. 282, 103–110. https://doi.org/10.1016/j.bbr.2014.12.056

Coomes, E., Chan, E.S.L., Reiss, A.B., 2011. Methotrexate in Atherogenesis and Cholesterol Metabolism. Cholesterol 2011, 1–8. https://doi.org/10.1155/2011/503028

Cronstein, B.N., Sitkovsky, M., 2016. Adenosine and adenosine receptors in the pathogenesis and treatment of rheumatic diseases. Nat. Rev. Rheumatol. 13, 41–51. https://doi.org/10.1038/nrrheum.2016.178

Cunha, R.A., 2016. How does adenosine control neuronal dysfunction and neurodegeneration? J. Neurochem. 139, 1019–1055. https://doi.org/10.1111/jnc.13724

Dall'Igna, O.P., Fett, P., Gomes, M.W., Souza, D.O., Cunha, R.A., Lara, D.R., 2007. Caffeine and adenosine A2a receptor antagonists prevent β-amyloid (25–35)-induced cognitive deficits in mice. Exp. Neurol. 203, 241–245. https://doi.org/10.1016/j.expneurol.2006.08.008

Dalpiaz, A., Cacciari, B., Vicentini, C.B., Bortolotti, F., Spalluto, G., Federico, S., Pavan, B., Vincenzi, F., Borea, P.A., Varani, K., 2012. A novel conjugated agent between dopamine and an A2A adenosine receptor antagonist as a potential anti-Parkinson multitarget approach. Mol. Pharm. 9, 591–604. https://doi.org/10.1021/mp200489d

Dancevic, C.M., McCulloch, D.R., 2014. Current and emerging therapeutic strategies for preventing inflammation and aggrecanase-mediated cartilage destruction in arthritis. Arthritis Res. Ther. 16, 429.

David, M., Akerman, L., Ziv, M., Kadurina, M., Gospodinov, D., Pavlotsky, F., Yankova, R., Kouzeva, V., Ramon, M., Silverman, M.H., Fishman, P., 2012. Treatment of plaque-type psoriasis with oral CF101: data from an exploratory randomized phase 2 clinical trial. J. Eur. Acad. Dermatol. Venereol. 26, 361–7. https://doi.org/10.1111/j.1468-3083.2011.04078.x

Day, Y.-J., Marshall, M.A., Huang, L., McDuffie, M.J., Okusa, M.D., Linden, J., 2004. Protection from ischemic liver injury by activation of A2A adenosine receptors during reperfusion: inhibition of chemokine induction. Am. J. Physiol. Gastrointest. Liver Physiol. 286, G285-93. https://doi.org/10.1152/ajpgi.00348.2003

de Andrade, K.R., de Castro, G.R.W., Vicente, G., da Rosa, J.S., Nader, M., Pereira, I.A., Fröde, T.S., 2014. Evaluation of circulating levels of inflammatory and bone formation markers in axial spondyloarthritis. Int. Immunopharmacol. 21, 481–486. https://doi.org/10.1016/j.intimp.2014.05.031

Dean, L.E., Jones, G.T., MacDonald, A.G., Downham, C., Sturrock, R.D., Macfarlane, G.J., 2014. Global prevalence of ankylosing spondylitis. Rheumatology 53, 650–657. https://doi.org/10.1093/rheumatology/ket387

Du, L., Gao, Z.-G., Nithipatikom, K., IJzerman, A.P., van Veldhoven, J.P.D., Jacobson, K.A., Gross, G.J., Auchampach, J.A., 2012. Protection from Myocardial Ischemia/Reperfusion Injury by a Positive Allosteric Modulator of the A3 Adenosine Receptor. J. Pharmacol. Exp. Ther. 340, 210–217. https://doi.org/10.1124/jpet.111.187559

Du, X., Ou, X., Song, T., Zhang, W., Cong, F., Zhang, S., Xiong, Y., 2015. Adenosine A 2B receptor stimulates angiogenesis by inducing VEGF and eNOS in human microvascular endothelial cells. Exp. Biol. Med. 240, 1472–1479. https://doi.org/10.1177/1535370215584939

Eckle, T., Hartmann, K., Bonney, S., Reithel, S., Mittelbronn, M., Walker, L.A., Lowes, B.D., Han, J., Borchers, C.H., Buttrick, P.M., Kominsky, D.J., Colgan, S.P., Eltzschig, H.K., 2012. Adora2b-elicited Per2 stabilization promotes a HIF-dependent metabolic switch crucial for myocardial adaptation to ischemia. Nat. Med. 18, 774–782. https://doi.org/10.1038/nm.2728

El Yacoubi, M., Costentin, J., Vaugeois, J.-M., 2003. Adenosine A2A receptors and depression. Neurology 61, S82-7.

Eltzschig, H.K., Bonney, S.K., Eckle, T., 2013. Attenuating myocardial ischemia by targeting A2B adenosine receptors. Trends Mol. Med. 19, 345–354. https://doi.org/10.1016/j.molmed.2013.02.005

Elzein, E., Zablocki, J., 2008. A 1 adenosine receptor agonists and their potential therapeutic applications. Expert Opin. Investig. Drugs 17, 1901–1910. https://doi.org/10.1517/13543780802497284

Feoktistov, I., Biaggioni, I., 2011. Role of Adenosine A2B Receptors in Inflammation, in: Advances in Pharmacology (San Diego, Calif.). pp. 115–144. https://doi.org/10.1016/B978-0-12-385526-8.00005-9

Fernandez, L.G., Sharma, A.K., LaPar, D.J., Kron, I.L., Laubach, V.E., 2013. Adenosine A1 receptor activation attenuates lung ischemia-reperfusion injury. J. Thorac. Cardiovasc. Surg. 145, 1654–9. https://doi.org/10.1016/j.jtcvs.2013.01.006

Ferrante, C.J., Pinhal-Enfield, G., Elson, G., Cronstein, B.N., Hasko, G., Outram, S., Leibovich, S.J., 2013. The Adenosine-Dependent Angiogenic Switch of Macrophages to an M2-Like Phenotype is Independent of Interleukin-4 Receptor Alpha (IL-4Rα) Signaling. Inflammation 36, 921–931. https://doi.org/10.1007/s10753-013-9621-3

Ferrari, D., Gambari, R., Idzko, M., Müller, T., Albanesi, C., Pastore, S., La Manna, G., Robson, S.C., Cronstein, B., 2016. Purinergic signaling in scarring. FASEB J. 30, 3–12. https://doi.org/10.1096/fj.15-274563

Ferré, S., Bonaventura, J., Tomasi, D., Navarro, G., Moreno, E., Cortés, A., Lluís, C., Casadó, V., Volkow, N.D., 2016. Allosteric mechanisms within the adenosine A2A–dopamine D2 receptor heterotetramer. Neuropharmacology 104, 154–160. https://doi.org/10.1016/j.neuropharm.2015.05.028

Ferre, S., von Euler, G., Johansson, B., Fredholm, B.B., Fuxe, K., 1991. Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. Proc. Natl. Acad. Sci. U. S. A. 88, 7238–41.

Festugato, M., 2015. Adenosine: an endogenous mediator in the pathogenesis of psoriasis. An. Bras. Dermatol. 90, 862–7. https://doi.org/10.1590/abd1806-4841.20153689

Figler, R.A., Wang, G., Srinivasan, S., Jung, D.Y., Zhang, Z., Pankow, J.S., Ravid, K., Fredholm, B., Hedrick, C.C., Rich, S.S., Kim, J.K., LaNoue, K.F., Linden, J., 2011. Links between insulin resistance, adenosine A2B receptors, and inflammatory markers in mice and humans. Diabetes 60, 669–79. https://doi.org/10.2337/db10-1070

Fiocco, U., Oliviero, F., Sfriso, P., Calabrese, F., Lunardi, F., Scagliori, E., Rubaltelli, L., Stramare, R., Di Maggio, A., Nardacchione, R., Cozzi, L., Molena, B., Felicetti, M., Gazzola, K., Lo Nigro, A., Accordi, B., Roux-Lombard, P., Dayer, J.-M., Punzi, L., 2012.

Synovial biomarkers in psoriatic arthritis. J. Rheumatol. Suppl. 89, 61–4. https://doi.org/10.3899/jrheum.120246

Fishman, P., Bar-Yehuda, S., Liang, B.T., Jacobson, K.A., 2012. Pharmacological and therapeutic effects of A3 adenosine receptor agonists. Drug Discov. Today 17, 359–366. https://doi.org/10.1016/j.drudis.2011.10.007

Fishman, P., Bar-Yehuda, S., Madi, L., Rath-Wolfson, L., Ochaion, A., Cohen, S., Baharav, E., 2006. The PI3K-NF-kappaB signal transduction pathway is involved in mediating the anti-inflammatory effect of IB-MECA in adjuvant-induced arthritis. Arthritis Res. Ther. 8, R33. https://doi.org/10.1186/ar1887

Fishman, P., Bar-Yehuda, S., Synowitz, M., Powell, J.D., Klotz, K.N., Gessi, S., Borea, P.A., 2009. Adenosine Receptors and Cancer. pp. 399–441. https://doi.org/10.1007/978-3-540-89615-9_14

Fishman, P., Cohen, S., 2016. The A3 adenosine receptor (A3AR): therapeutic target and predictive biological marker in rheumatoid arthritis. Clin. Rheumatol. 35, 2359–2362. https://doi.org/10.1007/s10067-016-3202-4

Fishman, P., Cohen, S., Bar-Yehuda, S., 2013. Targeting the A3 adenosine receptor for glaucoma treatment (Review). Mol. Med. Rep. 7, 1723–5. https://doi.org/10.3892/mmr.2013.1413

Fishman, P., Luger, D., Ochaion, A., Cohen, S., Patokaa, R., Zozulya, G., Silver, P.B., de Morales, J.M.G.R., Caspi, R.R., Fishman, P., 2011. Inhibition of experimental auto-immune uveitis by the A3 adenosine receptor agonist CF101. Int. J. Mol. Med. 28, 727–31. https://doi.org/10.3892/ijmm.2011.753

Flogel, U., Burghoff, S., van Lent, P.L.E.M., Temme, S., Galbarz, L., Ding, Z., El-Tayeb, A., Huels, S., Bonner, F., Borg, N., Jacoby, C., Muller, C.E., van den Berg, W.B., Schrader, J., 2012. Selective Activation of Adenosine A2A Receptors on Immune Cells by a CD73-Dependent Prodrug Suppresses Joint Inflammation in Experimental Rheumatoid Arthritis. Sci. Transl. Med. 4, 146ra108-146ra108. https://doi.org/10.1126/scitranslmed.3003717

Ford, A., Castonguay, A., Cottet, M., Little, J.W., Chen, Z., Symons-Liguori, A.M., Doyle, T., Egan, T.M., Vanderah, T.W., De Konnick, Y., Tosh, D.K., Jacobson, K.A., Salvemini, D., 2015. Engagement of the GABA to KCC2 signaling pathway contributes to the analgesic effects of A3AR agonists in neuropathic pain. J. Neurosci. 35, 6057–67. https://doi.org/10.1523/JNEUROSCI.4495-14.2015

Fordyce, C.B., Gersh, B.J., Stone, G.W., Granger, C.B., 2015. Novel therapeutics in myocardial infarction: targeting microvascular dysfunction and reperfusion injury. Trends Pharmacol. Sci. 36, 605–616. https://doi.org/10.1016/j.tips.2015.06.004

Forrest, C.M., Harman, G., McMillan, R.B., Stoy, N., Stone, T.W., Darlington, L.G., n.d. Modulation of cytokine release by purine receptors in patients with rheumatoid arthritis. Clin. Exp. Rheumatol. 23, 89–92.

Fozard, J.R., Ellis, K.M., Villela Dantas, M.F., Tigani, B., Mazzoni, L., 2002. Effects of CGS 21680, a selective adenosine A2A receptor agonist, on allergic airways inflammation in the rat. Eur. J. Pharmacol. 438, 183–8.

Fredholm, B.B., 2010. Adenosine receptors as drug targets. Exp. Cell Res. 316, 1284–1288. https://doi.org/10.1016/j.yexcr.2010.02.004

Fredholm, B.B., Arslan, G., Halldner, L., Kull, B., Schulte, G., Wasserman, W., 2000. Structure and function of adenosine receptors and their genes. Naunyn. Schmiedebergs. Arch. Pharmacol. 362, 364–74.

Fredholm, B.B., IJzerman, A.P., Jacobson, K.A., Klotz, K.N., Linden, J., 2001. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol. Rev. 53, 527–52.

Fredholm, B.B., IJzerman, A.P., Jacobson, K.A., Linden, J., Müller, C.E., 2011. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors--an update. Pharmacol. Rev. 63, 1–34. https://doi.org/10.1124/pr.110.003285

Furst, D.E., Keystone, E.C., Fleischmann, R., Mease, P., Breedveld, F.C., Smolen, J.S., Kalden, J.R., Braun, J., Bresnihan, B., Burmester, G.R., De Benedetti, F., Dorner, T., Emery, P., Gibofsky, A., Kavanaugh, A., Kirkham, B., Schiff, M.H., Sieper, J., Singer, N., Van Riel, P.L.C.M., Weinblatt, M.E., Weisman, M.H., Winthrop, K., 2010a. Updated consensus statement on biological agents for the treatment of rheumatic diseases, 2009. Ann. Rheum. Dis. 69, i2–i29. https://doi.org/10.1136/ard.2009.123885

Fuxe, K., Guidolin, D., Agnati, L.F., Borroto-Escuela, D.O., 2015. Dopamine heteroreceptor complexes as therapeutic targets in Parkinson's disease. Expert Opin. Ther. Targets 19, 377–398. https://doi.org/10.1517/14728222.2014.981529

Gabriel, S.E., 2001. The epidemiology of rheumatoid arthritis. Rheum. Dis. Clin. North Am. 27, 269–81.

Gao, Z., Li, B.S., Day, Y.J., Linden, J., 2001. A3 adenosine receptor activation triggers phosphorylation of protein kinase B and protects rat basophilic leukemia 2H3 mast cells from apoptosis. Mol. Pharmacol. 59, 76–82.

Gazoni, L.M., Walters, D.M., Unger, E.B., Linden, J., Kron, I.L., Laubach, V.E., 2010. Activation of A1, A2A, or A3 adenosine receptors attenuates lung ischemia-reperfusion injury. J. Thorac. Cardiovasc. Surg. 140, 440–6. https://doi.org/10.1016/j.jtcvs.2010.03.002

Ge, Z.-D., van der Hoeven, D., Maas, J.E., Wan, T.C., Auchampach, J.A., 2010. A(3) adenosine receptor activation during reperfusion reduces infarct size through actions on bone marrow-derived cells. J. Mol. Cell. Cardiol. 49, 280–6. https://doi.org/10.1016/j.yjmcc.2010.01.018

Gessi, S., Fogli, E., Sacchetto, V., Merighi, S., Varani, K., Preti, D., Leung, E., MacLennan, S., Borea, P.A., 2010. Adenosine Modulates HIF-1, VEGF, IL-8, and Foam Cell Formation in a Human Model of Hypoxic Foam Cells. Arterioscler. Thromb. Vasc. Biol. 30, 90–97. https://doi.org/10.1161/ATVBAHA.109.194902

Gessi, S., Merighi, S., Borea, P.A., 2014. Targeting adenosine receptors to prevent inflammatory skin diseases. Exp. Dermatol. 23, 553–554. https://doi.org/10.1111/exd.12474

Gessi, S., Merighi, S., Fazzi, D., Stefanelli, A., Varani, K., Borea, P.A., 2011. Adenosine receptor targeting in health and disease. Expert Opin. Investig. Drugs 20, 1591–1609. https://doi.org/10.1517/13543784.2011.627853

Gessi, S., Merighi, S., Sacchetto, V., Simioni, C., Borea, P.A., 2011b. Adenosine receptors and cancer. Biochim. Biophys. Acta - Biomembr. 1808, 1400–1412. https://doi.org/10.1016/j.bbamem.2010.09.020

Gessi, S., Merighi, S., Stefanelli, A., Mirandola, P., Bonfatti, A., Fini, S., Sensi, A., Marci, R., Varani, K., Borea, P.A., Vesce, F., 2012. Downregulation of A1 and A2B adenosine receptors in human trisomy 21 mesenchymal cells from first-trimester chorionic villi. Biochim. Biophys. Acta - Mol. Basis Dis. 1822, 1660–1670. https://doi.org/10.1016/j.bbadis.2012.07.013

Gessi, S., Merighi, S., Varani, K., Borea, P.A., 2011a. Adenosine Receptors in Health and Disease, in: Advances in Pharmacology (San Diego, Calif.). pp. 41–75. https://doi.org/10.1016/B978-0-12-385526-8.00002-3

Gessi, S., Merighi, S., Varani, K., Cattabriga, E., Benini, A., Mirandola, P., Leung, E., Mac Lennan, S., Feo, C., Baraldi, S., Borea, P.A., 2007. Adenosine receptors in colon carcinoma tissues and colon tumoral cell lines: focus on the A(3) adenosine subtype. J. Cell. Physiol. 211, 826–36. https://doi.org/10.1002/jcp.20994

Gessi, S., Merighi, S., Varani, K., Leung, E., Mac Lennan, S., Borea, P.A., 2008. The A3 adenosine receptor: An enigmatic player in cell biology. Pharmacol. Ther. 117, 123–140. https://doi.org/10.1016/j.pharmthera.2007.09.002

Gessi, S., Varani, K., Merighi, S., Cattabriga, E., Iannotta, V., Leung, E., Baraldi, P.G., Borea, P.A., 2002. A(3) adenosine receptors in human neutrophils and promyelocytic HL60 cells: a pharmacological and biochemical study. Mol. Pharmacol. 61, 415–24.

Gessi, S., Varani, K., Merighi, S., Morelli, A., Ferrari, D., Leung, E., Baraldi, P.G., Spalluto, G., Borea, P.A., 2001. Pharmacological and biochemical characterization of A3 adenosine receptors in Jurkat T cells. Br. J. Pharmacol. 134, 116–26. https://doi.org/10.1038/sj.bjp.0704254

Gessi, S., Varani, K., Merighi, S., Ongini, E., Borea, P.A., 2000. A2A adenosine receptors in human peripheral blood cells. Br. J. Pharmacol. 129, 2–11. https://doi.org/10.1038/sj.bjp.0703045

Gharibi, B., Abraham, A.A., Ham, J., Evans, B.A., 2011. Adenosine receptor subtype expression and activation influence the differentiation of mesenchymal stem cells to osteoblasts and adipocytes. J. Bone Miner. Res. 26, 2112–2124. https://doi.org/10.1002/jbmr.424

Ghasemi-Rad, M., Attaya, H., Lesha, E., Vegh, A., Maleki-Miandoab, T., Nosair, E., Sepehrvand, N., Davarian, A., Rajebi, H., Pakniat, A., Fazeli, S.A., Mohammadi, A., 2015. Ankylosing spondylitis: A state of the art factual backbone. World J. Radiol. 7, 236–52. https://doi.org/10.4329/wjr.v7.i9.236

Golder, V., Schachna, L., 2013. Ankylosing spondylitis: an update. Aust. Fam. Physician 42, 780–4.

Goldman, N., Chen, M., Fujita, T., Xu, Q., Peng, W., Liu, W., Jensen, T.K., Pei, Y., Wang, F., Han, X., Chen, J.-F., Schnermann, J., Takano, T., Bekar, L., Tieu, K., Nedergaard, M., 2010. Adenosine A1 receptors mediate local anti-nociceptive effects of acupuncture. Nat. Neurosci. 13, 883–8. https://doi.org/10.1038/nn.2562

Gomes, C., Ferreira, R., George, J., Sanches, R., Rodrigues, D.I., Gonçalves, N., Cunha, R.A., 2013. Activation of microglial cells triggers a release of brain-derived neurotrophic factor (BDNF) inducing their proliferation in an adenosine A2A receptor-dependent manner: A2A receptor blockade prevents BDNF release and proliferation of microglia. J. Neuroinflammation 10, 780. https://doi.org/10.1186/1742-2094-10-16

Gomes, C.V., Kaster, M.P., Tomé, A.R., Agostinho, P.M., Cunha, R.A., 2011. Adenosine receptors and brain diseases: Neuroprotection and neurodegeneration. Biochim. Biophys. Acta - Biomembr. 1808, 1380–1399. https://doi.org/10.1016/j.bbamem.2010.12.001

Gorter, S.L., Bijlsma, J.W., Cutolo, M., Gomez-Reino, J., Kouloumas, M., Smolen, J.S., Landewe, R., 2010. Current evidence for the management of rheumatoid arthritis with glucocorticoids: a systematic literature review informing the EULAR recommendations for the management of rheumatoid arthritis. Ann. Rheum. Dis. 69, 1010–1014. https://doi.org/10.1136/ard.2009.127332

Gratacós, J., Collado, A., Filella, X., Sanmartí, R., Cañete, J., Llena, J., Molina, R., Ballesta, A., Muñoz-Gómez, J., 1994. Serum cytokines (IL-6, TNF-alpha, IL-1 beta and IFN-gamma) in ankylosing spondylitis: a close correlation between serum IL-6 and disease activity and severity. Br. J. Rheumatol. 33, 927–31.

Grenz, A., Bauerle, J.D., Dalton, J.H., Ridyard, D., Badulak, A., Tak, E., McNamee, E.N., Clambey, E., Moldovan, R., Reyes, G., Klawitter, J., Ambler, K., Magee, K., Christians, U., Brodsky, K.S., Ravid, K., Choi, D.-S., Wen, J., Lukashev, D., Blackburn, M.R., Osswald, H., Coe, I.R., Nürnberg, B., Haase, V.H., Xia, Y., Sitkovsky, M., Eltzschig, H.K., 2012. Equilibrative nucleoside transporter 1 (ENT1) regulates postischemic blood flow during acute kidney injury in mice. J. Clin. Invest. 122, 693–710. https://doi.org/10.1172/JCI60214

Grenz, A., Homann, D., Eltzschig, H.K., 2011. Extracellular Adenosine: A Safety Signal That Dampens Hypoxia-Induced Inflammation During Ischemia. Antioxid. Redox Signal. 15, 2221–2234. https://doi.org/10.1089/ars.2010.3665

Grenz, A., Osswald, H., Eckle, T., Yang, D., Zhang, H., Tran, Z.V., Klingel, K., Ravid, K., Eltzschig, H.K., 2008. The reno-vascular A2B adenosine receptor protects the kidney from ischemia. PLoS Med. 5, e137. https://doi.org/10.1371/journal.pmed.0050137

Haglund, E., Bremander, A.B., Petersson, I.F., Strombeck, B., Bergman, S., Jacobsson, L.T.H., Turkiewicz, A., Geborek, P., Englund, M., 2011. Prevalence of spondyloarthritis and its subtypes in southern Sweden. Ann. Rheum. Dis. 70, 943–948. https://doi.org/10.1136/ard.2010.141598

Hammarberg, C., Fredholm, B.B., Schulte, G., 2004. Adenosine A3 receptor-mediated regulation of p38 and extracellular-regulated kinase ERK1/2 via phosphatidylinositol-3'-kinase. Biochem. Pharmacol. 67, 129–34.

Harada, H., Asano, O., Hoshino, Y., Yoshikawa, S., Matsukura, M., Kabasawa, Y., Niijima, J., Kotake, Y., Watanabe, N., Kawata, T., Inoue, T., Horizoe, T., Yasuda, N.,

Minami, H., Nagata, K., Murakami, M., Nagaoka, J., Kobayashi, S., Tanaka, I., Abe, S., 2001. 2-Alkynyl-8-aryl-9-methyladenines as novel adenosine receptor antagonists: their synthesis and structure-activity relationships toward hepatic glucose production induced via agonism of the A(2B) receptor. J. Med. Chem. 44, 170–9.

Hargus, N.J., Jennings, C., Perez-Reyes, E., Bertram, E.H., Patel, M.K., 2012. Enhanced actions of adenosine in medial entorhinal cortex layer II stellate neurons in temporal lobe epilepsy are mediated via A1-receptor activation. Epilepsia 53, 168–176. https://doi.org/10.1111/j.1528-1167.2011.03337.x

Harish, A., Hohana, G., Fishman, P., Arnon, O., Bar-Yehuda, S., 2003. A3 adenosine receptor agonist potentiates natural killer cell activity. Int. J. Oncol. 23, 1245–9.

Hart, M.L., Grenz, A., Gorzolla, I.C., Schittenhelm, J., Dalton, J.H., Eltzschig, H.K., 2011. Hypoxia-inducible factor-1α-dependent protection from intestinal ischemia/reperfusion injury involves ecto-5'-nucleotidase (CD73) and the A2B adenosine receptor. J. Immunol. 186, 4367–74. https://doi.org/10.4049/jimmunol.0903617

Haskó, G., Cronstein, B., 2013. Regulation of Inflammation by Adenosine. Front. Immunol. 4, 85. https://doi.org/10.3389/fimmu.2013.00085

Haskó, G., Linden, J., Cronstein, B., Pacher, P., 2008. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. Nat. Rev. Drug Discov. 7, 759–770. https://doi.org/10.1038/nrd2638

Hasko, G., Pacher, P., 2012. Regulation of Macrophage Function by Adenosine. Arterioscler. Thromb. Vasc. Biol. 32, 865–869. https://doi.org/10.1161/ATVBAHA.111.226852

He, W., Mazumder, A., Wilder, T., Cronstein, B.N., 2013. Adenosine regulates bone metabolism via A1, A2A, and A2B receptors in bone marrow cells from normal humans and patients with multiple myeloma. FASEB J. 27, 3446–3454. https://doi.org/10.1096/fj.13-231233

Hodgson, R.A., Bertorelli, R., Varty, G.B., Lachowicz, J.E., Forlani, A., Fredduzzi, S., Cohen-Williams, M.E., Higgins, G.A., Impagnatiello, F., Nicolussi, E., Parra, L.E., Foster, C., Zhai, Y., Neustadt, B.R., Stamford, A.W., Parker, E.M., Reggiani, A., Hunter, J.C., 2009. Characterization of the potent and highly selective A2A receptor antagonists preladenant and SCH 412348 [7-[2-[4-2,4-difluorophenyl]-1-piperazinyl]ethyl]-2-(2-furanyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine] in rodent models of movement disorders and depression. J. Pharmacol. Exp. Ther. 330, 294–303. https://doi.org/10.1124/jpet.108.149617

Hoegl, S., Brodsky, K.S., Blackburn, M.R., Karmouty-Quintana, H., Zwissler, B., Eltzschig, H.K., 2015. Alveolar Epithelial A2B Adenosine Receptors in Pulmonary Protection during Acute Lung Injury. J. Immunol. 195, 1815–1824. https://doi.org/10.4049/jimmunol.1401957

Hreggvidsdottir, H.S., Noordenbos, T., Baeten, D.L., 2014. Inflammatory pathways in spondyloarthritis. Mol. Immunol. 57, 28–37. https://doi.org/10.1016/j.molimm.2013.07.016

Hussain, A., Gharanei, A.M., Nagra, A.S., Maddock, H.L., 2014. Caspase Inhibition Via A3 Adenosine Receptors: A New Cardioprotective Mechanism Against Myocardial Infarction. Cardiovasc. Drugs Ther. 28, 19–32. https://doi.org/10.1007/s10557-013-6500-y

Iliodromitis, E.K., Andreadou, I., Iliodromitis, K., Dagres, N., 2014. Ischemic and Postischemic Conditioning of the Myocardium in Clinical Practice: Challenges, Expectations and Obstacles. Cardiology 129, 117–125. https://doi.org/10.1159/000362499

Imlach, W.L., Bhola, R.F., May, L.T., Christopoulos, A., Christie, M.J., 2015. A Positive Allosteric Modulator of the Adenosine A1 Receptor Selectively Inhibits Primary Afferent Synaptic Transmission in a Neuropathic Pain Model. Mol. Pharmacol. 88, 460–8. https://doi.org/10.1124/mol.115.099499

Impellizzeri, D., Di Paola, R., Esposito, E., Mazzon, E., Paterniti, I., Melani, A., Bramanti, P., Pedata, F., Cuzzocrea, S., 2011. CGS 21680, an agonist of the adenosine (A2A) receptor, decreases acute lung inflammation. Eur. J. Pharmacol. 668, 305–316. https://doi.org/10.1016/j.ejphar.2011.06.049

Ingersoll, S.A., Laroui, H., Kolachala, V.L., Wang, L., Garg, P., Denning, T.L., Gewirtz, A.T., Merlin, D., Sitaraman, S. V., 2012. A2BAR expression in non-immune cells plays an important role in the development of murine colitis. Dig. Liver Dis. 44, 819–826. https://doi.org/10.1016/j.dld.2012.05.013

Jacobs, J.W.G., 2012. Optimal use of non-biologic therapy in the treatment of rheumatoid arthritis. Rheumatology 51, iv3-iv8. https://doi.org/10.1093/rheumatology/kes083

Janes, K., Esposito, E., Doyle, T., Cuzzocrea, S., Tosh, D.K., Jacobson, K.A., Salvemini, D., 2014. A3 adenosine receptor agonist prevents the development of paclitaxel-induced neuropathic pain by modulating spinal glial-restricted redox-dependent signaling pathways. Pain 155, 2560–2567. https://doi.org/10.1016/j.pain.2014.09.016

Janes, K., Symons-Liguori, A.M., Jacobson, K.A., Salvemini, D., 2016. Identification of A3 adenosine receptor agonists as novel non-narcotic analgesics. Br. J. Pharmacol. 173, 1253–67. https://doi.org/10.1111/bph.13446

Janes, K., Wahlman, C., Little, J.W., Doyle, T., Tosh, D.K., Jacobson, K.A., Salvemini, D., 2015. Spinal neuroimmune activation is independent of T-cell infiltration and attenuated by A3 adenosine receptor agonists in a model of oxaliplatin-induced peripheral neuropathy. Brain. Behav. Immun. 44, 91–99. https://doi.org/10.1016/j.bbi.2014.08.010

Jenner, P., Mori, A., Hauser, R., Morelli, M., Fredholm, B.B., Chen, J.F., 2009. Adenosine, adenosine A2A antagonists, and Parkinson's disease. Parkinsonism Relat. Disord. 15, 406–413. https://doi.org/10.1016/j.parkreldis.2008.12.006

Johnston-Cox, H.A., Ravid, K., 2011. Adenosine and blood platelets. Purinergic Signal. 7, 357–365. https://doi.org/10.1007/s11302-011-9220-4

Johnston-Cox, H., Eisenstein, A.S., Koupenova, M., Carroll, S., Ravid, K., 2014. The Macrophage A2b Adenosine Receptor Regulates Tissue Insulin Sensitivity. PLoS One 9, e98775. https://doi.org/10.1371/journal.pone.0098775

Johnston-Cox, H., Koupenova, M., Yang, D., Corkey, B., Gokce, N., Farb, M.G., LeBrasseur, N., Ravid, K., 2012. The A2b Adenosine Receptor Modulates Glucose

Homeostasis and Obesity. PLoS One 7, e40584. https://doi.org/10.1371/journal.pone.0040584

Jörg, M., May, L.T., Mak, F.S., Lee, K.C.K., Miller, N.D., Scammells, P.J., Capuano, B., 2015. Synthesis and Pharmacological Evaluation of Dual Acting Ligands Targeting the Adenosine A 2A and Dopamine D 2 Receptors for the Potential Treatment of Parkinson's Disease. J. Med. Chem. 58, 718–738. https://doi.org/10.1021/jm501254d

Joseph, A., Brasington, R., Kahl, L., Ranganathan, P., Cheng, T.P., Atkinson, J., 2010. Immunologic rheumatic disorders. J. Allergy Clin. Immunol. 125, S204-15. https://doi.org/10.1016/j.jaci.2009.10.067

Kachroo, A., Schwarzschild, M.A., 2012. Adenosine A2A receptor gene disruption protects in an α-synuclein model of Parkinson's disease. Ann. Neurol. 71, 278–82. https://doi.org/10.1002/ana.22630

Kaster, M.P., Machado, N.J., Silva, H.B., Nunes, A., Ardais, A.P., Santana, M., Baqi, Y., Müller, C.E., Rodrigues, A.L.S., Porciúncula, L.O., Chen, J.F., Tomé, Â.R., Agostinho, P., Canas, P.M., Cunha, R.A., 2015. Caffeine acts through neuronal adenosine A 2A receptors to prevent mood and memory dysfunction triggered by chronic stress. Proc. Natl. Acad. Sci. 112, 7833–7838. https://doi.org/10.1073/pnas.1423088112

Kay, A.B., Phipps, S., Robinson, D.S., 2004. A role for eosinophils in airway remodelling in asthma. Trends Immunol. 25, 477–82. https://doi.org/10.1016/j.it.2004.07.006

Keller, C., Webb, A., Davis, J., 2003. Cytokines in the seronegative spondyloarthropathies and their modification by TNF blockade: a brief report and literature review. Ann. Rheum. Dis. 62, 1128–32.

Khoa, N.D., Montesinos, M.C., Reiss, A.B., Delano, D., Awadallah, N., Cronstein, B.N., 2001. Inflammatory cytokines regulate function and expression of adenosine A(2A) receptors in human monocytic THP-1 cells. J. Immunol. 167, 4026–32.

Kiesman, W.F., Elzein, E., Zablocki, J., 2009. A1 Adenosine Receptor Antagonists, Agonists, and Allosteric Enhancers, in: Handbook of Experimental Pharmacology. pp. 25–58. https://doi.org/10.1007/978-3-540-89615-9_2

Kobow, K., Blümcke, I., 2012. The emerging role of DNA methylation in epileptogenesis. Epilepsia 53, 11–20. https://doi.org/10.1111/epi.12031

Koda, K., Salazar-Rodriguez, M., Corti, F., Chan, N.Y.-K., Estephan, R., Silver, R.B., Mochly-Rosen, D., Levi, R., 2010. Aldehyde Dehydrogenase Activation Prevents Reperfusion Arrhythmias by Inhibiting Local Renin Release From Cardiac Mast Cells. Circulation 122, 771–781. https://doi.org/10.1161/CIRCULATIONAHA.110.952481

Koeppen, M., Eckle, T., Eltzschig, H.K., 2011. The hypoxia–inflammation link and potential drug targets. Curr. Opin. Anaesthesiol. 24, 363–369. https://doi.org/10.1097/ACO.0b013e32834873fd

Koizumi, S., Odashima, M., Otaka, M., Jin, M., Linden, J., Watanabe, S., Ohnishi, H., 2009. Attenuation of gastric mucosal inflammation induced by indomethacin through activation of the A2A adenosine receptor in rats. J. Gastroenterol. 44, 419–425. https://doi.org/10.1007/s00535-009-0028-8

Kolachala, V.L., Vijay–Kumar, M., Dalmasso, G., Yang, D., Linden, J., Wang, L., Gewirtz, A., Ravid, K., Merlin, D., Sitaraman, S. V., 2008. A2B Adenosine Receptor Gene Deletion Attenuates Murine Colitis. Gastroenterology 135, 861–870. https://doi.org/10.1053/j.gastro.2008.05.049

Konrad, F.M., Neudeck, G., Vollmer, I., Ngamsri, K.C., Thiel, M., Reutershan, J., 2013. Protective effects of pentoxifylline in pulmonary inflammation are adenosine receptor A2A dependent. FASEB J. 27, 3524–35. https://doi.org/10.1096/fj.13-228122

Koupenova, M., Johnston-Cox, H., Vezeridis, A., Gavras, H., Yang, D., Zannis, V., Ravid, K., 2012. A2b Adenosine Receptor Regulates Hyperlipidemia and Atherosclerosis. Circulation 125, 354–363. https://doi.org/10.1161/CIRCULATIONAHA.111.057596

Kretschmar, C., Oyarzún, C., Villablanca, C., Jaramillo, C., Alarcón, S., Perez, G., Díaz-Encarnación, M.M., Pastor-Anglada, M., Garrido, W., Quezada, C., San Martín, R., 2016. Reduced Adenosine Uptake and Its Contribution to Signaling that Mediates Profibrotic Activation in Renal Tubular Epithelial Cells: Implication in Diabetic Nephropathy. PLoS One 11, e0147430. https://doi.org/10.1371/journal.pone.0147430

Krügel, U., 2016. Purinergic receptors in psychiatric disorders. Neuropharmacology 104, 212–225. https://doi.org/10.1016/j.neuropharm.2015.10.032

Kuhn, K.A., Kulik, L., Tomooka, B., Braschler, K.J., Arend, W.P., Robinson, W.H., Holers, V.M., 2006. Antibodies against citrullinated proteins enhance tissue injury in experimental autoimmune arthritis. J. Clin. Invest. 116, 961–73. https://doi.org/10.1172/JCI25422

Lane, J.R., Jaakola, V.-P., IJzerman, A.P., 2011. The Structure of the Adenosine Receptors, in: Advances in Pharmacology (San Diego, Calif.). pp. 1–40. https://doi.org/10.1016/B978-0-12-385526-8.00001-1

LaPar, D.J., Laubach, V.E., Emaminia, A., Crosby, I.K., Hajzus, V.A., Sharma, A.K., Sumner, H.M., Webb, D. V, Lau, C.L., Kron, I.L., 2011. Pretreatment strategy with adenosine A2A receptor agonist attenuates reperfusion injury in a preclinical porcine lung transplantation model. J. Thorac. Cardiovasc. Surg. 142, 887–94. https://doi.org/10.1016/j.jtcvs.2011.06.015

Laurent, C., Burnouf, S., Ferry, B., Batalha, V.L., Coelho, J.E., Baqi, Y., Malik, E., Mariciniak, E., Parrot, S., Van der Jeugd, A., Faivre, E., Flaten, V., Ledent, C., D'Hooge, R., Sergeant, N., Hamdane, M., Humez, S., Müller, C.E., Lopes, L. V, Buée, L., Blum, D., 2016. A2A adenosine receptor deletion is protective in a mouse model of Tauopathy. Mol. Psychiatry 21, 97–107. https://doi.org/10.1038/mp.2014.151

Lee, H.-S., Chung, H.-J., Lee, H.W., Jeong, L.S., Lee, S.K., 2011. Suppression of inflammation response by a novel A3 adenosine receptor agonist thio-Cl-IB-MECA through inhibition of Akt and NF-κB signaling. Immunobiology 216, 997–1003. https://doi.org/10.1016/j.imbio.2011.03.008

León-Navarro, D.A., Albasanz, J.L., Martín, M., 2015. Hyperthermia-induced seizures alter adenosine A 1 and A 2A receptors and 5′-nucleotidase activity in rat cerebral cortex. J. Neurochem. 134, 395–404. https://doi.org/10.1111/jnc.13130

Li, L., Huang, L., Ye, H., Song, S.P., Bajwa, A., Lee, S.J., Moser, E.K., Jaworska, K., Kinsey, G.R., Day, Y.J., Linden, J., Lobo, P.I., Rosin, D.L., Okusa, M.D., 2012. Dendritic cells tolerized with adenosine A2AR agonist attenuate acute kidney injury. J. Clin. Invest. 122, 3931–3942. https://doi.org/10.1172/JCI63170

Li, W., Silva, H.B., Real, J., Wang, Y.-M., Rial, D., Li, P., Payen, M.-P., Zhou, Y., Muller, C.E., Tomé, A.R., Cunha, R.A., Chen, J.-F., 2015. Inactivation of adenosine A2A receptors reverses working memory deficits at early stages of Huntington's disease models. Neurobiol. Dis. 79, 70–80. https://doi.org/10.1016/j.nbd.2015.03.030

Linden, J., 2005. Adenosine in Tissue Protection and Tissue Regeneration. Mol. Pharmacol. 67, 1385–1387. https://doi.org/10.1124/mol.105.011783

Linden, J., Cekic, C., 2012. Regulation of Lymphocyte Function by Adenosine. Arterioscler. Thromb. Vasc. Biol. 32, 2097–2103. https://doi.org/10.1161/ATVBAHA.111.226837

Little, J.W., Ford, A., Symons-Liguori, A.M., Chen, Z., Janes, K., Doyle, T., Xie, J., Luongo, L., Tosh, D.K., Maione, S., Bannister, K., Dickenson, A.H., Vanderah, T.W., Porreca, F., Jacobson, K.A., Salvemini, D., 2015. Endogenous adenosine A3 receptor activation selectively alleviates persistent pain states. Brain 138, 28–35. https://doi.org/10.1093/brain/awu330

Loram, L.C., Taylor, F.R., Strand, K.A., Harrison, J.A., Rzasalynn, R., Sholar, P., Rieger, J., Maier, S.F., Watkins, L.R., 2013. Intrathecal injection of adenosine 2A receptor agonists reversed neuropathic allodynia through protein kinase (PK)A/PKC signaling. Brain. Behav. Immun. 33, 112–22. https://doi.org/10.1016/j.bbi.2013.06.004

Luongo, L., Guida, F., Imperatore, R., Napolitano, F., Gatta, L., Cristino, L., Giordano, C., Siniscalco, D., Di Marzo, V., Bellini, G., Petrelli, R., Cappellacci, L., Usiello, A., de Novellis, V., Rossi, F., Maione, S., 2014. The A1 adenosine receptor as a new player in microglia physiology. Glia 62, 122–132. https://doi.org/10.1002/glia.22592

Lusardi, T.A., Akula, K.K., Coffman, S.Q., Ruskin, D.N., Masino, S.A., Boison, D., 2015. Ketogenic diet prevents epileptogenesis and disease progression in adult mice and rats. Neuropharmacology 99, 500–509. https://doi.org/10.1016/j.neuropharm.2015.08.007

Machado, N.J., Simões, A.P., Silva, H.B., Ardais, A.P., Kaster, M.P., Garção, P., Rodrigues, D.I., Pochmann, D., Santos, A.I., Araújo, I.M., Porciúncula, L.O., Tomé, Â.R., Köfalvi, A., Vaugeois, J.-M., Agostinho, P., El Yacoubi, M., Cunha, R.A., Gomes, C.A., 2017. Caffeine Reverts Memory But Not Mood Impairment in a Depression-Prone Mouse Strain with Up-Regulated Adenosine A2A Receptor in Hippocampal Glutamate Synapses. Mol. Neurobiol. 54, 1552–1563. https://doi.org/10.1007/s12035-016-9774-9

Maia, L., de Mendonça, A., 2002. Does caffeine intake protect from Alzheimer's disease? Eur. J. Neurol. 9, 377–82.

Maksymowych, W.P., 2012. Biomarkers in spondyloarthritis: From pathophysiology to disease assessment. Jt. Bone Spine 79, 4–6. https://doi.org/10.1016/j.jbspin.2011.09.018

Mandili, G., Alchera, E., Merlin, S., Imarisio, C., Chandrashekar, B.R., Riganti, C., Bianchi, A., Novelli, F., Follenzi, A., Carini, R., 2015. Mouse hepatocytes and LSEC

proteome reveal novel mechanisms of ischemia/reperfusion damage and protection by A2aR stimulation. J. Hepatol. 62, 573–80. https://doi.org/10.1016/j.jhep.2014.10.007

Mantell, S., Jones, R., Trevethick, M., 2010. Design and application of locally delivered agonists of the adenosine A 2A receptor. Expert Rev. Clin. Pharmacol. 3, 55–72. https://doi.org/10.1586/ecp.09.57

Maraula, G., Traini, C., Mello, T., Coppi, E., Galli, A., Pedata, F., Pugliese, A.M., 2013. Effects of oxygen and glucose deprivation on synaptic transmission in rat dentate gyrus: Role of A2A adenosine receptors. Neuropharmacology 67, 511–520. https://doi.org/10.1016/j.neuropharm.2012.12.002

Martire, A., Pepponi, R., Domenici, M.R., Ferrante, A., Chiodi, V., Popoli, P., 2013. BDNF prevents NMDA-induced toxicity in models of Huntington's disease: the effects are genotype specific and adenosine A 2A receptor is involved. J. Neurochem. 125, 225–235. https://doi.org/10.1111/jnc.12177

Masino, S.A., Kawamura, M., Ruskin, D.N., 2014. Adenosine Receptors and Epilepsy, in: International Review of Neurobiology. pp. 233–255. https://doi.org/10.1016/B978-0-12-801022-8.00011-8

Masino, S.A., Kawamura, M., Ruskin, D.N., Geiger, J.D., Boison, D., 2012. Purines and neuronal excitability: Links to the ketogenic diet. Epilepsy Res. 100, 229–238. https://doi.org/10.1016/j.eplepsyres.2011.07.014

Masino, S.A., Li, T., Theofilas, P., Sandau, U.S., Ruskin, D.N., Fredholm, B.B., Geiger, J.D., Aronica, E., Boison, D., 2011. A ketogenic diet suppresses seizures in mice through adenosine A1 receptors. J. Clin. Invest. 121, 2679–2683. https://doi.org/10.1172/JCI57813

Matos, M., Augusto, E., Santos-Rodrigues, A. Dos, Schwarzschild, M.A., Chen, J.-F., Cunha, R.A., Agostinho, P., 2012. Adenosine A2A receptors modulate glutamate uptake in cultured astrocytes and gliosomes. Glia 60, 702–716. https://doi.org/10.1002/glia.22290

Mazzon, E., Esposito, E., Impellizzeri, D., DI Paola, R., Melani, A., Bramanti, P., Pedata, F., Cuzzocrea, S., 2011. CGS 21680, an agonist of the adenosine (A2A) receptor, reduces progression of murine type II collagen-induced arthritis. J. Rheumatol. 38, 2119–29. https://doi.org/10.3899/jrheum.110111

McColl, S.R., St-Onge, M., Dussault, A.-A., Laflamme, C., Bouchard, L., Boulanger, J., Pouliot, M., 2006. Immunomodulatory impact of the A2A adenosine receptor on the profile of chemokines produced by neutrophils. FASEB J. 20, 187–9. https://doi.org/10.1096/fj.05-4804fje

McInnes, I.B., Schett, G., 2011. The pathogenesis of rheumatoid arthritis. N. Engl. J. Med. 365, 2205–19. https://doi.org/10.1056/NEJMra1004965

McIntosh, V.J., Lasley, R.D., 2012. Adenosine Receptor-Mediated Cardioprotection. J. Cardiovasc. Pharmacol. Ther. 17, 21–33. https://doi.org/10.1177/1074248410396877

Mediero, A., Cronstein, B.N., 2013. Adenosine and bone metabolism. Trends Endocrinol. Metab. 24, 290–300. https://doi.org/10.1016/j.tem.2013.02.001

Mediero, A., Frenkel, S.R., Wilder, T., He, W., Mazumder, A., Cronstein, B.N., 2012. Adenosine A2A Receptor Activation Prevents Wear Particle-Induced Osteolysis. Sci. Transl. Med. 4, 135ra65-135ra65. https://doi.org/10.1126/scitranslmed.3003393

Mediero, A., Perez-Aso, M., Cronstein, B.N., 2013. Activation of adenosine A(2A) receptor reduces osteoclast formation via PKA- and ERK1/2-mediated suppression of NFκB nuclear translocation. Br. J. Pharmacol. 169, 1372–88. https://doi.org/10.1111/bph.12227

Mediero, A., Wilder, T., Perez-Aso, M., Cronstein, B.N., 2015. Direct or indirect stimulation of adenosine A2A receptors enhances bone regeneration as well as bone morphogenetic protein-2. FASEB J. 29, 1577–1590. https://doi.org/10.1096/fj.14-265066

Melani, A., Pugliese, A.M., Pedata, F., 2014. Adenosine Receptors in Cerebral Ischemia. pp. 309–348. https://doi.org/10.1016/B978-0-12-801022-8.00013-1

Merighi, S., Benini, A., Mirandola, P., Gessi, S., Varani, K., Simioni, C., Leung, E., Maclennan, S., Baraldi, P.G., Borea, P.A., 2007. Caffeine inhibits adenosine-induced accumulation of hypoxia-inducible factor-1alpha, vascular endothelial growth factor, and interleukin-8 expression in hypoxic human colon cancer cells. Mol. Pharmacol. 72, 395–406. https://doi.org/10.1124/mol.106.032920

Merighi, S., Borea, P.A., Gessi, S., 2015a. Adenosine receptors and diabetes: Focus on the A2B adenosine receptor subtype. Pharmacol. Res. 99, 229–236. https://doi.org/10.1016/j.phrs.2015.06.015

Merighi, S., Borea, P.A., Stefanelli, A., Bencivenni, S., Castillo, C.A., Varani, K., Gessi, S., 2015b. A2a and A2b adenosine receptors affect HIF-1α signaling in activated primary microglial cells. Glia 63, 1933–1952. https://doi.org/10.1002/glia.22861

Methner, C., Schmidt, K., Cohen, M. V., Downey, J.M., Krieg, T., 2010. Both A2a and A2b adenosine receptors at reperfusion are necessary to reduce infarct size in mouse hearts. AJP Hear. Circ. Physiol. 299, H1262–H1264. https://doi.org/10.1152/ajpheart.00181.2010

Minor, T.R., Hanff, T.C., 2015. Adenosine signaling in reserpine-induced depression in rats. Behav. Brain Res. 286, 184–91. https://doi.org/10.1016/j.bbr.2015.02.032

Mitchell, S., Vargas, J., Hoffmann, A., 2016. Signaling via the NFκB system. Wiley Interdiscip. Rev. Syst. Biol. Med. 8, 227–241. https://doi.org/10.1002/wsbm.1331

Mohsenin, A., Mi, T., Xia, Y., Kellems, R.E., Chen, J.-F., Blackburn, M.R., 2007. Genetic removal of the A2A adenosine receptor enhances pulmonary inflammation, mucin production, and angiogenesis in adenosine deaminase-deficient mice. Am. J. Physiol. Lung Cell. Mol. Physiol. 293, L753-61. https://doi.org/10.1152/ajplung.00187.2007

Monnet, D., Kadi, A., Izac, B., Lebrun, N., Letourneur, F., Zinovieva, E., Said-Nahal, R., Chiocchia, G., Breban, M., 2012. Association between the IL-1 family gene cluster and spondyloarthritis. Ann. Rheum. Dis. 71, 885–890. https://doi.org/10.1136/annrheumdis-2011-200439

Montesinos, M.C., Desai-Merchant, A., Cronstein, B.N., 2015. Promotion of Wound Healing by an Agonist of Adenosine A2A Receptor Is Dependent on Tissue Plasminogen Activator. Inflammation 38, 2036–2041. https://doi.org/10.1007/s10753-015-0184-3

Montesinos, M.C., Desai, A., Delano, D., Chen, J.-F., Fink, J.S., Jacobson, M.A., Cronstein, B.N., 2003. Adenosine A2A or A3 receptors are required for inhibition of inflammation by methotrexate and its analog MX-68. Arthritis Rheum. 48, 240–7. https://doi.org/10.1002/art.10712

Montesinos, M.C., Yap, J.S., Desai, A., Posadas, I., McCrary, C.T., Cronstein, B.N., 2000. Reversal of the antiinflammatory effects of methotrexate by the nonselective adenosine receptor antagonists theophylline and caffeine: evidence that the antiinflammatory effects of methotrexate are mediated via multiple adenosine receptors in rat adjuvant arthritis. Arthritis Rheum. 43, 656–63. https://doi.org/10.1002/1529-0131(200003)43:3<656::AID-ANR23>3.0.CO;2-H

Naganuma, M., Wiznerowicz, E.B., Lappas, C.M., Linden, J., Worthington, M.T., Ernst, P.B., 2006. Cutting edge: Critical role for A2A adenosine receptors in the T cell-mediated regulation of colitis. J. Immunol. 177, 2765–9.

Nascimento, F.P., Macedo-Júnior, S.J., Pamplona, F.A., Luiz-Cerutti, M., Córdova, M.M., Constantino, L., Tasca, C.I., Dutra, R.C., Calixto, J.B., Reid, A., Sawynok, J., Santos, A.R.S., 2015. Adenosine A1 Receptor-Dependent Antinociception Induced by Inosine in Mice: Pharmacological, Genetic and Biochemical Aspects. Mol. Neurobiol. 51, 1368–1378. https://doi.org/10.1007/s12035-014-8815-5

Navarro, G., Borroto-Escuela, D.O., Fuxe, K., Franco, R., 2016. Purinergic signaling in Parkinson's disease. Relevance for treatment. Neuropharmacology 104, 161–168. https://doi.org/10.1016/j.neuropharm.2015.07.024

Nazir, S.A., Khan, J.N., Mahmoud, I.Z., Greenwood, J.P., Blackman, D.J., Kunadian, V., Been, M., Abrams, K.R., Wilcox, R., Adgey, A.J., McCann, G.P., Gershlick, A.H., 2014. The REFLO-STEMI trial comparing intracoronary adenosine, sodium nitroprusside and standard therapy for the attenuation of infarct size and microvascular obstruction during primary percutaneous coronary intervention: study protocol for a randomised controlled trial. Trials 15, 371. https://doi.org/10.1186/1745-6215-15-371

Niccoli, G., Rigattieri, S., De Vita, M.R., Valgimigli, M., Corvo, P., Fabbiocchi, F., Romagnoli, E., De Caterina, A.R., La Torre, G., Lo Schiavo, P., Tarantino, F., Ferrari, R., Tomai, F., Olivares, P., Cosentino, N., D'Amario, D., Leone, A.M., Porto, I., Burzotta, F., Trani, C., Crea, F., 2013. Open-Label, Randomized, Placebo-Controlled Evaluation of Intracoronary Adenosine or Nitroprusside After Thrombus Aspiration During Primary Percutaneous Coronary Intervention for the Prevention of Microvascular Obstruction in Acute Myocardial Infarction. JACC Cardiovasc. Interv. 6, 580–589. https://doi.org/10.1016/j.jcin.2013.02.009

Niccoli, G., Spaziani, C., Crea, F., 2014. Left Ventricular Remodeling and 1-Year Clinical Follow-Up of the REOPEN-AMI Trial. J. Am. Coll. Cardiol. 63, 1454–1455. https://doi.org/10.1016/j.jacc.2013.10.042

Noort, A.R., Tak, P.P., Tas, S.W., 2015. Non-canonical NF-κB signaling in rheumatoid arthritis: Dr Jekyll and Mr Hyde? Arthritis Res. Ther. 17, 15. https://doi.org/10.1186/s13075-015-0527-3

Ntantie, E., Gonyo, P., Lorimer, E.L., Hauser, A.D., Schuld, N., McAllister, D., Kalyanaraman, B., Dwinell, M.B., Auchampach, J.A., Williams, C.L., 2013. An

adenosine-mediated signaling pathway suppresses prenylation of the GTPase Rap1B and promotes cell scattering. Sci. Signal. 6, ra39. https://doi.org/10.1126/scisignal.2003374

Ochaion, A., Bar-Yehuda, S., Cohen, S., Amital, H., Jacobson, K.A., Joshi, B. V, Gao, Z.G., Barer, F., Patoka, R., Del Valle, L., Perez-Liz, G., Fishman, P., 2008. The A3 adenosine receptor agonist CF502 inhibits the PI3K, PKB/Akt and NF-kappaB signaling pathway in synoviocytes from rheumatoid arthritis patients and in adjuvant-induced arthritis rats. Biochem. Pharmacol. 76, 482–94. https://doi.org/10.1016/j.bcp.2008.05.032

Ochaion, A., Bar-Yehuda, S., Cohen, S., Barer, F., Patoka, R., Amital, H., Reitblat, T., Reitblat, A., Ophir, J., Konfino, I., Chowers, Y., Ben-Horin, S., Fishman, P., 2009. The anti-inflammatory target A3 adenosine receptor is over-expressed in rheumatoid arthritis, psoriasis and Crohn's disease. Cell. Immunol. 258, 115–122. https://doi.org/10.1016/j.cellimm.2009.03.020

Ochaion, A., Bar-Yehuda, S., Cohn, S., Del Valle, L., Perez-Liz, G., Madi, L., Barer, F., Farbstein, M., Fishman-Furman, S., Reitblat, T., Reitblat, A., Amital, H., Levi, Y., Molad, Y., Mader, R., Tishler, M., Langevitz, P., Zabutti, A., Fishman, P., 2006. Methotrexate enhances the anti-inflammatory effect of CF101 via up-regulation of the A3 adenosine receptor expression. Arthritis Res. Ther. 8, R169. https://doi.org/10.1186/ar2078

Odashima, M., Bamias, G., Rivera-Nieves, J., Linden, J., Nast, C.C., Moskaluk, C.A., Marini, M., Sugawara, K., Kozaiwa, K., Otaka, M., Watanabe, S., Cominelli, F., 2005. Activation of A2A adenosine receptor attenuates intestinal inflammation in animal models of inflammatory bowel disease. Gastroenterology 129, 26–33.

Oertel, W., Schulz, J.B., 2016. Current and experimental treatments of Parkinson disease: A guide for neuroscientists. J. Neurochem. 139, 325–337. https://doi.org/10.1111/jnc.13750

Ohta, A., Sitkovsky, M., 2001. Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. Nature 414, 916–920. https://doi.org/10.1038/414916a

Ohta, A., Sitkovsky, M., 2009. The adenosinergic immunomodulatory drugs. Curr. Opin. Pharmacol. 9, 501–506. https://doi.org/10.1016/j.coph.2009.05.005

Ohta, A., Sitkovsky, M., 2014. Extracellular Adenosine-Mediated Modulation of Regulatory T Cells. Front. Immunol. 5, 304. https://doi.org/10.3389/fimmu.2014.00304

Orr, A.G., Hsiao, E.C., Wang, M.M., Ho, K., Kim, D.H., Wang, X., Guo, W., Kang, J., Yu, G.-Q., Adame, A., Devidze, N., Dubal, D.B., Masliah, E., Conklin, B.R., Mucke, L., 2015. Astrocytic adenosine receptor A2A and Gs-coupled signaling regulate memory. Nat. Neurosci. 18, 423–434. https://doi.org/10.1038/nn.3930

Otsuguro, K., Tomonari, Y., Otsuka, S., Yamaguchi, S., Kon, Y., Ito, S., 2015. An adenosine kinase inhibitor, ABT-702, inhibits spinal nociceptive transmission by adenosine release via equilibrative nucleoside transporters in rat. Neuropharmacology 97, 160–170. https://doi.org/10.1016/j.neuropharm.2015.05.035

Panther, E., Idzko, M., Herouy, Y., Rheinen, H., Gebicke-Haerter, P.J., Mrowietz, U., Dichmann, S., Norgauer, J., 2001. Expression and function of adenosine receptors in human dendritic cells. FASEB J. 15, 1963–70. https://doi.org/10.1096/fj.01-0169com

Patel, L., Thaker, A., 2014. The effects of adenosine A2B receptor inhibition on VEGF and nitric oxide axis-mediated renal function in diabetic nephropathy. Ren. Fail. 36, 916–24. https://doi.org/10.3109/0886022X.2014.900404

Patel, R.A.G., Glover, D.K., Broisat, A., Kabul, H.K., Ruiz, M., Goodman, N.C., Kramer, C.M., Meerdink, D.J., Linden, J., Beller, G.A., 2009. Reduction in myocardial infarct size at 48 hours after brief intravenous infusion of ATL-146e, a highly selective adenosine A2A receptor agonist. AJP Hear. Circ. Physiol. 297, H637–H642. https://doi.org/10.1152/ajpheart.00705.2008

Pedata, F., Dettori, I., Coppi, E., Melani, A., Fusco, I., Corradetti, R., Pugliese, A.M., 2016. Purinergic signalling in brain ischemia. Neuropharmacology 104, 105–30. https://doi.org/10.1016/j.neuropharm.2015.11.007

Pedata, F., Pugliese, A.M., Coppi, E., Dettori, I., Maraula, G., Cellai, L., Melani, A., 2014. Adenosine A2A Receptors Modulate Acute Injury and Neuroinflammation in Brain Ischemia. Mediators Inflamm. 2014, 1–16. https://doi.org/10.1155/2014/805198

Peleli, M., Hezel, M., Zollbrecht, C., Persson, A.E.G., Lundberg, J.O., Weitzberg, E., Fredholm, B.B., Carlström, M., 2015. In adenosine A2B knockouts acute treatment with inorganic nitrate improves glucose disposal, oxidative stress, and AMPK signaling in the liver. Front. Physiol. 6, 222. https://doi.org/10.3389/fphys.2015.00222

Peres, R.S., Liew, F.Y., Talbot, J., Carregaro, V., Oliveira, R.D., Almeida, S.L., França, R.F.O., Donate, P.B., Pinto, L.G., Ferreira, F.I.S., Costa, D.L., Demarque, D.P., Gouvea, D.R., Lopes, N.P., Queiroz, R.H.C., Silva, J.S., Figueiredo, F., Alves-Filho, J.C., Cunha, T.M., Ferreira, S.H., Louzada-Junior, P., Cunha, F.Q., 2015. Low expression of CD39 on regulatory T cells as a biomarker for resistance to methotrexate therapy in rheumatoid arthritis. Proc. Natl. Acad. Sci. 112, 2509–2514. https://doi.org/10.1073/pnas.1424792112

Perez-Aso, M., Feig, J.L., Aránzazu, M., Cronstein, B.N., Cronstein, B.N., 2013. Adenosine A2A Receptor and TNF-α Regulate the Circadian Machinery of the Human Monocytic THP-1 Cells. Inflammation 36, 152–162. https://doi.org/10.1007/s10753-012-9530-x

Perretti, M., Leroy, X., Bland, E.J., Montero-Melendez, T., 2015. Resolution Pharmacology: Opportunities for Therapeutic Innovation in Inflammation. Trends Pharmacol. Sci. 36, 737–755. https://doi.org/10.1016/j.tips.2015.07.007

Peterson, J.D., Goldberg, J.A., Surmeier, D.J., 2012. Adenosine A2a receptor antagonists attenuate striatal adaptations following dopamine depletion. Neurobiol. Dis. 45, 409–416. https://doi.org/10.1016/j.nbd.2011.08.030

Polosa, R., Blackburn, M.R., 2009. Adenosine receptors as targets for therapeutic intervention in asthma and chronic obstructive pulmonary disease. Trends Pharmacol. Sci. 30, 528–535. https://doi.org/10.1016/j.tips.2009.07.005

Poth, J.M., Brodsky, K., Ehrentraut, H., Grenz, A., Eltzschig, H.K., 2013. Transcriptional control of adenosine signaling by hypoxia-inducible transcription factors during ischemic or inflammatory disease. J. Mol. Med. (Berl). 91, 183–93. https://doi.org/10.1007/s00109-012-0988-7

Preti, D., Baraldi, P.G., Moorman, A.R., Borea, P.A., Varani, K., 2015. History and Perspectives of A2A Adenosine Receptor Antagonists as Potential Therapeutic Agents. Med. Res. Rev. 35, 790–848. https://doi.org/10.1002/med.21344

Prevoo, M.L., van't Hof, M.A., Kuper, H.H., van Leeuwen, M.A., van de Putte, L.B., van Riel, P.L., 1995. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum. 38, 44–8.

Quinn, M.A., Emery, P., n.d. Window of opportunity in early rheumatoid arthritis: possibility of altering the disease process with early intervention. Clin. Exp. Rheumatol. 21, S154-7.

Quiñonez-Flores, C.M., González-Chávez, S.A., Del Río Nájera, D., Pacheco-Tena, C., 2016. Oxidative Stress Relevance in the Pathogenesis of the Rheumatoid Arthritis: A Systematic Review. Biomed Res. Int. 2016, 1–14. https://doi.org/10.1155/2016/6097417

Quiroz, C., Gulyani, S., Ruiqian, W., Bonaventura, J., Cutler, R., Pearson, V., Allen, R.P., Earley, C.J., Mattson, M.P., Ferré, S., 2016. Adenosine receptors as markers of brain iron deficiency: Implications for Restless Legs Syndrome. Neuropharmacology 111, 160–168. https://doi.org/10.1016/j.neuropharm.2016.09.002

Ralph, J.A., McEvoy, A.N., Kane, D., Bresnihan, B., FitzGerald, O., Murphy, E.P., 2005. Modulation of orphan nuclear receptor NURR1 expression by methotrexate in human inflammatory joint disease involves adenosine A2A receptor-mediated responses. J. Immunol. 175, 555–65.

Rauck, R.L., North, J., Eisenach, J.C., 2015. Intrathecal clonidine and adenosine: effects on pain and sensory processing in patients with chronic regional pain syndrome. Pain 156, 88–95. https://doi.org/10.1016/j.pain.0000000000000007

Reeves, J.J., Harris, C.A., Hayes, B.P., Butchers, P.R., Sheehan, M.J., 2000. Studies on the effects of adenosine A3 receptor stimulation on human eosinophils isolated from non-asthmatic or asthmatic donors. Inflamm. Res. 49, 666–72. https://doi.org/10.1007/s000110050644

Ren, W., Tu, W., Jiang, S., Cheng, R., Du, Y., 2012. Electroacupuncture improves neuropathic pain: Adenosine, adenosine 5'-triphosphate disodium and their receptors perhaps change simultaneously. Neural Regen. Res. 7, 2618–23. https://doi.org/10.3969/j.issn.1673-5374.2012.33.007

Reveille, J.D., 2011. Epidemiology of spondyloarthritis in North America. Am. J. Med. Sci. 341, 284–6. https://doi.org/10.1097/MAJ.0b013e31820f8c99

Reveille, J.D., Weisman, M.H., 2013. The epidemiology of back pain, axial spondyloarthritis and HLA-B27 in the United States. Am. J. Med. Sci. 345, 431–6.

Rihl, M., Kellner, H., Kellner, W., Barthel, C., Yu, D.T.Y., Tak, P.P., Zeidler, H., Baeten, D., 2008. Identification of interleukin-7 as a candidate disease mediator in spondylarthritis. Arthritis Rheum. 58, 3430–3435. https://doi.org/10.1002/art.23998

Romagnoli, R., Baraldi, P.G., Carrion, M.D., Cara, C.L., Cruz-Lopez, O., Iaconinoto, M.A., Preti, D., Shryock, J.C., Moorman, A.R., Vincenzi, F., Varani, K., Andrea Borea, P., 2008. Synthesis and biological evaluation of 2-amino-3-(4-chlorobenzoyl)-4-[N-

(substituted) piperazin-1-yl]thiophenes as potent allosteric enhancers of the A1 adenosine receptor. J. Med. Chem. 51, 5875–9. https://doi.org/10.1021/jm800586p

Romagnoli, R., Baraldi, P.G., Tabrizi, M.A., Gessi, S., Borea, P.A., Merighi, S., 2010. Allosteric enhancers of A1 adenosine receptors: state of the art and new horizons for drug development. Curr. Med. Chem. 17, 3488–502.

Ross, G.W., Abbott, R.D., Petrovitch, H., Morens, D.M., Grandinetti, A., Tung, K.H., Tanner, C.M., Masaki, K.H., Blanchette, P.L., Curb, J.D., Popper, J.S., White, L.R., n.d. Association of coffee and caffeine intake with the risk of Parkinson disease. JAMA 283, 2674–9.

Roux, C.H., Saraux, A., Le Bihan, E., Fardellone, P., Guggenbuhl, P., Fautrel, B., Masson, C., Chary-Valckenaere, I., Cantagrel, A., Juvin, R., Flipo, R.-M., Euller-Ziegler, L., Coste, J., Guillemin, F., 2007. Rheumatoid arthritis and spondyloarthropathies: geographical variations in prevalence in France. J. Rheumatol. 34, 117–22.

Rudich, N., Dekel, O., Sagi-Eisenberg, R., 2015. Down-regulation of the A3 adenosine receptor in human mast cells upregulates mediators of angiogenesis and remodeling. Mol. Immunol. 65, 25–33. https://doi.org/10.1016/j.molimm.2014.12.015

Rudwaleit, M., Siegert, S., Yin, Z., Eick, J., Thiel, A., Radbruch, A., Sieper, J., Braun, J., 2001. Low T cell production of TNFalpha and IFNgamma in ankylosing spondylitis: its relation to HLA-B27 and influence of the TNF-308 gene polymorphism. Ann. Rheum. Dis. 60, 36–42.

Rudwaleit, M., van der Heijde, D., Landewe, R., Listing, J., Akkoc, N., Brandt, J., Braun, J., Chou, C.T., Collantes-Estevez, E., Dougados, M., Huang, F., Gu, J., Khan, M.A., Kirazli, Y., Maksymowych, W.P., Mielants, H., Sorensen, I.J., Ozgocmen, S., Roussou, E., Valle-Onate, R., Weber, U., Wei, J., Sieper, J., 2009. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. Ann. Rheum. Dis. 68, 777–783. https://doi.org/10.1136/ard.2009.108233

Rüsing, D., Müller, C.E., Verspohl, E.J., 2006. The impact of adenosine and A(2B) receptors on glucose homoeostasis. J. Pharm. Pharmacol. 58, 1639–45. https://doi.org/10.1211/jpp.58.12.0011

Ryzhov, S., Biktasova, A., Goldstein, A.E., Zhang, Q., Biaggioni, I., Dikov, M.M., Feoktistov, I., 2013. Role of JunB in Adenosine A2B Receptor-Mediated Vascular Endothelial Growth Factor Production. Mol. Pharmacol. 85, 62–73. https://doi.org/10.1124/mol.113.088567

Ryzhov, S., Goldstein, A.E., Novitskiy, S. V., Blackburn, M.R., Biaggioni, I., Feoktistov, I., 2012. Role of A2B Adenosine Receptors in Regulation of Paracrine Functions of Stem Cell Antigen 1-Positive Cardiac Stromal Cells. J. Pharmacol. Exp. Ther. 341, 764–774. https://doi.org/10.1124/jpet.111.190835

Ryzhov, S., Sung, B.H., Zhang, Q., Weaver, A., Gumina, R.J., Biaggioni, I., Feoktistov, I., 2014. Role of adenosine A2B receptor signaling in contribution of cardiac mesenchymal stem-like cells to myocardial scar formation. Purinergic Signal. 10, 477–486. https://doi.org/10.1007/s11302-014-9410-y

Ryzhov, S., Zaynagetdinov, R., Goldstein, A.E., Novitskiy, S. V, Blackburn, M.R., Biaggioni, I., Feoktistov, I., 2008. Effect of A2B adenosine receptor gene ablation on adenosine-dependent regulation of proinflammatory cytokines. J. Pharmacol. Exp. Ther. 324, 694–700. https://doi.org/10.1124/jpet.107.131540

Ryzhov, S., Zhang, Q., Biaggioni, I., Feoktistov, I., 2013. Adenosine A2B Receptors on Cardiac Stem Cell Antigen (Sca)-1–Positive Stromal Cells Play a Protective Role in Myocardial Infarction. Am. J. Pathol. 183, 665–672. https://doi.org/10.1016/j.ajpath.2013.05.012

Salvatore, C.A., Tilley, S.L., Latour, A.M., Fletcher, D.S., Koller, B.H., Jacobson, M.A., 2000. Disruption of the A(3) adenosine receptor gene in mice and its effect on stimulated inflammatory cells. J. Biol. Chem. 275, 4429–34.

Santos, C., Lunet, N., Azevedo, A., de Mendonça, A., Ritchie, K., Barros, H., 2010. Caffeine Intake is Associated with a Lower Risk of Cognitive Decline: A Cohort Study from Portugal. J. Alzheimer's Dis. 20, S175–S185. https://doi.org/10.3233/JAD-2010-091303

Saraux, A., Guillemin, F., Guggenbuhl, P., Roux, C.H., Fardellone, P., Le Bihan, E., Cantagrel, A., Chary-Valckenaere, I., Euller-Ziegler, L., Flipo, R.-M., Juvin, R., Behier, J.-M., Fautrel, B., Masson, C., Coste, J., 2005. Prevalence of spondyloarthropathies in France: 2001. Ann. Rheum. Dis. 64, 1431–5. https://doi.org/10.1136/ard.2004.029207

Sarkar, S., Fox, D.A., 2010. Targeting IL-17 and Th17 Cells in Rheumatoid Arthritis. Rheum. Dis. Clin. North Am. 36, 345–366. https://doi.org/10.1016/j.rdc.2010.02.006

Sawynok, J., 2016. Adenosine receptor targets for pain. Neuroscience 338, 1–18. https://doi.org/10.1016/j.neuroscience.2015.10.031

Sawynok, J., Reid, A.R., 2012. Caffeine inhibits antinociception by acetaminophen in the formalin test by inhibiting spinal adenosine A1 receptors. Eur. J. Pharmacol. 674, 248–254. https://doi.org/10.1016/j.ejphar.2011.10.036

Sawynok, J., Reid, A.R., Liu, J., 2013. Spinal and peripheral adenosine A1 receptors contribute to antinociception by tramadol in the formalin test in mice. Eur. J. Pharmacol. 714, 373–378. https://doi.org/10.1016/j.ejphar.2013.07.012

Schafer, P., 2012. Apremilast mechanism of action and application to psoriasis and psoriatic arthritis. Biochem. Pharmacol. 83, 1583–1590. https://doi.org/10.1016/j.bcp.2012.01.001

Schingnitz, U., Hartmann, K., MacManus, C.F., Eckle, T., Zug, S., Colgan, S.P., Eltzschig, H.K., 2010. Signaling through the A2B Adenosine Receptor Dampens Endotoxin-Induced Acute Lung Injury. J. Immunol. 184, 5271–5279. https://doi.org/10.4049/jimmunol.0903035

Schmidt, A., Böhmer, A., Antunes, C., Schallenberger, C., Porciúncula, L., Elisabetsky, E., Lara, D., Souza, D., 2009. Anti-nociceptive properties of the xanthine oxidase inhibitor allopurinol in mice: role of A 1 adenosine receptors. Br. J. Pharmacol. 156, 163–172. https://doi.org/10.1111/j.1476-5381.2008.00025.x

Schnurr, M., Toy, T., Shin, A., Hartmann, G., Rothenfusser, S., Soellner, J., Davis, I.D., Cebon, J., Maraskovsky, E., 2004. Role of adenosine receptors in regulating chemotaxis

and cytokine production of plasmacytoid dendritic cells. Blood 103, 1391–7. https://doi.org/10.1182/blood-2003-06-1959

Senolt, L., Vencovský, J., Pavelka, K., Ospelt, C., Gay, S., 2009. Prospective new biological therapies for rheumatoid arthritis. Autoimmun. Rev. 9, 102–7. https://doi.org/10.1016/j.autrev.2009.03.010

Seo, S., Koeppen, M., Bonney, S., Gobel, M., Thayer, M., Harter, P.N., Ravid, K., Eltzschig, H.K., Mittelbronn, M., Walker, L., Eckle, T., 2015. Differential Tissue-Specific Function of Adora2b in Cardioprotection. J. Immunol. 195, 1732–1743. https://doi.org/10.4049/jimmunol.1402288

Sevigny, C.P., Li, L., Awad, A.S., Huang, L., McDuffie, M., Linden, J., Lobo, P.I., Okusa, M.D., 2007. Activation of adenosine 2A receptors attenuates allograft rejection and alloantigen recognition. J. Immunol. 178, 4240–9.

Sieper, J., Rudwaleit, M., Khan, M.A., Braun, J., 2006. Concepts and epidemiology of spondyloarthritis. Best Pract. Res. Clin. Rheumatol. 20, 401–17. https://doi.org/10.1016/j.berh.2006.02.001

Silverman, M.H., Strand, V., Markovits, D., Nahir, M., Reitblat, T., Molad, Y., Rosner, I., Rozenbaum, M., Mader, R., Adawi, M., Caspi, D., Tishler, M., Langevitz, P., Rubinow, A., Friedman, J., Green, L., Tanay, A., Ochaion, A., Cohen, S., Kerns, W.D., Cohn, I., Fishman-Furman, S., Farbstein, M., Yehuda, S.B., Fishman, P., 2008. Clinical evidence for utilization of the A3 adenosine receptor as a target to treat rheumatoid arthritis: data from a phase II clinical trial. J. Rheumatol. 35, 41–8.

Sitkovsky, M. V., 2009. T regulatory cells: hypoxia-adenosinergic suppression and re-direction of the immune response. Trends Immunol. 30, 102–108. https://doi.org/10.1016/j.it.2008.12.002

Sitkovsky, M. V, Lukashev, D., Apasov, S., Kojima, H., Koshiba, M., Caldwell, C., Ohta, A., Thiel, M., 2004. Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. Annu. Rev. Immunol. 22, 657–82. https://doi.org/10.1146/annurev.immunol.22.012703.104731

Smith, A.P., 2009. Caffeine, cognitive failures and health in a non-working community sample. Hum. Psychopharmacol. Clin. Exp. 24, 29–34. https://doi.org/10.1002/hup.991

Smolen, J.S., Aletaha, D., Bijlsma, J.W.J., Breedveld, F.C., Boumpas, D., Burmester, G., Combe, B., Cutolo, M., de Wit, M., Dougados, M., Emery, P., Gibofsky, A., Gomez-Reino, J.J., Haraoui, B., Kalden, J., Keystone, E.C., Kvien, T.K., McInnes, I., Martin-Mola, E., Montecucco, C., Schoels, M., van der Heijde, D., van der Heijde, D., T2T Expert Committee, 2010a. Treating rheumatoid arthritis to target: recommendations of an international task force. Ann. Rheum. Dis. 69, 631–637. https://doi.org/10.1136/ard.2009.123919

Smolen, J.S., Landewe, R., Breedveld, F.C., Dougados, M., Emery, P., Gaujoux-Viala, C., Gorter, S., Knevel, R., Nam, J., Schoels, M., Aletaha, D., Buch, M., Gossec, L., Huizinga, T., Bijlsma, J.W.J.W., Burmester, G., Combe, B., Cutolo, M., Gabay, C., Gomez-Reino, J., Kouloumas, M., Kvien, T.K., Martin-Mola, E., McInnes, I., Pavelka, K., van Riel, P., Scholte, M., Scott, D.L., Sokka, T., Valesini, G., van Vollenhoven, R., Winthrop, K.L., Wong, J., Zink, A., van der Heijde, D., 2010b. EULAR recommendations for the

management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. Ann. Rheum. Dis. 69, 964–975. https://doi.org/10.1136/ard.2009.126532

Sokka, T., n.d. Increases in use of methotrexate since the 1980s. Clin. Exp. Rheumatol. 28, S13-20.

Soriano, A., Ventura, R., Molero, A., Hoen, R., Casadó, V., Cortés, A., Fanelli, F., Albericio, F., Lluís, C., Franco, R., Royo, M., 2009. Adenosine A2A receptor-antagonist/dopamine D2 receptor-agonist bivalent ligands as pharmacological tools to detect A2A-D2 receptor heteromers. J. Med. Chem. 52, 5590–602. https://doi.org/10.1021/jm900298c

Sowa, N.A., Street, S.E., Vihko, P., Zylka, M.J., 2010a. Prostatic Acid Phosphatase Reduces Thermal Sensitivity and Chronic Pain Sensitization by Depleting Phosphatidylinositol 4,5-Bisphosphate. J. Neurosci. 30, 10282–10293. https://doi.org/10.1523/JNEUROSCI.2162-10.2010

Sowa, N.A., Voss, M.K., Zylka, M.J., 2010b. Recombinant ecto-5'-nucleotidase (CD73) has long lasting antinociceptive effects that are dependent on adenosine A1 receptor activation. Mol. Pain 6, 20. https://doi.org/10.1186/1744-8069-6-20

Squadrito, F., Bitto, A., Altavilla, D., Arcoraci, V., De Caridi, G., De Feo, M.E., Corrao, S., Pallio, G., Sterrantino, C., Minutoli, L., Saitta, A., Vaccaro, M., Cucinotta, D., 2014. The effect of PDRN, an adenosine receptor A2A agonist, on the healing of chronic diabetic foot ulcers: results of a clinical trial. J. Clin. Endocrinol. Metab. 99, E746-53. https://doi.org/10.1210/jc.2013-3569

Sritheran, D., Leung, Y.Y., 2015. Making the next steps in psoriatic arthritis management: current status and future directions. Ther. Adv. Musculoskelet. Dis. 7, 173–186. https://doi.org/10.1177/1759720X15595966

Stamp, L.K., Hazlett, J., Roberts, R.L., Frampton, C., Highton, J., Hessian, P.A., 2012. Adenosine receptor expression in rheumatoid synovium: a basis for methotrexate action. Arthritis Res. Ther. 14, R138. https://doi.org/10.1186/ar3871

Stemmer, S.M., Benjaminov, O., Medalia, G., Ciuraru, N.B., Silverman, M.H., Bar-Yehuda, S., Fishman, S., Harpaz, Z., Farbstein, M., Cohen, S., Patoka, R., Singer, B., Kerns, W.D., Fishman, P., 2013. CF102 for the treatment of hepatocellular carcinoma: a phase I/II, open-label, dose-escalation study. Oncologist 18, 25–6. https://doi.org/10.1634/theoncologist.2012-0211

Sun, C.-X., Zhong, H., Mohsenin, A., Morschl, E., Chunn, J.L., Molina, J.G., Belardinelli, L., Zeng, D., Blackburn, M.R., 2006. Role of A2B adenosine receptor signaling in adenosine-dependent pulmonary inflammation and injury. J. Clin. Invest. 116, 2173–2182. https://doi.org/10.1172/JCI27303

Suzuki, A., Yamada, R., Chang, X., Tokuhiro, S., Sawada, T., Suzuki, M., Nagasaki, M., Nakayama-Hamada, M., Kawaida, R., Ono, M., Ohtsuki, M., Furukawa, H., Yoshino, S., Yukioka, M., Tohma, S., Matsubara, T., Wakitani, S., Teshima, R., Nishioka, Y., Sekine, A., Iida, A., Takahashi, A., Tsunoda, T., Nakamura, Y., Yamamoto, K., 2003. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are

associated with rheumatoid arthritis. Nat. Genet. 34, 395–402. https://doi.org/10.1038/ng1206

Świerkot, J., Sokolik, R., Czarny, A., Zaczyńska, E., Nowak, B., Chlebicki, A., Korman, L., Madej, M., Wojtala, P., Lubiński, Ł., Wiland, P., 2015. Activity of JAK/STAT and NF-kB in patients with axial spondyloarthritis. Postepy Hig. Med. Dosw. (Online) 69, 1291–8.

Takedachi, M., Oohara, H., Smith, B.J., Iyama, M., Kobashi, M., Maeda, K., Long, C.L., Humphrey, M.B., Stoecker, B.J., Toyosawa, S., Thompson, L.F., Murakami, S., 2012. CD73-generated adenosine promotes osteoblast differentiation. J. Cell. Physiol. 227, 2622–2631. https://doi.org/10.1002/jcp.23001

Taylor, W.J., Robinson, P.C., 2013. Classification Criteria: Peripheral Spondyloarthropathy and Psoriatic Arthritis. Curr. Rheumatol. Rep. 15, 317. https://doi.org/10.1007/s11926-013-0317-3

Theofilas, P., Brar, S., Stewart, K.-A., Shen, H.-Y., Sandau, U.S., Poulsen, D., Boison, D., 2011. Adenosine kinase as a target for therapeutic antisense strategies in epilepsy. Epilepsia 52, 589–601. https://doi.org/10.1111/j.1528-1167.2010.02947.x

Tian, Y., Marshall, M., French, B.A., Linden, J., Yang, Z., 2015. The infarct-sparing effect of IB-MECA against myocardial ischemia/reperfusion injury in mice is mediated by sequential activation of adenosine A3 and A2A receptors. Basic Res. Cardiol. 110, 16. https://doi.org/10.1007/s00395-015-0473-x

Tosh, D.K., Padia, J., Salvemini, D., Jacobson, K.A., 2015. Efficient, large-scale synthesis and preclinical studies of MRS5698, a highly selective A3 adenosine receptor agonist that protects against chronic neuropathic pain. Purinergic Signal. 11, 371–387. https://doi.org/10.1007/s11302-015-9459-2

Trevethick, M.A., Mantell, S.J., Stuart, E.F., Barnard, A., Wright, K.N., Yeadon, M., 2008. Treating lung inflammation with agonists of the adenosine A2A receptor: promises, problems and potential solutions. Br. J. Pharmacol. 155, 463–74. https://doi.org/10.1038/bjp.2008.329

Trifilieff, P., Rives, M.-L., Urizar, E., Piskorowski, R., Vishwasrao, H., Castrillon, J., Schmauss, C., Slättman, M., Gullberg, M., Javitch, J., 2011. Detection of antigen interactions ex vivo by proximity ligation assay: endogenous dopamine D2-adenosine A2A receptor complexes in the striatum. Biotechniques 51, 111–8. https://doi.org/10.2144/000113719

Trueblood, K.E., Mohr, S., Dubyak, G.R., 2011. Purinergic regulation of high-glucose-induced caspase-1 activation in the rat retinal Muller cell line rMC-1. AJP Cell Physiol. 301, C1213–C1223. https://doi.org/10.1152/ajpcell.00265.2011

Tyebji, S., Saavedra, A., Canas, P.M., Pliassova, A., Delgado-García, J.M., Alberch, J., Cunha, R.A., Gruart, A., Pérez-Navarro, E., 2015. Hyperactivation of D1 and A2A receptors contributes to cognitive dysfunction in Huntington's disease. Neurobiol. Dis. 74, 41–57. https://doi.org/10.1016/j.nbd.2014.11.004

Vallon, V., Osswald, H., 2009. Adenosine Receptors and the Kidney, in: Handbook of Experimental Pharmacology. pp. 443–470. https://doi.org/10.1007/978-3-540-89615-9_15

van der Helm-van Mil, A.H.M., Verpoort, K.N., Breedveld, F.C., Huizinga, T.W.J., Toes, R.E.M., de Vries, R.R.P., 2006. The HLA–DRB1 shared epitope alleles are primarily a risk factor for anti–cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. Arthritis Rheum. 54, 1117–1121. https://doi.org/10.1002/art.21739

van der Hoeven, D., Wan, T.C., Auchampach, J.A., 2008. Activation of the A3 Adenosine Receptor Suppresses Superoxide Production and Chemotaxis of Mouse Bone Marrow Neutrophils. Mol. Pharmacol. 74, 685–696. https://doi.org/10.1124/mol.108.048066

van der Linden, S.M., Valkenburg, H.A., de Jongh, B.M., Cats, A., 1984. The risk of developing ankylosing spondylitis in HLA-B27 positive individuals. A comparison of relatives of spondylitis patients with the general population. Arthritis Rheum. 27, 241–9.

van Hulst, L.T.C., Hulscher, M.E.J.L., van Riel, P.L.C.M., 2011. Achieving tight control in rheumatoid arthritis. Rheumatology (Oxford). 50, 1729–31. https://doi.org/10.1093/rheumatology/ker325

Vandooren, B., Yeremenko, N., Noordenbos, T., Bras, J., Tak, P.P., Baeten, D., 2009. Mediators of structural remodeling in peripheral spondylarthritis. Arthritis Rheum. 60, 3534–45. https://doi.org/10.1002/art.27251

Varani, K., Abbracchio, M.P., Cannella, M., Cislaghi, G., Giallonardo, P., Mariotti, C., Cattabriga, E., Cattabeni, F., Borea, P.A., Squitieri, F., Cattaneo, E., 2003a. Aberrant A2A receptor function in peripheral blood cells in Huntington's disease. FASEB J. 17, 2148–50. https://doi.org/10.1096/fj.03-0079fje

Varani, K., Bachoud-Lévi, A.-C., Mariotti, C., Tarditi, A., Abbracchio, M.P., Gasperi, V., Borea, P.A., Dolbeau, G., Gellera, C., Solari, A., Rosser, A., Naji, J., Handley, O., Maccarrone, M., Peschanski, M., DiDonato, S., Cattaneo, E., 2007. Biological abnormalities of peripheral A(2A) receptors in a large representation of polyglutamine disorders and Huntington's disease stages. Neurobiol. Dis. 27, 36–43. https://doi.org/10.1016/j.nbd.2007.03.011

Varani, K., Caramori, G., Vincenzi, F., Adcock, I., Casolari, P., Leung, E., Maclennan, S., Gessi, S., Morello, S., Barnes, P.J., Ito, K., Chung, K.F., Cavallesco, G., Azzena, G., Papi, A., Borea, P.A., 2006. Alteration of adenosine receptors in patients with chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. 173, 398–406. https://doi.org/10.1164/rccm.200506-869OC

Varani, K., Caramori, G., Vincenzi, F., Tosi, A., Barczyk, A., Contoli, M., Casolari, P., Triggiani, M., Hansel, T., Leung, E., MacLennan, S., Barnes, P.J., Chung, K.F., Adcock, I., Papi, A., Borea, P.A., 2010a. Oxidative/nitrosative stress selectively altered A2B adenosine receptors in chronic obstructive pulmonary disease. FASEB J. 24, 1192–1204. https://doi.org/10.1096/fj.09-139485

Varani, K., Laghi-Pasini, F., Camurri, A., Capecchi, P.L., Maccherini, M., Diciolla, F., Ceccatelli, L., Lazzerini, P.E., Ulouglu, C., Cattabeni, F., Borea, P.A., Abbracchio, M.P., 2003b. Changes of peripheral A2A adenosine receptors in chronic heart failure and cardiac transplantation. FASEB J. 17, 280–2. https://doi.org/10.1096/fj.02-0543fje

Varani, K., Maniero, S., Vincenzi, F., Targa, M., Stefanelli, A., Maniscalco, P., Martini, F., Tognon, M., Borea, P.A., 2011a. A3 Receptors Are Overexpressed in Pleura from Patients

with Mesothelioma and Reduce Cell Growth via Akt/Nuclear Factor-κB Pathway. Am. J. Respir. Crit. Care Med. 183, 522–530. https://doi.org/10.1164/rccm.201006-0980OC

Varani, K., Massara, A., Vincenzi, F., Tosi, A., Padovan, M., Trotta, F., Borea, P.A., 2009. Normalization of A2A and A3 adenosine receptor up-regulation in rheumatoid arthritis patients by treatment with anti-tumor necrosis factor α but not methotrexate. Arthritis Rheum. 60, 2880–2891. https://doi.org/10.1002/art.24794

Varani, K., Padovan, M., Govoni, M., Vincenzi, F., Trotta, F., Borea, P.A., 2010b. The role of adenosine receptors in rheumatoid arthritis. Autoimmun. Rev. 10, 61–64. https://doi.org/10.1016/j.autrev.2010.07.019

Varani, K., Padovan, M., Vincenzi, F., Targa, M., Trotta, F., Govoni, M., Borea, P., 2011b. A2A and A3 adenosine receptor expression in rheumatoid arthritis: upregulation, inverse correlation with disease activity score and suppression of inflammatory cytokine and metalloproteinase release. Arthritis Res. Ther. 13, R197. https://doi.org/10.1186/ar3527

Varani, K., Vincenzi, F., Targa, M., Paradiso, B., Parrilli, A., Fini, M., Lanza, G., Borea, P.A., 2013. The stimulation of A3 adenosine receptors reduces bone-residing breast cancer in a rat preclinical model. Eur. J. Cancer 49, 482–491. https://doi.org/10.1016/j.ejca.2012.06.005

Varani, K., Vincenzi, F., Tosi, A., Gessi, S., Casetta, I., Granieri, G., Fazio, P., Leung, E., MacLennan, S., Granieri, E., Borea, P.A., 2010c. A2A adenosine receptor overexpression and functionality, as well as TNF-alpha levels, correlate with motor symptoms in Parkinson's disease. FASEB J. 24, 587–98. https://doi.org/10.1096/fj.09-141044

Varani, K., Vincenzi, F., Tosi, A., Targa, M., Masieri, F., Ongaro, A., De Mattei, M., Massari, L., Borea, P., 2010d. Expression and functional role of adenosine receptors in regulating inflammatory responses in human synoviocytes. Br. J. Pharmacol. 160, 101–115. https://doi.org/10.1111/j.1476-5381.2010.00667.x

Venken, K., Elewaut, D., 2015. New immune cells in spondyloarthritis: Key players or innocent bystanders? Best Pract. Res. Clin. Rheumatol. 29, 706–14. https://doi.org/10.1016/j.berh.2016.02.002

Viana da Silva, S., Haberl, M.G., Zhang, P., Bethge, P., Lemos, C., Gonçalves, N., Gorlewicz, A., Malezieux, M., Gonçalves, F.Q., Grosjean, N., Blanchet, C., Frick, A., Nägerl, U.V., Cunha, R.A., Mulle, C., 2016. Early synaptic deficits in the APP/PS1 mouse model of Alzheimer's disease involve neuronal adenosine A2A receptors. Nat. Commun. 7, 11915. https://doi.org/10.1038/ncomms11915

Vincenzi, F., Corciulo, C., Targa, M., Casetta, I., Gentile, M., Granieri, E., Borea, P.A., Popoli, P., Varani, K., 2013a. A2A adenosine receptors are up-regulated in lymphocytes from amyotrophic lateral sclerosis patients. Amyotroph. Lateral Scler. Front. Degener. 14, 406–413. https://doi.org/10.3109/21678421.2013.793358

Vincenzi, F., Corciulo, C., Targa, M., Merighi, S., Gessi, S., Casetta, I., Gentile, M., Granieri, E., Borea, P.A., Varani, K., 2013b. Multiple sclerosis lymphocytes upregulate A 2A adenosine receptors that are antiinflammatory when stimulated. Eur. J. Immunol. 43, 2206–2216. https://doi.org/10.1002/eji.201343314

Vincenzi, F., Padovan, M., Targa, M., Corciulo, C., Giacuzzo, S., Merighi, S., Gessi, S., Govoni, M., Borea, P.A., Varani, K., 2013c. A2A Adenosine Receptors Are Differentially Modulated by Pharmacological Treatments in Rheumatoid Arthritis Patients and Their Stimulation Ameliorates Adjuvant-Induced Arthritis in Rats. PLoS One 8, e54195. https://doi.org/10.1371/journal.pone.0054195

Vincenzi, F., Targa, M., Corciulo, C., Gessi, S., Merighi, S., Setti, S., Cadossi, R., Goldring, M.B., Borea, P.A., Varani, K., 2013d. Pulsed Electromagnetic Fields Increased the Anti-Inflammatory Effect of A2A and A3 Adenosine Receptors in Human T/C-28a2 Chondrocytes and hFOB 1.19 Osteoblasts. PLoS One 8, e65561. https://doi.org/10.1371/journal.pone.0065561

Vincenzi, F., Targa, M., Romagnoli, R., Merighi, S., Gessi, S., Baraldi, P.G., Borea, P.A., Varani, K., 2014. TRR469, a potent A(1) adenosine receptor allosteric modulator, exhibits anti-nociceptive properties in acute and neuropathic pain models in mice. Neuropharmacology 81, 6–14. https://doi.org/10.1016/j.neuropharm.2014.01.028

Vindeirinho, J., Santiago, A.R., Cavadas, C., Ambrósio, A.F., Santos, P.F., 2016. The Adenosinergic System in Diabetic Retinopathy. J. Diabetes Res. 2016, 1–8. https://doi.org/10.1155/2016/4270301

Wallace, K.L., Linden, J., 2010. Adenosine A2A receptors induced on iNKT and NK cells reduce pulmonary inflammation and injury in mice with sickle cell disease. Blood 116, 5010–5020. https://doi.org/10.1182/blood-2010-06-290643

Wan, T.C., Tosh, D.K., Du, L., Gizewski, E.T., Jacobson, K.A., Auchampach, J.A., 2011. Polyamidoamine (PAMAM) dendrimer conjugate specifically activates the A3 adenosine receptor to improve post-ischemic/reperfusion function in isolated mouse hearts. BMC Pharmacol. 11, 11. https://doi.org/10.1186/1471-2210-11-11

Wang, J.-Y., Li, H., Zhang, L., Ma, C.-M., Wang, J.-L., Lai, X.-S., Zhou, S.-F., 2014. Adenosine as a probing tool for the mechanistic study of acupuncture treatment. Clin. Exp. Pharmacol. Physiol. 41, 933–939. https://doi.org/10.1111/1440-1681.12304

Williams-Karnesky, R.L., Sandau, U.S., Lusardi, T.A., Lytle, N.K., Farrell, J.M., Pritchard, E.M., Kaplan, D.L., Boison, D., 2013. Epigenetic changes induced by adenosine augmentation therapy prevent epileptogenesis. J. Clin. Invest. 123, 3552–63. https://doi.org/10.1172/JCI65636

Wilson, C.N., Nadeem, A., Spina, D., Brown, R., Page, C.P., Mustafa, S.J., 2009. Adenosine Receptors and Asthma. pp. 329–362. https://doi.org/10.1007/978-3-540-89615-9_11

Woods, L.T., Ajit, D., Camden, J.M., Erb, L., Weisman, G.A., 2016. Purinergic receptors as potential therapeutic targets in Alzheimer's disease. Neuropharmacology 104, 169–179. https://doi.org/10.1016/j.neuropharm.2015.10.031

Xu, K., Di Luca, D.G., Orrú, M., Xu, Y., Chen, J.-F., Schwarzschild, M.A., 2016. Neuroprotection by caffeine in the MPTP model of parkinson's disease and its dependence on adenosine A2A receptors. Neuroscience 322, 129–137. https://doi.org/10.1016/j.neuroscience.2016.02.035

Yamada, K., Kobayashi, M., Kanda, T., 2014a. Involvement of Adenosine A2A Receptors in Depression and Anxiety, in: International Review of Neurobiology. pp. 373–393. https://doi.org/10.1016/B978-0-12-801022-8.00015-5

Yamada, K., Kobayashi, M., Shiozaki, S., Ohta, T., Mori, A., Jenner, P., Kanda, T., 2014b. Antidepressant activity of the adenosine A2A receptor antagonist, istradefylline (KW-6002) on learned helplessness in rats. Psychopharmacology (Berl). 231, 2839–2849. https://doi.org/10.1007/s00213-014-3454-0

Yang, T., Gao, X., Sandberg, M., Zollbrecht, C., Zhang, X.-M., Hezel, M., Liu, M., Peleli, M., Lai, E.-Y., Harris, R.A., Persson, A.E.G., Fredholm, B.B., Jansson, L., Carlström, M., 2015. Abrogation of adenosine A1 receptor signalling improves metabolic regulation in mice by modulating oxidative stress and inflammatory responses. Diabetologia 58, 1610–1620. https://doi.org/10.1007/s00125-015-3570-3

Yasuda, N., Inoue, T., Horizoe, T., Nagata, K., Minami, H., Kawata, T., Hoshino, Y., Harada, H., Yoshikawa, S., Asano, O., Nagaoka, J., Murakami, M., Abe, S., Kobayashi, S., Tanaka, I., 2003. Functional characterization of the adenosine receptor contributing to glycogenolysis and gluconeogenesis in rat hepatocytes. Eur. J. Pharmacol. 459, 159–66.

Yoon, M.H., Bae, H.B., Choi, J. Il, Kim, S.J., Chung, S.T., Kim, C.M., 2006. Roles of adenosine receptor subtypes in the antinociceptive effect of intrathecal adenosine in a rat formalin test. Pharmacology 78, 21–6. https://doi.org/10.1159/000094762

Young, H.W.J., Molina, J.G., Dimina, D., Zhong, H., Jacobson, M., Chan, L.-N.L., Chan, T.-S., Lee, J.J., Blackburn, M.R., 2004. A3 adenosine receptor signaling contributes to airway inflammation and mucus production in adenosine deaminase-deficient mice. J. Immunol. 173, 1380–9.

Zaynagetdinov, R., Ryzhov, S., Goldstein, A.E., Yin, H., Novitskiy, S. V, Goleniewska, K., Polosukhin, V. V, Newcomb, D.C., Mitchell, D., Morschl, E., Zhou, Y., Blackburn, M.R., Peebles, R.S., Biaggioni, I., Feoktistov, I., 2010. Attenuation of chronic pulmonary inflammation in A2B adenosine receptor knockout mice. Am. J. Respir. Cell Mol. Biol. 42, 564–71. https://doi.org/10.1165/rcmb.2008-0391OC

Zhai, P., Sciarretta, S., Galeotti, J., Volpe, M., Sadoshima, J., 2011. Differential Roles of GSK-3 During Myocardial Ischemia and Ischemia/Reperfusion. Circ. Res. 109, 502–511. https://doi.org/10.1161/CIRCRESAHA.111.249532

Zhou, Y., Mohsenin, A., Morschl, E., Young, H.W.J., Molina, J.G., Ma, W., Sun, C.-X., Martinez-Valdez, H., Blackburn, M.R., 2009. Enhanced airway inflammation and remodeling in adenosine deaminase-deficient mice lacking the A2B adenosine receptor. J. Immunol. 182, 8037–46. https://doi.org/10.4049/jimmunol.0900515

Zhou, Y., Murthy, J.N., Zeng, D., Belardinelli, L., Blackburn, M.R., 2010. Alterations in Adenosine Metabolism and Signaling in Patients with Chronic Obstructive Pulmonary Disease and Idiopathic Pulmonary Fibrosis. PLoS One 5, e9224. https://doi.org/10.1371/journal.pone.0009224

Zimmerman, M.A., Grenz, A., Tak, E., Kaplan, M., Ridyard, D., Brodsky, K.S., Mandell, M.S., Kam, I., Eltzschig, H.K., 2013. Signaling through hepatocellular A2B adenosine receptors dampens ischemia and reperfusion injury of the liver. Proc. Natl. Acad. Sci. 110, 12012–12017. https://doi.org/10.1073/pnas.1221733110

Zochling, J., van der Heijde, D., Burgos-Vargas, R., Collantes, E., Davis, J.C., Dijkmans, B., Dougados, M., Géher, P., Inman, R.D., Khan, M.A., Kvien, T.K., Leirisalo-Repo, M., Olivieri, I., Pavelka, K., Sieper, J., Stucki, G., Sturrock, R.D., van der Linden, S., Wendling, D., Böhm, H., van Royen, B.J., Braun, J., 'ASsessment in AS' international working group, European League Against Rheumatism, 2006. ASAS/EULAR recommendations for the management of ankylosing spondylitis. Ann. Rheum. Dis. 65, 442–52. https://doi.org/10.1136/ard.2005.041137

Zylka, M.J., 2011. Pain-relieving prospects for adenosine receptors and ectonucleotidases. Trends Mol. Med. 17, 188–196. https://doi.org/10.1016/j.molmed.2010.12.006

CURRICULUM VITAE

Date of birth: 20th October 1988
e-mail: annalisa.ravani@gmail.com

Education:

- Scholarship period from April 2012 to September 2012 at the Karolinska Institutet, Department of Neurosciences, Stockholm, Sweden;
- Degree on 18th October 2012 in Pharmacy, at the University of Ferrara (100/110). Thesis entitled: "The D2R/NTS1 heteromer and schizophrenia: a possibile new target for antipsychotic drugs", tutor Prof. Luca Ferraro;
- Temporary research associate from June 2014 to May 2017 at the University of Ferrara, Department of Medical Science, Section of Pharmacology;
- PhD student from November 2014 to October 2017 in Molecular Medicine and Pharmacology, Department of Medical Sciences, Pharmacology section, University of Ferrara, tutor Prof. Katia Varani.

Publications:

Borroto-Escuela DO, **Ravani A**, Tarakanov AO , Brito I, Narvaez M, Romero-Fernandez W, Corrales F, Agnati LF, Tanganelli S, Ferraro L, Fuxe K. "Dopamine D2 receptor signaling dynamics of dopamine D2-neurotensin 1 receptor heteromers." (2013) Biochemical and Biophysical Research Communications, 435, 140–146.

Ferraro L, Beggiato S, Borroto-Escuela DO, **Ravani A**, O'Connor WT, Tomasini MC, Borelli AC, Agnati LF, Antonelli T, Tanganelli S, Fuxe K. "Neurotensin NTS1-Dopamine D2 Receptor-Receptor Interactions in Putative Receptor Heteromers:Relevance for Parkinson's Disease and Schizophrenia." (2014) Curr Protein Pept Sci.

Preti D, Baraldi PG, Saponaro G, Romagnoli R, Aghazadeh Tabrizi M, Baraldi S, Cosconati S, Bruno A, Novellino E, Vincenzi F, **Ravani A**, Borea PA, Varani K. "Design, synthesis, and biological evaluation of novel 2-((2-(4-(substituted)phenylpiperazin-1-yl)ethyl)amino)-5'-N-ethylcarboxamidoaden osines as potent and selective agonists of the A2A adenosine receptor." (2015) J Med Chem, 58:3253-67.

Varano F, Catarzi D, Squarcialupi L, Betti M, Vincenzi F, **Ravani A**, Varani K, Dal Ben D, Thomas A, Volpini R, Colotta V. "Exploring the 7-oxo-thiazolo[5,4-d]pyrimidine core for the design of new human adenosine A3 receptor antagonists. Synthesis, molecular modeling studies and pharmacological evaluation." (2015) Eur J Med Chem, 96:105-21.

Squarcialupi L, Catarzi D, Varano F, Betti M, Falsini M, Vincenzi F, **Ravani A**, Ciancetta A, Varani K, Moro S, Colotta V. "Structural refinement of pyrazolo[4,3-d]pyrimidine derivatives to obtain highly potent and selective antagonists for the human A(3) adenosine receptor." (2015) Eur J Med Chem, 108:117-133.

Varani K, Vincenzi F, Targa M, **Ravani A**, Bastia E, Storoni L, Brambilla S, Almirante N, Impagnatiello F. "Repeated Dosing with NCX1404, a Nitric Oxide-Donating Pregabalin, Re-establishes Normal Nociceptive Responses in Mice with Streptozotocin-Induced Painful Diabetic Neuropathy." (2016) J Pharmacol Exp Ther, 357:240-247.

Aghazadeh Tabrizi M, Baraldi PG, Ruggiero E, Saponaro G, Baraldi S, Poli G, Tuccinardi T, **Ravani A**, Vincenzi F, Borea PA, Varani K. "Synthesis and structure activity relationship investigation of triazolo[1,5-a]pyrimidines as CB2 cannabinoid receptor inverse agonists". (2016) Eur J Med Chem, 113:11-27.

Bortoluzzi A, Vincenzi F, Govoni M, Padovan M, **Ravani A**, Borea PA, Varani K. "A2A adenosine receptor upregulation correlates with disease activity in patients with systemic lupus erythematosus." (2016) Arthritis Res Ther. 18:192.

Vincenzi F, **Ravani A**, Pasquini S, Merighi S, Gessi S, Romagnoli R, Baraldi PG, Borea PA, Varani K. "Positive allosteric modulation of A(1) adenosine receptors as a novel and promising therapeutic strategy for anxiety." (2016) Neuropharmacology, 111:283-292.

Varano F, Catarzi D, Vincenzi F, Betti M, Falsini M, **Ravani A**, Borea PA, Colotta V, Varani K. "Design, Synthesis, and Pharmacological Characterization of 2-(2-Furanyl)thiazolo[5,4-d]pyrimidine-5,7-diamine Derivatives: New Highly Potent A(2A) Adenosine Receptor Inverse Agonists with Antinociceptive Activity." (2016) J Med Chem, 59:10564-10576.

Vincenzi F, **Ravani A**, Pasquini S, Merighi S, Gessi S, Setti S, Cadossi R, Borea PA, Varani K. "Pulsed Electromagnetic Field Exposure Reduces Hypoxia and Inflammation Damage in Neuron-Like and Microglial Cells." (2017) J Cell Physiol, 232:1200-1208.

Squarcialupi L, Betti M, Catarzi D, Varano F, Falsini M, **Ravani A**, Pasquini S, Vincenzi F, Salmaso V, Sturlese M, Varani K, Moro S, Colotta V. "The role of 5-arylalkylamino- and 5-piperazino- moieties on the 7-aminopyrazolo[4,3-d]pyrimidine core in affecting adenosine A(1) and A(2A) receptor affinity and selectivity profiles." (2017) J Enzyme Inhib Med Chem, 32:248-263.

Sartori M, Vincenzi F, **Ravani A**, Cepollaro S, Martini L, Varani K, Fini M, Tschon M. "RAW 264.7 co-cultured with ultra-high molecular weight polyethylene particles spontaneously differentiate into osteoclasts: an in vitro model of periprosthetic osteolysis." (2017) J Biomed Mater Res A, 105:510-520.

Varani K, Vincenzi F, **Ravani A**, Pasquini S, Merighi S, Gessi S, Setti S, Cadossi M, Borea PA, Cadossi R. "Adenosine Receptors as a Biological Pathway for the Anti-Inflammatory and Beneficial Effects of Low Frequency Low Energy Pulsed Electromagnetic Fields." (2017) Mediators Inflamm. 2017:2740963.

Ravani A, Vincenzi F, Bortoluzzi A, Padovan M, Pasquini S, Gessi S, Merighi S, Borea PA, Govoni M, Varani K. "Role and Function of A(2A) and A_3 Adenosine Receptors in Patients with Ankylosing Spondylitis, Psoriatic Arthritis and Rheumatoid Arthritis." (2017) Int J Mol Sci. 18(4).

Tschon M, Veronesi F, Contartese D, Sartori M, Martini L, Vincenzi F, **Ravani A**, Varani K, Fini M. "Effects of pulsed electromagnetic fields and platelet rich plasma in preventing osteoclastogenesis in an in vitro model of osteolysis." (2017) J Cell Physiol. [Epub ahead of print] PubMed PMID: 28786478.

Congress Participations:

Ravani Annalisa, Vincenzi Fabrizio, Pasquini Silvia, Gessi Stefania, Merighi Stefania, Borea Pier Andrea and Varani Katia "Pharmacological characterization of adenosine receptors in systemic rheumatic diseases". Annual meeting of Purine Club, Rome, 15th January 2015.

Vincenzi Fabrizio, **Ravani Annalisa**, Merighi Stefania, Gessi Stefania, Borea Pier Andrea and Varani Katia, "Binding characterization and analgesic properties of novel A_1 adenosine receptor allosteric modulators". 37° SIF National Congress - Nuovi orizzonti della ricerca farmacologica: tra etica e scienza, Naples, 27-30 October 2015.

Vincenzi Fabrizio, **Ravani Annalisa**, Merighi Stefania, Gessi Stefania, Borea Pier Andrea and Varani Katia, "Role of A_{2A} adenosine receptors in multiple sclerosis patients". 37° SIF National Congress - Nuovi orizzonti della ricerca farmacologica: tra etica e scienza, Naples, 27-30 October 2015.

Vincenzi Fabrizio, Varano Flavia, Catarzi Daniela, **Ravani Annalisa**, Battistello Enrica, Pasquini Silvia, Gessi Stefania, Merighi Stefania, Borea Pier Andrea and Varani Katia, "Pharmacological characterization of new highly potent A_{2A} adenosine receptor inverse agonists with anti-nociceptive activity." Selected as the best poster in the section "Nervous system and receptor structure", VII Joint Italian-German Purine Club Meeting, Rome, 21-22 July 2017.

Varani Katia, Vincenzi Fabrizio, **Ravani Annalisa,** Pasquini Silvia, Merighi Stefania, Gessi Stefania, Setti Stefania, Cadossi Matteo, Borea Pier Andrea and Cadossi Ruggero, "Pulsed electromagnetic fields treatment mediates anti-inflammatory and chondroprotective effects through adenosine receptor pathway". 38° SIF National Congress – Farmaci- salute e qualità della vita, Rimini, 25-28 October 2017.

Vincenzi Fabrizio, **Ravani Annalisa,** Pasquini Silvia, Merighi Stefania, Gessi Stefania, Battistello Enrica, Romagnoli Romeo, Baraldi Pier Giovanni, Borea Pier Andrea and Varani Katia, "Allosteric modulation of A_1 adenosine receptors as a novel and promising therapeutic strategy for anxiety". 38° SIF National Congress - Farmaci- salute e qualità della vita, Rimini, 25-28 October 2017.

Vincenzi Fabrizio, Varano Flavia, **Ravani Annalisa,** Pasquini Silvia, Merighi Stefania, Gessi Stefania, Catarzi Daniela, Colotta Vittoria, Borea Pier Andrea and Varani Katia, "Anti-nociceptive effects of novel highly potent A2A adenosine receptor inverse agonists". 38° SIF National Congress - Farmaci- salute e qualità della vita, Rimini, 25-28 October 2017.

Merighi Stefania, Bencivenni Serena, Battistello Enrica, Vincenzi Fabrizio, Pasquini Silvia, **Ravani Annalisa,** Varani Katia, Borea Pier Andrea and Gessi Stefania, "A_{2B} adenosine receptors stimulate IL-6 production in primary murine microglia through p38 MAPK kinase pathway". 38° SIF National Congress - Farmaci- salute e qualità della vita, Rimini, 25-28 October 2017.

ACKNOWLEDGEMENTS

The research described in this thesis was carried out in the Laboratory of Cellular and Molecular Pharmacology, Institute of Pharmacology, Department of Medical Sciences, University of Ferrara.

I wish to express my sincere gratitude to my supervisor Professor Katia Varani for her support, her valuable advices and constant encouragement.

Special thanks are due to Fabrizio, Silvia and Serena for giving me support and friendship.

I would like to thank my parents and all my family.