We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Pharmacology of Adenosine Receptors and Their Signaling Role in Immunity and Inflammation

Fernanda da Rocha Lapa,
Sérgio José Macedo Júnior, Murilo Luiz Cerutti and
Adair Roberto Soares Santos

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/57206>

1. Introduction

Since the late 1920s, the importance of the physiological role of adenosine triphosphate (ATP) and its metabolites, mainly adenosine, has been clear. In 1970, strong evidence now suggests ATP as a neurotransmitter in nonadrenergic, noncholinergic (NANC) nerves supplying the gut. Initially, the hypothesis of purinergic transmission encountered some resistance, however, this concept is now widely accepted and purines are considered powerful extracellular messengers in peripheral and central nervous system and to non-neuronal cells, including immune, and inflammatory cells. Implicit in the purinergic hypothesis was the presence of purinoceptors. The first evidence for the existence of adenosine receptors, responsible for the physiological effects of adenosine, was only published in the beginning of the 1970s. Throughout the 1990s and 2000s adenosine receptors were cloned, and the mechanisms of signal transduction mediated by these receptors were described. Currently, it is well known that adenosine activates four G protein-coupled receptors named A_1 , A_{2A} , A_{2B} and A_3 . The A_1 and A_3 receptors preferentially interact with members of the $G_{i/o}$ family of G proteins, lowering the intracellular levels of cyclic adenosine monophosphate (cAMP), whereas the A_{2A} and A_{2B} receptors interact with members of the G_s family of G proteins, elevating of intracellular cAMP.

Throughout the 1980s and 1990s, several studies demonstrated that ATP metabolites, especially adenosine, are important signaling molecules, and adenosine receptors are important molecular targets in inflammation and immunity. During inflammation, the generation of the appropriate immune response can itself cause considerable damage and thus requires effective regulation. It has been suggested that this regulation of the immune system requires the

sensing of specific signals called “danger signals”. Thus, in the context of purinergic signaling, it has been proposed that the regulation of the immune system requires at least two ‘danger’ signals, the first (ATP) indicating the presence of danger from pathogens or other injurious events and leading to the activation of immune cells and defensive effector function and the second (adenosine) indicating the danger from overactive immune cells and triggering the downregulation of the proinflammatory activities of the immune system. These discoveries increased the interest in the purinergic signaling pathways, and there was an increase in publications studying the effects of adenosine and inosine in inflammation and immunity (Figure 1 A and B).

Substantial evidence demonstrates that adenosine receptors are expressed in most inflammatory cells and may therefore modulate different steps involved in inflammatory and immune responses. Moreover, several recent studies have indicated that inosine, a metabolite of adenosine, once believed to be an inert metabolite, can exert many immunomodulatory actions through adenosine receptors, mainly the A_2 and A_3 receptors. In this regard, adenosine receptors have become potential therapeutic targets for the treatment of several pathologies in which inflammatory modulation is a key component. In this regard, adenosine and inosine can be considered molecules with valuable therapeutic potential, performing the desired effect with minimal side effects, such as those present in therapy with adenosine (Adenocard®) for the treatment of paroxysmal supraventricular tachycardia (facial flushing, chest pressure, hyperventilation, dizziness, numbness and tingling). Moreover, no studies clearly demonstrate the side effects of inosine. However, it is important to warn that the purines can play deleterious effects not observed clinically, depending on the target tissue and the receptor that is activated according to figure 3.

Thus, this chapter was designed to highlight the importance of ATP metabolites, especially adenosine and inosine, and their modulatory effect on inflammation through the activation of adenosine receptors. In addition, we aimed to provide updated information about the pharmacology of adenosine receptors, especially about its proinflammatory versus anti-inflammatory effects. Furthermore, we are able to clarify the overall effect of adenosine and inosine in different inflammatory diseases. Finally, we intend to present a short overview concerning the advances in drug development targeting adenosine receptors.

2. The ATP metabolic pathways

Carbohydrates, lipids and proteins, also called “metabolic fuels” are constantly being oxidized to provide energy. Glucose is generally the primary energy source for cellular metabolism. It is catabolized by the following three main processes: glycolysis, the tricarboxylic acid (TCA or Krebs) cycle and oxidative phosphorylation, which lead to the production of ATP, the final energy-rich product that is used in many different active processes in an organism. Macromolecule synthesis, muscle contraction, active transport of ions and thermogenesis are some of the key processes that require energy. Since the mid-1920s, when ATP was discovered as a substrate used in muscle contraction, knowledge about this high-energy molecule has

constantly been expanded. The literature has shown that many aspects of cellular metabolism are directly linked to the production and consumption of ATP and has also emphasized its importance in purinergic signaling mechanisms. In this context, taking into account the importance of ATP in the maintenance of homeostasis and the evidence indicating the role of its metabolites in the control of immunity and inflammation, understanding ATP metabolism in the body has become more imperative [1].

2.1. The degradation of ATP and formation of ADP, adenosine and inosine

In situations of high energy demand such as inflammation and hypoxia, ATP may be converted into adenosine monophosphate (AMP) in the intracellular environment through a reaction dependent on ATPase and adenylate kinase. AMP can be converted into adenosine by the intracellular enzyme 5-nucleotidase and thereafter can be transported to the extracellular environment via bidirectional nucleoside transporters. ATP in the extracellular environment can activate P2 -type receptors in the surroundings or generate adenosine via ecto-5-nucleotidase (NT5'E), the primary enzyme responsible for ATP metabolism under physiological conditions [2]. In the extracellular space, ATP and ADP are converted to AMP through the ectonucleoside triphosphate diphosphohydrolase-1 (CD39). The second step for extracellular adenosine formation is the ecto-5'-nucleotidase (CD73) conversion of extracellular AMP into adenosine. During inflammation and hypoxia, an increase in the activity and expression of adenosine deaminase and in its binding partner, CD26, has also been demonstrated [3]. This increase promotes adenosine conversion into inosine within seconds, terminates adenosine signaling and can thus initiate inosine signaling. Inosine can be converted into hypoxanthines and uric acid (UA) by purine nucleoside phosphorylase (PNP) and xanthine oxidase (XO), respectively [4, 5]. Current studies using mice lacking the CD39 and CD73 genes have revealed the importance of these enzymes in contributing to extracellular adenosine generation in different organs and situations [5]. In agreement with those studies, certain CD39 polymorphisms increase ATP and ADP, lowering extracellular adenosine levels, which can lead to increased susceptibility to inflammatory pathological conditions such as inflammatory bowel disease (IBD) and multiple sclerosis (MS) [6, 7]. Furthermore, the loss-of-function mutation of CD73 in humans is suggested to be the basis for the development of peripheral arterial calcifications, indicating that adenosine generation can be vasoprotective [8, 9]. Currently, it is known that after adenosine is released from cells or generated in extracellular space, it diffuses into the surroundings, where it binds to adenosine receptors (A_1 , A_{2A} , A_{2B} and A_3) on adjacent cells. Finally, after adenosine generation and receptor activation, adenosine diffuses away from the receptor and is rapidly transported into the intracellular space mainly through equilibrate nucleoside transporters (ENT-1 and ENT-2) [5, 10, 11].

3. ATP metabolites as danger signals: the role of adenosine and inosine

During inflammation, infection or hypoxia, the generation of the appropriate immune response can itself cause considerable damage and thus requires effective regulation [12]. It has been suggested that this regulation of the immune system requires the sensing of specific

signals called “danger signals” [13-15]. Although the immune response can be activated by recognizing the signatures of foreign pathogens, collectively called pathogen-associated molecular patterns (PAMPs), it is also able to respond to endogenous host molecules to trigger inflammatory responses. Most of these are produced as a result of cell death or injury or by tumor cells; they include degradation products of the extracellular matrix (ECM), heat-shock proteins and high-mobility group box 1 (HMGB1) proteins, UA crystals, amyloid- β and oxidized LDL (Ox-LDL), which act as stimulators for pattern recognition receptors (PRRs) and have been referred to as danger-associated molecular patterns (DAMPs) [15, 16].

Extracellular ATP and UA are well-characterized dangers signals, likely released from cells as a consequence of cell damage or nonapoptotic cell death. The exposure of local cells to elevated extracellular ATP and monosodium urate crystals has been described as proinflammatory because it activates P2X7 receptors, NALP3 (a member of NOD-like receptors, also called cryopyrin) and caspase-1 [17-19]. This activation leads to the processing and release of interleukin 1 β (IL-1 β) and results in inflammation [14, 20]. Furthermore, the elevation of extracellular ATP has been demonstrated to guide circulating neutrophils to the inflammatory microenvironment and can function as a “find-me signal” to attract inflammatory cells (particularly phagocytes) and direct the inflammatory response [12, 21].

Recent studies have shown that in situations of inflammation, trauma or hypoxia when extracellular ATP concentrations are elevated, there is an increased expression of ectonucleotidases that rapidly convert ATP/ ADP into adenosine, terminating the proinflammatory effects of ATP [12]. Thus, in the context of purinergic signaling, it has been proposed that the regulation of the immune system requires at least two ‘danger’ signals, the first (ATP) indicating the presence of danger from pathogens or other injurious events and leading to the activation of immune cells and defensive effector function and the second (adenosine) indicating the danger from overactive immune cells and triggering the downregulation of the proinflammatory activities of the immune system [13, 22].

The effects of adenosine in different tissues may depend upon the repertoire of adenosine receptors present on the cell surfaces [22]. In this context, the A_{2A} and A_{2B} receptors have been described as the receptors most involved in the control of immunity and inflammation [13, 22, 23]. By binding to A_{2A} and A_{2B} receptors, adenosine triggers cAMP elevation in T cells, which results in the activation of CREB/ATF (cAMP-responsive element (CRE)-binding protein/activating transcription factor), an immunosuppressive mechanism [12, 23, 24]. This activation has been shown to trigger Treg cell activation, the production of anti-inflammatory cytokines such as TGF- β and IL-10 and inhibit the functional response of TCR- activated T effector cells, reducing the secretion of IL-2 and IFN- γ [24]. Moreover, similar to adenosine, the metabolite inosine is also known to exert wide ranging anti-inflammatory effects, which include inhibition of proinflammatory cytokines, chemokine production and protection from septic shock, colitis and acute lung injury [25-27]. Some studies have shown that inosine can stimulate adenosine A_{2A} receptors and is protective in models of concanavalin A-induced liver damage, endotoxin-induced sepsis [28, 29] and TNBS-induced colitis [30]. In this context, although there is no description of inosine as a danger signal in the current literature, some studies have described inosine as tissue protective. Taking into account that it can activate the same sensors (receptors)

as adenosine, we speculate that inosine might be an additional danger signal that could work with adenosine to dampen inflammation.

In summary, the immunosuppressive effects of adenosine have been broadly described in the literature as a “retaliatory metabolite” [31] or an “engineer” of inflammation [22], indicating that adenosine can manipulate the intensity and the time course of inflammatory process in vivo and suggesting biochemical control of immunity. These events appear to be biologically coordinated and may constitute a homeostatic mechanism of tissue integrity. Therefore, the failure of this protective mechanism may contribute to beginning and perpetuating chronic inflammation response.

Figure 1. The number of publications regarding the investigation of adenosine and inosine effects in inflammation and immunity since the 1960s.

4. Adenosine receptors and inflammation: proinflammatory versus anti-inflammatory effects

4.1. A₁ adenosine receptors

The adenosine A₁ receptor is coupled to the G_{i/o} family of G proteins, lowering the intracellular levels of cAMP [32, 33]. Activation of A₁ receptors leads to increased intracellular Ca⁺² levels due to the stimulation of phospholipase C, which in turn promotes the cleavage of phosphatidylinositol 4,5-bisphosphate (PIP₂) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP₃) (Figure 2). Moreover, the enhancement of intracellular calcium can activate certain enzymes, such as protein kinase C (PKC), phospholipase D (PLD), phospholipase A₂ (PLA₂) and others [10, 33].

The recently reported crystal structure confirms that adenosine receptors display the typical topology of GPCRs, a common central core domain consisting of seven transmembrane (TM) helices numbered from 1 to 7 that are composed of 20-27 amino acids and that are largely α -helical. The TM domains are also slightly bent and linked by three intracellular (IL-1, IL-2 and IL-3) and three extracellular (EL1, EL2, and EL3) loops [34]. The A₁ receptor amino acid sequence varies from 324 to 328 residues [35]. In 1992, Ijzerman and colleagues highlighted the role of two histidine residues in ligand binding, one located in TM6 and one in TM7 [36]. This result was in agreement with the first mutagenesis data on adenosine receptors, published in the same year, performed on bovine A₁ receptors, which demonstrated that residues His251 (6.52) and His278 (7.43) were important for ligand binding. The same study also revealed that the mutation of histidine residues to leucine led to a decrease in ligand affinity, especially His278 [37]. In 1994, two mutagenesis studies suggested a role of residue 270 (7.35, isoleucine or methionine in the bovine/canine receptor) in the binding of N⁶-adenine substituted compounds (A₁ receptor agonists) and the importance of residue 277 (7.42, threonine in human/bovine receptor) in the recognition and interaction of the adenosine ribose moiety [38, 39]. It has also been demonstrated that the Pro25Leu (1.48) mutation and substitution of Leu88 (3.33), Thr91 (3.36), and Gln92 (3.37) by alanine reduces the affinity for N⁶-unsubstituted adenosine derivatives [39]. In the same study, it was also demonstrated that the Gly14Thr (1.37) mutation increases the receptor's affinity for agonists, suggesting the constitutively active form of this mutant receptor [40]. Finally, analysis of the Thr277Ala (7.42) mutation by an allosteric enhancer suggested an allosteric role for this residue [41].

There is no consensus regarding the effects of A₁ receptors in the inflammatory response; some studies suggest proinflammatory effects, while others suggest anti-inflammatory effects. The A₁ receptors are expressed in leukocytes. At submicromolar adenosine concentrations, the activation of these receptors in human neutrophils produces a proinflammatory response by promoting chemotaxis and adherence to the endothelium [42, 43]. In lymphocytes, A₁ receptor antagonism contributes to adenosine's anti-inflammatory effects by reducing the expression of intracellular adhesion molecule-1 (ICAM-1), production of IL-12 and IFN- γ and lymphocyte proliferation [44]. In addition to these data, various studies with selective agonists and antagonists demonstrated the proinflammatory effects of A₁ receptors in different inflammatory models, some of which are summarized here. During acute pancreatitis induced with cerulein or taurocholate in rats, the selective A₁ receptors agonist CCPA (2-chloro-N⁶-cyclopentyladenosine) produced an increase in leukocyte infiltration and interstitial edema in pancreatic tissue, which was attenuated by FK-838 (6-oxo-3-(2-phenylpyrazolo[1,5- α]pyridin-3-yl)-1(6*H*)-pyridazinebutanoic acid), a selective A₁ receptor antagonist [45]. A₁ receptor antagonism was also protective in the lungs; treatment with DPCPX (8-cyclopentyl-1,3-dipropylxanthine), an A₁ receptor antagonist, prevented endothelial damage, neutrophil migration and alveolar injury in a model of ischemia reperfusion in the lungs [46]. In another interesting study, the A₁ receptor antagonist DPSPX (1,3-dipropyl-8-*p*-sulfophenylxanthine) decreased the area of cardiac necrosis and improved ventricular function in a canine model of myocardial ischemia reperfusion, most likely due to inhibition of neutrophil chemoattraction [43]. Pretreatment with KW3902, a selective A₁ receptor antagonist, preserved hepatic architecture, decreasing the infiltration of neutrophils into hepatic tissue in a hepatic ischemia

reperfusion injury model in dogs [47]. Collectively, these findings demonstrate that the A₁ receptor is an important target in inflammation and that antagonists may be efficacious as anti-inflammatory drugs.

In opposition to such data, studies using pharmacological (selective A₁ receptor agonist or antagonists) and genetic tools (knockout animals) have shown that activation of the A₁ receptor can promote anti-inflammatory effects. CCPA, an A₁ receptor agonist, presented a protective effect in a mouse model of renal ischemia reperfusion, an effect reverted by DPCPX, a selective A₁ receptor [48]. Studies performed with knockout mice confirmed the renal protective effects of the A₁ receptor [49] and also revealed the protective effects of this receptor in other tissues and inflammatory conditions because the absence of the A₁ receptor promotes proinflammatory effects in the lungs, enhancing leukocyte migration and levels of cytokines, including IL-4 and IL-13 [50]. In the central nervous system (CNS), A₁ receptor knockout animals exhibited severe demyelination and axonal injury, involving the activation of macrophages and microglial cells [51]. In sepsis induced in mice, the A₁ receptor knockout animals had a higher degree of renal dysfunction induced by higher release of pro-inflammatory cytokines [52]. A previous study described the mechanism by which activation of A₁ receptor leads to anti-inflammatory effects, which involves phosphorylation of ERK, MAPK and Akt (Figure 2), all of which are involved in the upregulation of cytoprotective genes and also increase the phosphorylation of heat shock protein (HSP) 27, a molecular chaperone that prevents the denaturation and aggregation of cellular proteins, a cytoprotective effect [53]. In summary, the lack of consensus regarding the existence of the proinflammatory and anti-inflammatory effects of the adenosine A₁ receptor could be explained assuming that the A₁ receptor can activate intracellular signaling pathways that result in tissue injury or protection, through proinflammatory or anti-inflammatory effects, respectively, because the activated pathways depend on the following:

- the species/tissue/organ and the stage/progression of injury;
- Predominant inflammatory cell type as a function of species;
- Intracellular signaling and desensitization mechanisms as a function of species or cell/tissue/organ.

4.2. A_{2A} adenosine receptors

Most A_{2A} receptors are coupled to the G_s protein family. A subset, preferentially located in the striatum, is coupled to the G_{oif} protein family. It is well established that the biological effects triggered by A_{2A} receptors are due to enhancement of cAMP production followed by adenylate cyclase activation. The increase of the cAMP level stimulates cAMP-dependent kinase (PKA) (Figure 2), which, in turn, activates several pathways through calcium channels, potassium channels, cAMP responsive element-binding (CREB), mitógeno-activated protein kinase (MAPK) and phospholipase C (PLC) activation [23, 54].

The A_{2A} receptor structure is very similar to other adenosine receptors. However, it differs in the four disulfide links observed at the extracellular level, which are critical for the packing and stabilization of the restricted conformation of the seven transmembrane helices. Another difference concerns the A_{2A} receptor length of the C-terminal region, which consists of

approximately 120 residues [55]. In 1995, Kim et al. published the results of site directed mutagenesis experiments on A_{2A} receptors [56], revealing the essential role of some residues for ligand interaction, particularly Phe182 (5.43), Asn253 (6.55), Ile274 (7.39), and Ser281 (7.46). The key role of His250 (6.52), Ser277 (7.42) and His278 (7.43) was also confirmed. After 1995, several mutagenesis studies revealed some of the amino acids residues involved in direct or indirect interaction with ligands or in allosteric regulation. It was observed that the conserved Glu 1.39 is critical for agonist but not antagonist binding [57, 58]. Glu13 (1.39) and His278 (7.43) were found to be critical for the allosteric regulation of A_{2A} receptors [59], and Gln89 (3.37) was suggested to play an indirect role in ligand binding, while Ser281 (7.46) mutation to asparagine improved agonist affinity [60]. Additional studies were carried out to analyze the role of loop residues of A_{2A} receptors, revealing the importance of Glu151 and Glu169 (EL2) for ligand binding [61]. Unlike A₁ receptors, there is a considerable consensus regarding the effects of A_{2A} receptors on the inflammatory response. A broad range of investigations using *in vivo* and/or *in vitro* approaches have provided evidence that A_{2A} receptor activation limits inflammation and tissue damage, therefore playing an anti-inflammatory role [13, 31]. The A_{2A} receptor signaling in suppressing inflammation is related to cAMP-increased levels, which also have a well-known immunosuppressive effect on immune cells. Corroborating these data, the fact that A_{2A} receptors are expressed on most cells of the immune system is crucial to its anti-inflammatory properties [62]. Thus, the anti-inflammatory properties of these receptors are due, at least in part, to the prevention of the activation of effector cells of the immune system. For example, they may prevent neutrophil migration and interfere in the activity of proteins on the surface of neutrophils and endothelial cells that are crucial to the adhesion and migration of the former into the inflammatory site. In these sense, A_{2A} receptor activation inhibits the adherence of *N*-formyl methionyl-leucyl-phenylalanine (fMLP)-activated neutrophils to the endothelium [63] and downregulates Mac-1 [64], β 2-integrin [65] and L-selectin [66]. Furthermore, activation of the A_{2A} receptors also downregulates the activity of other endothelial cell surface proteins, including vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) [67], α 4/ β 1 integrin (VLA4) [68] and platelet cell adhesion molecule [69]. On the other hand, the anti-inflammatory properties resulting from A_{2A} receptor activation are due to the reduction of the release of inflammatory mediators such as IL-12, INF γ , TNF- α , and IL-4 from important immunomodulatory cells such as neutrophils, monocytes, dendritic cells and T lymphocytes [70, 71].

Many of the anti-inflammatory effects of A_{2A} receptors are mediated by adenosine itself. It is known that inflammatory tissue damage is accompanied by the accumulation of extracellular adenosine in inflamed sites due to its release from non-immune and immune cells. Because endogenous adenosine levels are elevated during an inflammatory process and endogenous adenosine can activate A_{2A} receptors to attenuate inflammation and tissue damage, strategies that aim to foment adenosine production and increase its availability to activate A_{2A} receptors present extraordinary anti-inflammatory potential. In this regard, an interesting study demonstrated that the immunosuppressive effects of activated Treg lymphocytes could be in part related to adenosine production in the extracellular environment through both CD39 (an ectonucleoside triphosphate diphosphohydrolase that converts ATP and ADP to AMP) and CD73 (an ectonucleoside that converts AMP to adenosine) expressed on the surface of these

cells. Moreover, the Treg immunosuppressive effects have been shown to be modulated by A_{2A} receptors [72]. In a model of ischemia reperfusion liver injury, treatment with ATL146e (4-(3-[6-amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9H-purin-2-yl]-prop-2-ynyl)- cyclohexanecarboxylic acid methyl ester), an agonist of A_{2A} receptors, was associated with decreased inflammation and protection from liver damage. When A_{2A} receptor knockout mice were subjected to the same insult, the effectiveness of ATL146e was lost [73]. Later, similar approaches showed the protective effect of the A_{2A} receptor in models of myocardial infarction, acute lung injury and spinal cord compression injury [74] [75, 76].

In another study, the same A_{2A} receptor agonist, ATL146e, was associated with decreased leukocyte infiltration, inflammatory mediator production and necrosis in a model of inflammatory bowel disease [77]. In a model of LPS-induced lung injury, treatment with the A_{2A} receptor agonist ATL202 was associated with decreased recruitment of neutrophils to the lung together with reduced cytokine levels and pulmonary edema, whereas A_{2A} receptor knockout mice treated with LPS showed an increase in neutrophil recruitment [75]. In a mouse model of allergic lung inflammation, treatment with an A_{2A} receptor (CGS-21680) agonist resulted in diminished pulmonary inflammation [78]. On the other hand, A_{2A} receptor knockout mice have been shown to have higher lung inflammation compared to wild-type mice [79].

4.3. A_{2B} adenosine receptors

The A_{2B} receptor couples to Gi-type G proteins, leading to the inhibition of adenylate cyclase upon receptor activation [80]. In some cell systems, such as HEK-293 and HMC-1 mast cells, A_{2B} receptors are also coupled to phospholipase C via the action of Gq proteins in increasing intracellular Ca²⁺ levels [81-83]. The A_{2B} receptor has also been described to be involved in the ERK1/2 [80, 83, 84] and p38 MAPK pathways in mast cells (Figure 2) [85]. Furthermore, a link between A_{2B} receptor signaling and the arachidonic acid signal transduction pathway leading to vasoconstriction has been described [83, 86]. Among the four adenosine receptors, A_{2B} is the least well characterized receptor, mainly due to the lack of suitably specific ligands [87].

The A_{2B} receptor has low affinity for most agonists, so only some agonists are useful, including the non-selective and related adenosine derivative NECA [88] and the highly selective A_{2B} agonist BAY60-6583 [89]. Conversely, highly selective A_{2B} antagonists have been developed, such CVT-6883 and an A_{2B}-specific antagonist radioligand, [3H]PSB-603, which has high potency and specificity across species, including rodents and humans [90]. The A_{2B} receptors have a closely related structure to A_{2A} receptors, and sequence analysis of the human A_{2A} and A_{2B} receptors show an overall identity of 58% and a similarity of 73%. The most conserved residues are found within the seven transmembrane domains. [83]. In contrast to the A_{2A} receptor, the A_{2B} receptor possesses the longest extracellular loop 2 (ECL2) of all four adenosine receptor subtypes, with four cysteine residues – the highest number found in any GPCR – of which three (C154, C167, C171) are homologous to the three (C146, C159, C166) found in the A_{2A} receptor [83]. These cysteine residues are involved in disulfide bonds formed between the ECL and transmembrane domains (TM) of GPCRs and have been reported to play an important role in ligand binding affinity and receptor stability and function.

Figure 2. A brief summary of the role of the purinergic system in inflammation. A₁, adenosine A₁ receptor; A_{2A}, adenosine A_{2A} receptor; A_{2B}, adenosine A_{2B} receptor; A₃, adenosine A₃ receptor; AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate; ERK1/2, extracellular signal-regulated kinases 1 and 2; IP₃, inositol triphosphate; MAPK, mitogen-activated protein kinase; PKB, protein kinase B; PLC, phospholipase C; PI3K, Phosphatidylinositol 3-kinase; ATP, adenosine triphosphate; AMP, adenosine monophosphate; HXT, hypoxanthine; AK, adenosine kinase; ADA, adenosine deaminase; PNP, purine nucleoside phosphorylase; ENTs, equilibrate nucleoside transporters; CD 39, ectonucleoside triphosphate diphosphohydrolase; Inhibition → ; Activation - - →

Studies of mutagenesis, X-ray receptor analysis and radioligand binding have shown that C78 in transmembrane domain 3 (TMD3) and C171 in ECL2 form an important disulfide bond related to ligand-binding affinity and receptor expression levels [83, 91, 92]. In agreement with these data, in a mutagenesis model in which the complete ECL2 of human A_{2B} was exchanged for the ECL2 of the A_{2A} receptor, the mutant A_{2B} (ECL2-A_{2A}) receptor mice had increased affinity and selectivity for A_{2B} agonists and antagonists as well as receptor-mediated cAMP accumulation, indicating that ECL2 is a ligand binding site [93]. ECL2 also seems to contribute to the low affinity of ligands to A_{2B} receptors because it has been shown to have between 4 and 10 more amino acids than A_{2A} receptors. The longer ECL2 may, in some cases, partially block the entrance of ligands into the binding pocket and explain why A_{2B} receptors typically show lower affinity for adenosine and adenosine derivatives (agonists) than A_{2A} receptors, which have a shorter ECL2, which may facilitate the entrance of ligands to the binding pocket [83]. In addition, residues Thr42, Val54 (2.51) and Phe84 (3.31) are also involved in the binding site;

mutation to alanine, leucine or serine, respectively, decreased agonist binding [94]. As mentioned above, in many tissues, A_{2B} receptors are considered low-affinity receptors with mostly low expression levels, so adenosine needs to reach higher concentrations to activate them [83, 95].

During hypoxia, a critical role of HIF-1 in the transcriptional induction of the adenosine A_{2B} receptor has been suggested. In such conditions, as in $A_{2B}^{-/-}$ receptor mice, the presence of A_{2B} receptors attenuates hypoxia-induced increases in vascular leakage, mainly in the lungs, a protective effect [95]. Accordingly, Yang and colleagues reported that A_{2B} -null mice present augmentation of proinflammatory cytokine levels, such as TNF- α , upregulation of vascular adhesion proteins and leukocyte migration in response to LPS-induced acute inflammation. Conversely, in adenosine-deficient mice and bleomycin-induced lung inflammation, the antagonist CVT-6883 attenuated pulmonary inflammation [96]. Moreover, A_{2B} activation resulted in an increase in IL-8 [85], IL-1 β , IL-3, IL-4, IL-13 and IgE leading to mast cell and Th2 and B lymphocyte activation [97]. Mice treated with CVT-6883 and MRS-1754 or A_{2B} -null mice developed less severe experimental autoimmune encephalomyelitis (EAE) with reduced IL-6 release and Th17 cell differentiation [98].

In spite of the structural similarity, adenosine has been recognized as the natural ligand of A_{2B} receptors, but inosine was not [99, 100]. However, a recent study conducted by our group has shown evidence that inosine can reduce both acute pleural inflammation and allergic lung inflammation through a mechanism that involves both A_{2A} and A_{2B} receptors, suggesting a regulatory role of inosine and A_2 in these process [11, 101]. Taking into account the lack of information about the A_{2B} receptor and its involvement in several inflammatory diseases, it may be a candidate target for future therapeutic intervention

4.4. A_3 adenosine receptors

Studies have demonstrated that both adenosine and inosine can activate A_3 receptors in vitro and therefore can directly modulate its activation and biological effects [99, 100]. Similar to other adenosine receptors, the A_3 receptors is a GPCR with seven transmembrane domains (TM). It is coupled to classical second-messenger pathways such as inhibition of adenylyl cyclase, stimulation of PLC and calcium mobilization [33, 102-104].

In the heart, A_3 mediates cardioprotective effects through the activation of K_{ATP} channels that are coupled to RhoA–phospholipase D signaling, mediating the protection of cardiac myocytes from ischemia [104]. With regard to cardiac protection, signaling through cAMP response element-binding protein (CREB)-Bcl2 pathways after A_3 receptor activation has also been described [104]. In addition, like other adenosine receptors, A_3 receptors are coupled to MAPK and lead to stimulation of extracellular signal-regulated kinases (ERK1/2), which relates A_3 receptor activation to cell growth, survival, death and differentiation [33, 80].

A_3 receptor activation in melanoma cells stimulates PI3K-dependent phosphorylation of protein kinase B (PKB/Akt), leading to the reduction of basal levels of ERK1/2 phosphorylation, which in turn inhibits cell proliferation (Figure 2) [105]. Consistent with these data, treatment with IB-MECA induced inhibition of tumor growth [33, 104], although in the hypoxic condi-

tions that occur in solid tumors, A₃ activation mediates angiogenesis and cell survival through increased HIF-1 α and VEGF production [106]. Moreover, the A₃ receptor is involved in adenosine and inosine induced-mast cell degranulation [99, 107]; inhibition of endotoxin-induced neutrophil granulation and TNF- α production [108, 109]; reduction of T cell tumoricidal activity and enhancement of natural killer cell cytotoxicity [104]; reduction of neuropathic pain [110]; and augmentation of bone marrow cells proliferation favoring myeloprotection [111]. One of the characteristics of the A₃ receptor is the rapid (within a few minutes) desensitization through phosphorylation by G-protein-coupled receptor kinase 2 (GRK2) at the intracellular threonine residues within the C-terminal domain after exposure to an agonist, which can limit the agonist's effect [112–114].

A₃ receptors exhibit the lowest degree of identity among species compared with other adenosine receptor subtypes [115]. In a study that used a combination of mutagenesis, radioligand binding, functional activity and molecular modeling approaches, a mutation of TMD3 His95 (3.37), which is conserved in A₃ receptors in various species including humans, sheep and rats, resulted in a decreased affinity of agonists and antagonists and is therefore considered critical to ligand binding. The same was observed when His272 (7.43) and Asn250 (6.55) were mutated. Moreover, residues Tyr243 (6.48) and Lys152 (ECL2) were needed only for antagonist binding [115], and Trp243 (TM6) is involved in the functional activation of the A₃ receptor based on the impairment of the coupling of the receptor to the G protein in the Trp243 mutant [115, 116]. Furthermore, molecular modeling suggested that Trp243 is in the binding pocket and might occupy a strategic position as a switch in the TM6-mediated structural transition from the resting to the active state [115]. Given the involvement of A₃ receptors in important pathological process, as described above, several studies evaluating the structure-activity relationships of agonists and antagonists with A₃ receptors have been conducted to identify new potential drugs for the treatment of deleterious diseases.

5. Adenosine and inosine regulation on leukocyte function

The action of adenosine and inosine on the immune system is determined by their bioavailability and adenosine receptor expression in immune cells. The rapid release of adenosine in response to tissue-disturbing stimuli such as hypoxia, ischemia, inflammation or trauma and the rapid conversion of adenosine into inosine have been reported to modulate the function of leukocytes, a basic constituent of immune system [117]. In this section, we discuss adenosine and inosine modulation of immune cells and the corresponding involvement of adenosine receptors.

Neutrophils are the first immune cells recruited to inflamed sites by a combination of chemo-attractant cytokines and adhesive interactions between leukocytes and the vascular endothelium [118, 119]. Adenosine mainly acts on A_{2A} receptors, signaling through cAMP-PKA-dependent pathways. It decreases neutrophil activation, neutrophil-mediated injury to endothelial cells, production of reactive oxygen species, PAF, and leukotriene B₄, secretion of cytokines such as TNF- α and chemokines such as MIP-1 α /CCL3, MIP-1 β /CCL4,

MIP-2alpha/CXCL2 and MIP-3alpha/CCL20 and expression of adhesion molecules such as selectins and integrins [31, 120-128]. Similar to adenosine, inosine also interferes with neutrophil activation by blocking formyl-Met-Leu-Phe-induced superoxide generation [31, 100], neutrophil migration and release of the proinflammatory cytokines TNF- α and IL-1 β during acute inflammation, acting through A_{2A} and A_{2B} receptors [11]. A₃ and A_{2A} receptors are involved in the reduction of superoxide anion generation [127, 129]. A₃ receptors can also direct neutrophil migration [130]. Interestingly, the adenosine interaction with A₁ and A₃ receptors induces G-CSF production, which leads to a stimulatory effect on bone marrow cells, suggesting that adenosine is a chemoprotective agent that could restore the number of leukocytes and neutrophils to normal levels after chemotherapy [131].

Macrophages and dendritic cells are phagocytes that are widely dispersed throughout the body at portals of microorganism entry [31]. They initiate an effective innate immune response against microbes by recognizing pathogen-associated molecular patterns (PAMPs) through pattern-recognition receptors (PRRs) [119, 132]. This response involves pathogen processing and is regulated by the secretion of several cytokines and activation of lymphocytes and other immune cells.

In this context, studies have demonstrated that adenosine inhibited TNF- α , IL-6 and IL-8 release from macrophages stimulated with thioglycollate or LPS via A_{2A} receptors, although the A_{2B} receptors seems to play an underlying inhibitory role that may contribute to anti-inflammatory action [133, 134]. Some studies have demonstrated that adenosine can increase IL-10 production and release through a mechanism involving adenosine A_{2A} receptor-CEBP β axis activation [135]. The augmentation of the production of IL-10 and the decrease in systemic endotoxin-induced levels of TNF- α , IL-12, MIP-1 α and IFN- γ have also been ascribed to inosine [136]. Current data have shown that activation of both A_{2A} and A₃ receptors inhibited IFN- γ and IL-12 release after TLR-4 stimulation by LPS [137-139]. Adenosine and inosine were described to decrease M1 activation and the release of mediators, reducing Th1 response, by interfering with TLR-4 activation. Moreover, there is growing evidence that A_{2A} receptor activation also reduces the TLR-2, 3, 7, and 9 responses in M1 macrophages, upregulating VEGF and IL-10 expression and therefore polarizing macrophages into an M2-like phenotype, called M2d, which favors an angiogenic switch and plays a protective role in ischemia [70, 140].

Adenosine also interferes with mature dendritic cell stimulation by producing a dose-dependent inhibition of TNF- α and IL-12 release, whereas it enhanced the secretion of IL-10, preventing tissue injury mediated by innate immune mediators during overwhelming immune response [141]. Furthermore, dendritic cells matured in the presence of adenosine had a reduced capacity to induce T helper 1 (Th1) polarization of naive CD4⁺ T lymphocytes, evidence that adenosine diminishes the capacity of dendritic cells (DCs) to initiate and amplify Th1 immune responses [141].

Mast cells are resident in all normal tissues, where they are believed to play an important role in tissue homeostasis, wound healing and host defense, particularly in terms of bacterial infection. When activated, they secrete the autacoid mediators histamine, prostaglandin (PG)D₂ and leukotriene (LT)C₄, which contribute to the pathophysiology of many diverse diseases including rhinitis and asthma [142-144].

Rodent and human mast cells express the A_{2A} , A_{2B} and A_3 receptors [145-149]. It has been reported that adenosine and inosine binding to A_3 receptors expressed in mast cell membranes induces degranulation and release of vasoactive mediators [99, 107]. Accordingly, the release of reactive mediators following A_3 activation in mast cells is directly related to the bronchoconstrictor effects observed after topical administration of adenosine in the airways of patients with asthma and chronic obstructive pulmonary disease [109, 150]. In contrast, inosine has no effect on airway caliber, indicating that bronchoconstriction is a specific response to adenosine [151]. Engagement of the A_3 receptors on rodent mast cells mediates degranulation and cell migration through a mechanism that involves phosphoinositide 3-kinase (PI3K) or protein C kinase (PKC) activation and an increase in intracellular Ca^{2+} [146, 147]. Moreover, A_1 and A_{2B} receptor activation has been related to the release of histamine, IL-8, IL-4 and IL-13 by mouse and human mast cells [97, 152-154]. In contrast to the A_1 and A_{2B} receptors, A_{2A} activation results in the suppression of histamine and tryptase release from human mast cells [155]. A_{2A} and A_{2B} receptors provide a balanced control mechanism for mast cell activation. It is possible that at low concentrations of adenosine, only the 'off' signal provided by the engagement of the higher affinity A_{2A} receptors prevails, thus downregulating mast-cell mediator release. Conversely, in situations in which high concentrations of adenosine are reached, such as in asthma and COPD [156], the low-affinity A_{2B} receptor becomes activated, resulting in significant mast cell degranulation [108, 109, 157]. Although the effects of adenosine regarding mast cell activation are well described, there is a lack of information regarding the effects of inosine on the activation of the A_3 receptor. Recently, a study from our group suggested that inosine can activate A_{2A} , A_{2B} and A_3 receptors and decrease mast cell migration during allergic pulmonary inflammation, suggesting that inosine can modulate allergic inflammation [101].

Lymphocytes cells play a vital role in the induction of adaptive immune responses and in steering them toward particular effector phenotypes [119]. Several pieces of evidence suggest that adenosine and inosine generated in the site of inflammation can modulate lymphocyte function [23, 24, 28]. Experimental data demonstrated that in ConA-induced liver injury (an *in vivo* model mediated by T cells), inosine inhibited hepatocyte apoptosis and reduced the accumulation of proinflammatory cytokines (e.g., TNF- α) and alanine transaminase in $A_3^{+/+}$ but not $A_3^{-/-}$ or $A_2^{-/-}$ mice, suggesting the endogenous inosine can influence inflammatory responses and indicating the importance of A_3 receptors in controlling liver injury [28].

The extracellular adenosine generated in inflammatory or hypoxic environments affects regulatory T cell lymphocytes (Treg) through the activation of A_{2A} receptors. Treg cells are a specialized population of $CD4^+$ T cells implicated in the regulation of immune responses, maintenance of immunological self-tolerance and protection from excessive inflammatory damage [24, 158]. A_{2A} receptor stimulation expanded the Treg population [159] coordinated by coexpressed CD39 ecto-ATPase/ADPase and CD73 ecto-5'-nucleotidase and generating adenosine pericellularly [72, 160]. The CD39- and CD73-mediated generation of extracellular adenosine might provide Treg cells with the capacity to directly inhibit DC and T effector cells by activating their respective cAMP-elevating A_{2A} receptors [160]. Consistent with these data, the A_{2A} receptor has been described to suppress the development of T-cell receptor (TCR) - stimulated naive T cells into both Th1 and Th2 cells [135], interfering with early development

as well as the late effector stages of Th1- and Th2-cell responses [135]. The activation of A_{2B} receptors seems to indirectly inhibit Th17 activation. A recent study using EAE in mice indicated that blocking A_{2B} receptors with specific antagonists, such as CVT-6883 and MRS-1754, alleviated the clinical symptoms of EAE and protected the CNS from immune system-mediated damage. Confirming this hypothesis, the deletion or blockade of A_{2B} receptors inhibited Th17 cell differentiation by blocking IL-6 production from APCs such as dendritic cells. The activation of phospholipase C β -protein kinase C and p38 MAPK pathways was found to be involved in A_{2B} -mediated IL-6 production, suggesting A_{2B} as a target for the development of anti-multiple sclerosis drugs and indicating that adenosine might participate in regulation of this pathology [98].

6. Overall effect of adenosine and inosine on inflammatory diseases

6.1. Asthma and COPD

A great deal of evidence suggests that adenosine plays a detrimental role in asthma and COPD and perhaps other chronic airway disorders [161]. The potential role of adenosine triphosphate and adenosine in the pathogenesis of asthma and COPD has been supported by the bronchoconstrictor effects observed after their topical administration in the airways of patients with these diseases and the lack of reaction in healthy subjects [150, 162-164]. However, there is some controversy about the role of adenosine during allergic reactions in the airways, such as asthma. When administrated topically, as mentioned above, it seems to trigger airway hyperactivity, but the administration of non-selective and selective agonists of different adenosine receptors can suppress (such as adenosine A_{2A} receptors) [165-167] or contribute with airway allergic inflammation, as described below.

Treatment with CGS21860, a selective A_{2A} receptor agonist, reduced the number of leukocytes in bronchoalveolar lavage fluid, protein content and eosinophil peroxidase activity in Brown Norway rats immunized and challenged with OVA, a compound similar to the glucocorticosteroid budesonide [165]. Moreover, treatment with NECA, a non-selective adenosine A_2 receptor agonist, reduced the total leukocyte infiltration and eosinophilia in a model of allergic airway inflammation [166]. Interestingly, inosine but not adenosine was described to reduce leukocyte infiltration into the lungs, Th2 pro-inflammatory cytokine levels and improve pulmonary mechanics in OVA-induced airway inflammation through a mechanism involving the A_{2A} and A_3 receptors [101]. Consistent with these data, inosine and its stable analogue INO-2002 were described to reduce LPS-induced airway inflammation [26, 168]. Several studies have hypothesized that the bronchial response to adenosine observed in asthma and COPD in humans can be attributed to an indirect mechanism involving mast cell activation, likely via A_{2B} or A_3 receptors and the release of mediators such as histamine and leukotriene (LT)C₄ [109, 150, 169]. These are low-affinity receptors that can modulate the deleterious effect of high concentrations of adenosine in chronic airways diseases [108, 109, 157]. Likely, adenosine signaling through the A_{2B} receptor also plays a role in asthma development, promoting the upregulation of pro-inflammatory cytokines, leukocyte migration and airway

remodeling (Figure 3) [170, 171]. Other studies have described a pro-fibrotic role for A_{2B} receptor signaling, which results in the differentiation of human pulmonary fibroblasts into collagen-producing myofibroblasts, increasing the production of the pro-fibrotic molecule fibronectin in alveolar epithelial cells [147, 161, 172]. These data demonstrate the potential involvement of A_{2B} receptors in the remodeling and fibrosis observed in asthma and COPD. Although the A_3 receptors appear to reduce inflammation and eosinophil activation in humans, in mice, A_3 activation induces mast cell degranulation and increases inflammation, activating eosinophils and mucus production [161, 173].

The literature data describes differences in mast cell expression between rodents and humans and maybe it can explain the different effects of adenosine in modulating these cells during asthma and allergy. The adenosine A_3 receptor that was expressed in mast cells in rodents [174], have recently been described to be expressed in human lung mast cells [175]. While the expression of adenosine A_{2A} and A_{2B} receptors in rats was not described; in mice, both were expressed in bone marrow derived mast cells. The A_{2A} receptors were also described in cardiac mast cells, in mice [174]. In humans, both receptors have been described in lung mast cells and in human mast cells line HMC-1 [174, 176]. The adenosine A_1 receptor were not described to be expressed in mice and humans but recent data have shown that agonists of adenosine A_1 receptor can potentiate human cultured mast cell activation, suggesting a modulatory effect [152, 177, 178].

The A_1 receptor is also described to have pro or anti-inflammatory effects on airway inflammation. Treatment with an A_1 receptor antagonist [179] or with antisense oligodeoxynucleotides targeting this receptor reduced the bronchoconstrictor responses in an allergic rabbit model [180]. Conversely, in knockout $A_1^{-/-}$ mice, an increase in transmigration of polymorphonuclear cells and microvascular permeability in comparison to wild type mice was observed in a model of LPS-induced lung injury, suggesting a possible protective effect of the A_1 receptor in airways [181]. The engagement of adenosine receptors on inflammatory and pulmonary cells appears to play an important role in regulating chronic lung disorders such as asthma and COPD, so the complete and full characterization of adenosine receptor subtype distribution in the airways and their specific role in the response to adenosine and inosine in health and disease is important for the development of new therapies to treat asthma and COPD [161].

6.2. Skin inflammation and wound healing

The skin is a highly specific immune defense organ. Physical, chemical or immune-specific insults rapidly evoke cellular responses, characterized by the increased expression of a wide range of pro-inflammatory mediators [182]. Controlling the extent of an immune response is thus a major challenge for maintaining skin integrity, which is of paramount importance for host survival [183]. In this context, several lines of evidence have shown that adenosine and adenosine receptors contribute to the regulation of skin inflammation.

A recent study showed that activated Treg cells can produce adenosine in a CD39-dependent manner and abrogated the ear-swelling reaction induced by 2,4,6-trinitro-1-chlorobenzene (TNCB), indicating a role of adenosine in the Treg cell-induced suppression of contact

hypersensitivity responses. Moreover, the same study demonstrated that adenosine's effects involve the impairment of effector T cell adhesion to inflamed endothelium and downregulation of E- and P- selectin in the vascular endothelium [184]. A complementary study of IL-10-deficient (IL-10^{-/-}) Tregs showed impaired adenosine production, which contributes with their inability to suppress contact hypersensitivity responses, indicating that the reduced suppressive effects observed may not be exclusively attributable to the lack of IL-10 production [185]. Several lines of evidence indicated that adenosine's effects on skin are mediated by the activation of adenosine receptors.

The activation of A₁ receptors has been demonstrated to decrease the numbers of circulating neutrophil granulocytes and ear swelling in a model of stress-induced contact hypersensitivity response [186]. In addition to receptor A₁ activation, activation of receptor A_{2A} was described to reduce leukocyte activation and to prevent ischemia reperfusion wound formation in a rat model of a pressure ulcer [187]. Evidence from experiments performed on A_{2A} receptor knockout mice and with CGS21680 demonstrated that the A_{2A} receptor is the main adenosine receptor subtype involved in wound healing [149, 188]. In addition, histological analysis of mice treated with the same agonist showed faster re-epithelialization and increased matrix deposition, fibroblast density and vascularity in the granulation tissue of the agonist treated wounds as soon as 3 days after injury [188]. A study from the same group revealed that treatment of human microvascular endothelial cells (HMVEC) with the selective A_{2A} receptor agonists CGS21680 and MRE0094 (Sonedenoson) favors vascular tube formation by cultured HMVEC and downregulated the antiangiogenic matrix protein thrombospondin 1 (TSP1) secretion by these cells, indicating that A_{2A} activation induces angiogenesis [189]. Furthermore, treatment with MRE0094 increased the rate of wound closure in comparison to recombinant human platelet-derived growth factor (Becaplermin gel), an agent currently used to promote the healing of diabetic ulcers, indicating the importance of A_{2A} receptors in wound healing [190]. Moreover, the A_{2A} and A_{2B} receptors were described to contribute to tissue formation because their activation leads to enhanced fibroblast and endothelial cell migration [191].

Adenosine seems to have a fibrogenic role in the skin. A study using ADA-deficient mice reported a direct fibrogenic effect of adenosine on the skin, and pharmacological treatment with the A_{2A} receptor antagonist ZM-241385 prevented the development of dermal fibrosis by reducing dermal collagen content and the expression of profibrotic cytokines and growth factors (Figure 3) [192]. The data mentioned above are interesting and strongly suggest that adenosine and A_{2A} receptors have important modulatory effects in skin homeostasis. Although several studies have shown a role for adenosine in the skin, nothing has been found regarding a role for inosine. Additional studies addressing the real role of the purinergic system in the skin would be extremely useful to improving wound management and care, as well as controlling chronic inflammatory diseases in skin.

6.3. Arthritis

Arthritis is the term used to designate a particular pathological condition that encompasses a constellation of more than 100 diseases, among which osteoarthritis (OA) and rheumatoid arthritis (RA) stand out. OA is the most common adult joint disease and is increasing in

frequency and severity, with an estimated US prevalence of more than 25 million affected adults [193]. It is characterized by gradual loss of articular cartilage and is therefore being considered a slowly progressing degenerative disease. The etiology of arthritis involves biochemical and genetic factors as well as repetitive mechanical injury, which has been proposed as the critical mechanisms contributing to alterations in the normal functional activities of chondrocytes, the main cellular component of hyaline cartilage, disrupting chondrocyte–matrix associations and culminating in the initiation and progression of OA [194].

In early OA, a transient proliferative chondrocytes response (clonal growth) occurs along with increased synthesis of the cartilage matrix as an early repair attempt and increased synthesis of catabolic cytokines (such as IL-1, TNF- α and IL-18) and matrix-degrading enzymes (such as metalloproteinases, especially collagenases, and aggrecanases). Fibroblast- and macrophage-like cells in the synovia also generated catabolic cytokines in response to breakdown products from the damaged cartilage. All these events contribute greatly to the local loss of proteoglycans and cleavage of type II collagen, which initially occurs at the cartilage surface, contributing to water content increases and loss of tensile strength in the cartilage matrix as the lesion progresses [195, 196]. It is characterized by several inflammatory cascades, which all lead towards a final common pathway in which persistent synovial inflammation and associated damage to articular cartilage and underlying bone are present [197].

One inflammatory cascade that deserves attention in the pathogenesis of RA is the overproduction and overexpression of TNF- α . Interactions between T and B lymphocytes, synovial-like fibroblasts and macrophages are likely to be involved in TNF- α and IL-6 overproduction, contributing to both synovial inflammation and joint destruction [198]. In addition to inflammatory cytokines, rheumatoid factors are key pathogenic markers of classic RA. In this case, the immunoglobulins IgM and IgA are directed against the Fc fragment of immunoglobulin IgG, resulting in the formation of immune complexes, which are able to activate the complement system, initiating an immune response [199]. Adenosine has a known therapeutic potential against inflammatory joint diseases; studies have demonstrated its ability to limit synoviocyte [200] and chondrocyte [201] inflammatory responses and to minimize articular damage in adjuvant-induced models of arthritis in rats [202].

In an interesting study, Tesch and colleagues demonstrated that endogenously produced adenosine regulates articular cartilage matrix homeostasis *in vitro*. In this study, authors showed that the depletion of endogenous adenosine through exposure to adenosine deaminase (ADA) in cartilage explants resulted in cartilage matrix degradation, involving matrix metalloproteinases-3 and -13 (MMP-3, MMP-13), prostaglandin E₂ (PGE₂), and nitric oxide (NO) release. In addition to these data, this study suggested that endogenously released adenosine can regulate chondrocyte production of matrix-degrading enzymes and matrix loss, an effect believed to be in part mediated via A_{2A} receptors, because N⁶-[2-(3,5-dimethoxyphenyl)-ethyl]adenosine (DPMA, an A_{2A} receptor selective agonist) was able to prevent the release of PGE₂, NO and glycosaminoglycan (GAG) (Figure 3) [203]. Another *in vitro* study demonstrated that adenosine, N⁶-methyladenosine (a substituted adenosine derivative that is resistant to breakdown by adenosine deaminase), DPMA and 5'-N-ethylcarboxamidoadeno-

sine (NECA, a non-selective adenosine receptor agonist) suppressed NO production by LPS-stimulated equine chondrocytes [201]. The addition of exogenous adenosine and erythro-9-(2-Hydroxy-3-nonyl) adenine hydrochloride (EHNA, an adenosine deaminase inhibitor) further suppressed NO production by LPS-stimulated chondrocytes [201]. These two studies clearly demonstrate the protective effect of adenosine against degradation of articular cartilage in vitro, suggesting its potential to prevent joint damage and hence its effectiveness in the prevention of joint diseases such as osteoarthritis.

In the second half of the 1980s, remarkable attention was focused on investigating the role played by ADA in RA pathophysiology, which provided the first evidence of the role of adenosine in RA. These studies showed increased levels of ADA activity in the synovial fluid from patients with seropositive rheumatoid arthritis, suggesting a local release of this catabolic enzyme by cells within joints [204]. Subsequent investigations were aimed at characterizing the activities of different ADA isoforms in tissues, cell homogenates and serum samples obtained from patients with rheumatic disorders. The highest level of enzyme activity was found in lymphocytes and monocytes from patients with rheumatoid arthritis, and ADA-2 was the isoform specifically expressed in monocytes [205]. Iwaki-Egawa and colleagues observed a significant positive correlation between high activity of ADA isoforms in the synovial fluid of rheumatic patients and metalloproteinase-9 (MMP-9), an enzyme critical to the regulation of the cell matrix composition [206]. These results suggest that the high activity of ADA in the synovial fluid of patients with RA and consequently the reduction in local levels of adenosine are directly related to the development and maintenance of RA. In support of this hypothesis, Forrest and colleagues demonstrated that adenosine is able to suppress the elevated levels of proinflammatory cytokines such as TNF- α and IL-1 β in RA patients and that this effect appears to be mediated by adenosine receptors [207].

Recent studies have shown adenosine A₃ receptor upregulation in RA patients, suggesting the potential of this receptor as a therapeutic target. In agreement with these results, after oral treatment with CF101, a selective A₃ receptor agonist, a marked decrease in RA clinical manifestations including inflammation, pannus formation, cartilage destruction and bone reabsorption and lysis was observed [208].

6.4. Ischemia

Ischemia is defined as a lack of blood supply to an organ or tissue, resulting in cellular oxygen deprivation. Although ischemia itself is a serious condition, the phenomenon that seems to be the definitive treatment for ischemia, reperfusion of the ischemic tissue, can promote further tissue injury, especially after prolonged ischemia [209]. Thus, the tissue can be injured by both ischemic and reperfusion processes and can be defined as ischemia reperfusion (IR) injury. IR injury involves a complex cascade of events including oxidative stress, inflammation and interactions among many cell types [209]. Furthermore, it has widespread clinical relevance and is encountered in a variety of surgical settings (e.g., transplantation, cardiopulmonary bypass and aneurysm repair) as well as non-surgical settings (e.g., myocardial infarction, stroke, hemorrhage, trauma and shock).

One of the potentially most striking features of IR injury involves the generation of reactive oxygen species (ROS) (e.g., superoxide, peroxynitrite, hydrogen peroxide, and hydroxyl radical) [210, 211], especially during reperfusion of ischemic tissue, which, in turn, initiates an inflammatory cascade resulting in direct oxidative injury to cells and stimulation of pro-inflammatory mediators such as cytokines, chemokines and cell-adhesion molecules. Briefly, circulating and resident leukocytes, such as neutrophils, lymphocytes and macrophages as well as tissue resident cells, such as dendritic cells, contribute to the immune response to IR injury, particularly infiltrating neutrophils, which can impose significant tissue injury through ROS generation and further release of cytokines and proteases [212].

Strong evidence has indicated that cellular responses to hypoxia include robust increases in extracellular adenosine and signaling events through adenosine receptors. The hypoxic adenosine response in acute injury settings is able to promote tissue adaptation during hypoxia, including restoration of normal oxygen levels, enhancing metabolic ischemia tolerance and dampening inflammation [213]. Preclinical studies have shown that adenosine signaling is beneficial in ischemic acute injury in the lung [214, 215], kidney [216], heart [217], gastrointestinal track [218] and liver [73]. Some studies have demonstrated that chronic elevations of adenosine can contribute to tissue fibrosis in different organs including the lungs [170, 219], liver [220], skin [221], kidney [222] and following transplants [223]. Studies using CD39 and CD73 knockout mice, which lack both the enzyme that converts ATP to ADP/AMP and the enzyme that converts AMP to adenosine, and inhibitors of these enzymes demonstrated enhanced inflammation and tissue injury in models of hypoxia and ischemic injury [6, 224] concurrent with reduced production of adenosine. These results suggest that elevated extracellular levels of adenosine may display a protective effect in models of hypoxia and ischemia injury. An interesting strategy used to enhance extracellular levels of adenosine is the prevention of adenosine uptake by equilibrate nucleoside transporters (ENTs) [225].

Recently, Grenz and colleagues demonstrated that treatment with dipyridamole, an inhibitor of ENTs, led to increased adenosine levels in association with tissue protection in a mouse model of ischemic acute kidney injury [226]. Furthermore, genetic deletion of ENTs resulted in selective protection in ENT1^{-/-} mice. A more detailed analysis using adenosine receptor-knockout mice exposed to acute kidney injury showed that renal protection promoted by ENT inhibitors involves the A_{2B} adenosine receptor. In addition, Eltzschig and colleagues demonstrated that the A_{2B} receptor serves anti-inflammatory and tissue-protective roles in various acute injury tissue models associated with hypoxic or ischemic injury including the heart [217], lung [227], intestine [218] and kidney [226] using genetic (A_{2B} receptor knockout mice) and pharmacological (A_{2B} receptor agonists) approaches. In summary, adenosine receptor signaling in hypoxic or ischemia-reperfusion injury varies depending on the receptor activation and cell/tissue type. In general, A₁ and A_{2A} receptor activation has been shown to be protective in the lung, kidney, heart and liver, whereas the role of the A_{2B} and A₃ receptors remains obscure. A considerable number of studies both *in vitro* and *in vivo* have demonstrated the potential of inosine in preventing injury in different models of hypoxia or ischemia.

In the early 1980s, it was demonstrated that infusion of 4 mM of inosine presented a protective effect in fetal mouse heart organ cultures deprived of oxygen, a model of ischemic-like injury

[228]. At the CNS level, purine catabolite concentrations were monitored for up to 15 h in the auditory and somatosensory cortices of cats using microdialysis/HPLC and hydrogen clearance following middle cerebral artery occlusion (MCAo). MCAo led to the release of inosine and its metabolite hypoxanthine from the ischemic cortex in stroke animals, which reached maximum levels 1-2 h after the onset of ischemia [229]. Cerebral infarct (stroke) often causes devastating and irreversible losses of function, in part because of the brain's limited capacity for anatomical reorganization. In an animal cerebral ischemia model, which results from infarction in the right dorsolateral cerebral cortex and underlying striatum, continuous infusion of inosine 50 mM into the cisterna magna using osmotic minipumps (0.25 μ L/h) stimulated neurons on the undamaged side of the brain to extend new projections to denervated areas of the midbrain and spinal cord and consequently improved performance on several behavioral measures in adult rats [230].

Taken together, these data suggest that inosine promotes neuroregeneration after stroke. An interesting study recently published Shen and colleagues demonstrated that intracerebroventricular administration of inosine (25 nmol/L in 25 μ L) before middle cerebral artery occlusion in rats resulted in a higher level of locomotor activity (lasting up to 2 weeks after stroke) and less cerebral infarction [231]. In addition, they indicated that coadministration of a selective A_3 receptor antagonist, MRS1191, significantly attenuated inosine-mediated protection. Moreover, in the electrophysiological study, inosine antagonized glutamate-induced excitation in cerebral cortical neurons. In summary, the authors proposed that inosine may inhibit glutamate postsynaptic responses and reduce cerebral infarction via the activation of the A_3 receptor, presenting a neuroprotective action (Figure 3) [231].

6.5. Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a common and lifelong disabling gastrointestinal disease that includes Crohn's disease (CD) and ulcerative colitis (UC) [232]. Worldwide UC incidence varies greatly, ranging from 0.5-24.5 /100,000 habitants, and CD ranges from 0.1-16/100.000 habitants, with the highest incidence in developed countries [233]. Genetic, environmental, and immunological factors interplay in a complex manner to contribute to the genesis of IBD. Generally, the presence of one or more genetic factors triggers an over-reaction of the host mucosal immune system to normal constituents of the mucosal microflora. This over-reaction involves either a Th1-type T cell-mediated inflammation in the case of Crohn's disease or a Th2-type T cell-mediated inflammation in ulcerative colitis. This inflammatory process leads to the release of multiple cytokines, including interferon (INF)- γ , tumor necrosis factor (TNF)- α , interleukin (IL)-1, IL-6, IL-8, IL-12, IL-13, IL-17, and monocyte chemotactic protein (MCP)-1 [234]. These cytokines are responsible for the attraction and activation of neutrophils, eosinophils, mast cells and macrophages, which, in turn, produce large amounts of unstable chemical species such as reactive oxygen species (ROS) or oxyradicals (i.e., superoxide anions, hydrogen peroxide, hydroxyl radicals, and peroxynitrite), mediators that contribute greatly to the tissue injury seen in IBDs [235, 236].

In early stages of immune or inflammatory response, significantly elevated extracellular concentrations of adenosine are anti-inflammatory and tissue protective. Thus, one of the most

widely used strategies to study the effects of adenosine on different inflammatory processes involves inhibition of adenosine catabolism, mainly through inhibition of adenosine deaminase (increasing adenosine levels) or direct activation of adenosine receptors. Antonioli and colleagues have demonstrated, using a model of 2,4-dinitrobenzenesulfonic acid (DNBS)-induced colitis, that treatment with 4-amino-2-(2-hydroxy-1-decyl) pyrazole[3,4-*d*]pyrimidine (APP; novel adenosine deaminase inhibitor) or erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride (EHNA; standard adenosine deaminase inhibitor) for up to 7 days was able to increase food intake and weight gain and ameliorate macroscopic and microscopic inflammatory colonic alterations with a concomitant reduction of mucosal and plasmatic pro-inflammatory mediators such as TNF- α and interleukin-6 [237]. In the same vein, Siegmund and colleagues showed that treatment with {4-amino-1-(5-amino-5-deoxy-1- β -d-ribofuranosyl)-3-bromo-pyrazol[3,4-*d*] pyrimidine} (GP515), an inhibitor of adenosine kinase, the enzyme responsible for the conversion of adenosine to AMP, resulted in a significant improvement of mucosal morphology and clinical score (weight loss, stool consistency, and bleeding) as well as decreased IFN- γ concentration in the colonic tissue in dextran sulfate sodium (DSS)-induced colitis [238]. These results clearly demonstrate that increased levels of adenosine can attenuate mucosal inflammation in experimental colitis. The activation of adenosine A_{2A} receptors seems to be one of the main mechanisms involved in the effects of adenosine (Figure 3).

Odashima and colleagues studied the anti-inflammatory effects of ATL-146e in acute and chronic rabbit formalin-immune complex models of colitis and the SAMP1/YitFc mouse model of spontaneous ileitis. ATL-146e (20 and 40 μ g/kg, i.p.) significantly reduced the acute and chronic inflammatory index and tissue necrosis and prevented mortality. Furthermore, TNF- α , IFN- γ and IL-4 concentrations were significantly suppressed with ATL-146e treatment in supernatants from cultures of mesenteric lymph node cells of SAMP1/YitFc mice. Thus, these results support important anti-inflammatory actions of ATL-146e in the intestine, including the suppression of lymphocyte-derived cytokine-mediated pro-inflammatory responses, suggesting that the activation of A_{2A} receptor-mediated signaling through selective agonists may be a novel therapeutic approach for patients with IBD [77]. To this end, Cavalcante and colleagues evaluated the effects of a new selective A_{2A} receptor agonist (ATL 313) on *Clostridium difficile* toxin A-induced injury in murine ileal loops. ATL 313 treatment directly into ileal loops significantly reduced toxin A-induced secretion and edema, prevented mucosal disruption, neutrophil infiltration, TNF- α production, adenosine deaminase activity and prevented toxin A-induced cell death. Based on these findings, the adenosine system may represent a promising target for therapies for inflammatory intestinal disorders, either by manipulating its metabolism or through direct activation of its receptors, especially A_{2A} receptors [239].

6.6. Multiple sclerosis

MS is an autoimmune disease mediated by T cells that is characterized by CNS demyelination and neurodegeneration [98]. There are many other diseases that are associated with inflammation of the CNS, including meningitis, encephalitis, among others. However, one of the most common is MS. It affects more than 2.5 million people around the world and is characterized by the loss of neurological function, which occurs due to axonal demyelination and

represents the main symptom of the disease. Recurrences, commonly associated with increased lymphocyte infiltration of the CNS, make the patient increasingly weak over time [240-242].

Studies in mice using an EAE model demonstrated that adenosine signaling modulates the development of EAE. A_{2B} receptor blockade with CVT-6883 and MRS-1754 (both specific antagonists of A_{2B}) promoted a reduction in the symptoms of EAE, thus protecting against CNS immune response damage. Moreover, both deletion and A_{2B} receptor blockade promoted inhibition of the differentiation of Th17 cells due to the reduction of IL-6 production by APCs (dendritic cells), suggesting that the A_{2B} receptor is a potential new target for "anti-multiple sclerosis" drug action [98]. Post-mortem analysis of brain tissue from patients diagnosed with MS showed the presence of cells with high expression of inducible nitric oxide synthase (iNOS) and nitrotyrosine in characteristic lesions of the disease [243, 244].

Figure 3. Adenosine deleterious and protective effects in inflammation and immunity. A_1 , adenosine A_1 receptor; A_{2A} , adenosine A_{2A} receptor; A_{2B} , adenosine A_{2B} receptor; A_3 , adenosine A_3 receptor; ATP, adenosine triphosphate; AMP, adenosine monophosphate; ADO, adenosine; CD 39, ectonucleoside triphosphate diphosphohydrolase.

Peroxynitrite, the end product of iNOS activity, which leads to the formation of NO and then to superoxide reaction, is highly reactive, causing a variety of toxic chemical changes in nerve tissue, including the nitration of tyrosine residues [245]. It has been previously described that UA, which is the final product of purine metabolism in humans, is a peroxynitrite scavenger [246], and for this reason UA therapy has become a potential method to alleviate the neuronal damage induced by peroxynitrite in MS treatment. Furthermore, inosine, an endogenous precursor of UA, seems to be an attractive candidate for the treatment of MS, given that patients who received inosine showed some evidence of clinical improvement and no sign of disease progression [247]. In addition, recent studies using adenosine receptor antagonists as well as

mice that were not capable of hydrolyzing adenosine from extracellular AMP (CD73^{-/-}), have suggested that blockade of adenosine receptors or CD73 deletion protected mice from EAE, decreasing lymphocyte infiltration in the CNS [248]. One very interesting note about the involvement of the adenosinergic system during EAE is that both CD73 and A_{2A} receptor presented increased expression in the choroid plexus [248, 249] in comparison to other regions of the CNS. This result shows that choroid plexus is higher permeable to lymphocytes than other regions, therefore, it is speculated that ATP, released as result of damage, and its conversion to adenosine represent a signal that regulates the entry of lymphocytes into the CNS [250, 251].

7. Adenosine receptors as drug targets: future directions for new drug development

There is increasing interest in the therapeutic potential of adenosinergic compounds (including receptor agonists and antagonists, enzyme inhibitors and others), and many adenosine compounds have been evaluated for therapeutic use. For a long time, adenosine itself was the only adenosine agonist used in humans. It is widely used in the treatment of paroxysmal supraventricular tachycardia (Adenocard®) due to the activation of A₁ receptor and is also used as a diagnostic tool for myocardial perfusion imaging (Adenoscan®) as a consequence of its A_{2A} receptor-activating effects, resulting in vasodilation [87].

Spinal administration of adenosine and adenosine analogs in humans also exhibited an analgesic effect. A phase I clinical safety study in healthy volunteers demonstrated that 1000 µg of adenosine given intrathecally led to a significant decrease in mustard oil-induced inflammatory pain and tourniquet-induced ischemic pain and decreased areas of secondary allodynia after skin inflammation with low side effects [252]. Recently, some studies have demonstrated new approaches regarding the development of allosteric modulators that enhance the potency of endogenous agonists and aim to minimize the side effects [253]. Allosteric enhancers of the adenosine A₁ receptor have been linked to anti-arrhythmic and anti-lipolytic activity and also have therapeutic potential as analgesics. Oral administration of the A₁ receptor-selective allosteric enhancer T-62 was shown to reduce hypersensitivity in carrageenan-inflamed rats and was approved for phase I clinical trials for neuropathic pain treatment [254]. As was previously discussed, various studies of selective agonists and antagonists demonstrated pro-inflammatory effects of A₁ receptors in different inflammatory models [47]. One interesting study showed a decrease in leukocyte infiltration and reduced lung edema in rats treated with the A₁ receptor antagonist L-97-1 [255]. Furthermore, the selective A_{2A} receptor agonists, apadenoson, binodenoson and sonedenoson have been considered candidates for clinical use in cardiovascular disorders [189, 256, 257]. These agonists are of interest as vasodilator agents in cardiac imaging [258] and as inflammation suppressors. Moreover, as reported by Press and Fozard (2010), two clinical trial applications from Santen Pharmaceuticals claim that use of agonist of adenosine receptors such as regadenoson and sonedenoson [259] is useful in the treatment of glaucoma.

The A_{2A} receptor agonist BVT.115959 from Biovitrum completed clinical trials for diabetic neuropathic pain, and it was well tolerated but did not significantly improve pain symptoms [260]. The primary indication claimed for A_{2A} receptor antagonists is in Parkinson's disease [261] because animal studies indicated that adenosine A_{2A} receptors are localized with dopamine D₂ receptors in the striatum and provide an antagonistic interaction between adenosine and dopamine [262]. Vernalis plc and Biogen Idec are currently profiling BIIB014 (V2006) in Phase II clinical trials for Parkinson's disease [263]. Patents for A_{2B} receptor antagonists generally claim asthma and allergic diseases as the primary indications, in line with current views on the receptor's role in vivo [163]. CVT-6883, an A_{2B}-adenosine receptor antagonist, is in clinical development for the treatment of asthma, and this antagonist significantly inhibits in vivo growth of B-16 tumors compared to taxol, as described in a recent review [259].

A₃ receptor selective agonists are also currently in clinical trials and exhibit nanomolar affinity to the receptor. In this context, CF101 (Can-Fite Biopharma) and CI-IB-MECA (CF102) are in trials for autoimmune inflammatory disorders and liver cancer, respectively. Two other A₃ receptor selective agonists, CP-608,039 and its N6-(2,5-dichlorobenzyl) analog, CP-532,903, were previously under development for cardioprotection [264, 265]. Allosteric modulators of the A₃ receptor have also been developed, such as imidazoquinolines (LUF6000), which have been shown to inhibit adjuvant-induced paw joint swelling in an arthritis rat model [265].

8. Conclusion

This chapter presented a general and updated review of the therapeutic potential of adenosinergic system (including receptor agonists and antagonists, enzyme inhibitors and others) in the control of inflammatory and immune responses. Recently, it is becoming clear that both adenosine and inosine play primordial roles in regulating the inflammatory process, working together for example as danger signals, in order to constitute a homeostatic mechanism of tissue integrity. Furthermore, adenosine as well as inosine effects are mediated by adenosine receptors and depending on the tissue or cells where they are expressed, pro-inflammatory or anti-inflammatory effects are observed. In this regard, several preclinical studies are conducted to clarify the role of these purines during the inflammatory response and to better understand the adenosine receptors activation which has become an interesting target to control inflammation. Currently, adenosine is used in the clinical setting, especially in emergency units, to convert sinus rhythm of paroxysmal supraventricular tachycardia (PSVT) to normal sinus rhythm, with excellent cost/effectiveness. Besides the differences in receptor expression between rodents and humans, the use of experimental animal models that can mimic the main features of inflammatory and immune diseases, the improvement of biochemical, genetic and molecular techniques have helped us to better establish a translation between preclinical and clinical effects of adenosine and inosine, and to develop and test selective agonists and antagonists of adenosine receptors that can be used in the future treatment of chronic diseases with inflammatory and immune features.

Author details

Fernanda da Rocha Lapa^{1,2,3}, Sérgio José Macedo Júnior^{1,2,3}, Murilo Luiz Cerutti^{2,3} and Adair Roberto Soares Santos^{2,3}

1 Department of Pharmacology, Universidade Federal de Santa Catarina, Brazil

2 Laboratory of Neurobiology of Pain and Inflammation, Universidade Federal de Santa Catarina, Brazil

3 Department of Physiological Sciences, Universidade Federal de Santa Catarina, Brazil

References

- [1] Bonora M, Patergnani S, Rimessi A, De Marchi E, Suski JM, Bononi A, et al. ATP synthesis and storage. *Purinergic Signal* 2012;8:343-57.
- [2] Sawynok J, Liu XJ. Adenosine in the spinal cord and periphery: release and regulation of pain. *Prog Neurobiol* 2003;69:313-40.
- [3] Eltzschig HK, Faigle M, Knapp S, Karhausen J, Ibla J, Rosenberger P, et al. Endothelial catabolism of extracellular adenosine during hypoxia: the role of surface adenosine deaminase and CD26. *Blood* 2006;108:1602-10.
- [4] Hasko G, Sitkovsky MV, Szabo C. Immunomodulatory and neuroprotective effects of inosine. *Trends Pharmacol Sci* 2004;25:152-7.
- [5] Fredholm BB, AP IJ, Jacobson KA, Linden J, Muller CE. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors--an update. *Pharmacol Rev* 2011;63:1-34.
- [6] Hart ML, Gorzolla IC, Schittenhelm J, Robson SC, Eltzschig HK. SP1-dependent induction of CD39 facilitates hepatic ischemic preconditioning. *J Immunol* 2010;184:4017-24.
- [7] Kohler D, Eckle T, Faigle M, Grenz A, Mittelbronn M, Laucher S, et al. CD39/ectonucleoside triphosphate diphosphohydrolase 1 provides myocardial protection during cardiac ischemia/reperfusion injury. *Circulation* 2007;116:1784-94.
- [8] Fletcher JM, Lonergan R, Costelloe L, Kinsella K, Moran B, O'Farrelly C, et al. CD39+Foxp3+ regulatory T Cells suppress pathogenic Th17 cells and are impaired in multiple sclerosis. *J Immunol* 2009;183:7602-10.
- [9] Friedman DJ, Kunzli BM, YI AR, Seigny J, Berberat PO, Enjyoji K, et al. From the Cover: CD39 deletion exacerbates experimental murine colitis and human polymor-

phisms increase susceptibility to inflammatory bowel disease. *Proc Natl Acad Sci U S A* 2009;106:16788-93.

- [10] Ralevic V, Burnstock G. Receptors for purines and pyrimidines. *Pharmacol Rev* 1998;50:413-92.
- [11] da Rocha Lapa F, da Silva MD, de Almeida Cabrini D, Santos AR. Anti-inflammatory effects of purine nucleosides, adenosine and inosine, in a mouse model of pleurisy: evidence for the role of adenosine A2 receptors. *Purinergic Signal* 2012;8:693-704.
- [12] Grenz A, Homann D, Eltzschig HK. Extracellular adenosine: a safety signal that dampens hypoxia-induced inflammation during ischemia. *Antioxid Redox Signal* 2011;15:2221-34.
- [13] Sitkovsky MV, Ohta A. The 'danger' sensors that STOP the immune response: the A2 adenosine receptors? *Trends Immunol* 2005;26:299-304.
- [14] Martinon F. Detection of immune danger signals by NALP3. *J Leukoc Biol* 2008;83:507-11.
- [15] Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol* 2010;11:373-84.
- [16] Rakoff-Nahoum S, Medzhitov R. Role of toll-like receptors in tissue repair and tumorigenesis. *Biochemistry (Mosc)* 2008;73:555-61.
- [17] Laliberte RE, Eggler J, Gabel CA. ATP treatment of human monocytes promotes caspase-1 maturation and externalization. *J Biol Chem* 1999;274:36944-51.
- [18] Communi D, Janssens R, Suarez-Huerta N, Robaye B, Boeynaems JM. Advances in signalling by extracellular nucleotides. the role and transduction mechanisms of P2Y receptors. *Cell Signal* 2000;12:351-60.
- [19] Riteau N, Baron L, Villeret B, Guillou N, Savigny F, Ryffel B, et al. ATP release and purinergic signaling: a common pathway for particle-mediated inflammasome activation. *Cell Death Dis* 2012;3:e403.
- [20] Trautmann A. Extracellular ATP in the immune system: more than just a "danger signal". *Sci Signal* 2009;2:pe6.
- [21] Elliott MR, Chekeni FB, Trampont PC, Lazarowski ER, Kadl A, Walk SF, et al. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature* 2009;461:282-6.
- [22] Sitkovsky MV. Use of the A(2A) adenosine receptor as a physiological immunosuppressor and to engineer inflammation in vivo. *Biochem Pharmacol* 2003;65:493-501.
- [23] Fredholm BB, Chern Y, Franco R, Sitkovsky M. Aspects of the general biology of adenosine A2A signaling. *Prog Neurobiol* 2007;83:263-76.

- [24] Sitkovsky MV. T regulatory cells: hypoxia-adenosinergic suppression and re-direction of the immune response. *Trends Immunol* 2009;30:102-8.
- [25] Liaudet L, Mabley JG, Soriano FG, Pacher P, Marton A, Hasko G, et al. Inosine reduces systemic inflammation and improves survival in septic shock induced by cecal ligation and puncture. *Am J Respir Crit Care Med* 2001;164:1213-20.
- [26] Liaudet L, Mabley JG, Pacher P, Virag L, Soriano FG, Marton A, et al. Inosine exerts a broad range of antiinflammatory effects in a murine model of acute lung injury. *Ann Surg* 2002;235:568-78.
- [27] Mabley JG, Pacher P, Liaudet L, Soriano FG, Hasko G, Marton A, et al. Inosine reduces inflammation and improves survival in a murine model of colitis. *Am J Physiol Gastrointest Liver Physiol* 2003;284:G138-44.
- [28] Gomez G, Sitkovsky MV. Differential requirement for A2a and A3 adenosine receptors for the protective effect of inosine in vivo. *Blood* 2003;102:4472-8.
- [29] Luscinskas FW, Ma S, Nusrat A, Parkos CA, Shaw SK. The role of endothelial cell lateral junctions during leukocyte trafficking. *Immunol Rev* 2002;186:57-67.
- [30] Rahimian R, Fakhfouri G, Daneshmand A, Mohammadi H, Bahremand A, Rasouli MR, et al. Adenosine A2A receptors and uric acid mediate protective effects of inosine against TNBS-induced colitis in rats. *Eur J Pharmacol* 2010;649:376-81.
- [31] Hasko G, Cronstein BN. Adenosine: an endogenous regulator of innate immunity. *Trends Immunol* 2004;25:33-9.
- [32] Burnstock G. Purine and pyrimidine receptors. *Cell Mol Life Sci* 2007;64:1471-83.
- [33] Jacobson KA, Gao ZG. Adenosine receptors as therapeutic targets. *Nat Rev Drug Discov* 2006;5:247-64.
- [34] Jaakola VP, Griffith MT, Hanson MA, Cherezov V, Chien EY, Lane JR, et al. The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist. *Science* 2008;322:1211-7.
- [35] Libert F, Van Sande J, Lefort A, Czernilofsky A, Dumont JE, Vassart G, et al. Cloning and functional characterization of a human A1 adenosine receptor. *Biochem Biophys Res Commun* 1992;187:919-26.
- [36] IJzerman AP, van der Wenden EM, van Galen PJ, Jacobson KA. Molecular modeling of adenosine receptors. The ligand binding site on the rat adenosine A2A receptor. *Eur J Pharmacol* 1994;268:95-104.
- [37] Olah ME, Ren H, Ostrowski J, Jacobson KA, Stiles GL. Cloning, expression, and characterization of the unique bovine A1 adenosine receptor. Studies on the ligand binding site by site-directed mutagenesis. *J Biol Chem* 1992;267:10764-70.

- [38] Tucker AL, Robeva AS, Taylor HE, Holeton D, Bockner M, Lynch KR, et al. A1 adenosine receptors. Two amino acids are responsible for species differences in ligand recognition. *J Biol Chem* 1994;269:27900-6.
- [39] Townsend-Nicholson A, Schofield PR. A threonine residue in the seventh transmembrane domain of the human A1 adenosine receptor mediates specific agonist binding. *J Biol Chem* 1994;269:2373-6.
- [40] de Ligt RA, Rivkees SA, Lorenzen A, Leurs R, AP IJ. A "locked-on," constitutively active mutant of the adenosine A1 receptor. *Eur J Pharmacol* 2005;510:1-8.
- [41] Kourounakis A, Visser C, de Groote M, AP IJ. Differential effects of the allosteric enhancer (2-amino-4,5-dimethyl-trienyl)[3-trifluoromethyl] phenyl]methanone (PD81,723) on agonist and antagonist binding and function at the human wild-type and a mutant (T277A) adenosine A1 receptor. *Biochem Pharmacol* 2001;61:137-44.
- [42] Bours MJ, Swennen EL, Di Virgilio F, Cronstein BN, Dagnelie PC. Adenosine 5'-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. *Pharmacol Ther* 2006;112:358-404.
- [43] Forman MB, Vitola JV, Velasco CE, Murray JJ, Dubey RK, Jackson EK. Sustained reduction in myocardial reperfusion injury with an adenosine receptor antagonist: possible role of the neutrophil chemoattractant response. *J Pharmacol Exp Ther* 2000;292:929-38.
- [44] Takahashi HK, Iwagaki H, Hamano R, Kanke T, Liu K, Sadamori H, et al. Effect of adenosine receptor subtypes stimulation on mixed lymphocyte reaction. *Eur J Pharmacol* 2007;564:204-10.
- [45] Satoh A, Shimosegawa T, Satoh K, Ito H, Kohno Y, Masamune A, et al. Activation of adenosine A1-receptor pathway induces edema formation in the pancreas of rats. *Gastroenterology* 2000;119:829-36.
- [46] Neely CF, Keith IM. A1 adenosine receptor antagonists block ischemia-reperfusion injury of the lung. *Am J Physiol* 1995;268:L1036-46.
- [47] Magata S, Taniguchi M, Suzuki T, Shimamura T, Fukai M, Furukawa H, et al. The effect of antagonism of adenosine A1 receptor against ischemia and reperfusion injury of the liver. *J Surg Res* 2007;139:7-14.
- [48] Lee HT, Gallos G, Nasr SH, Emala CW. A1 adenosine receptor activation inhibits inflammation, necrosis, and apoptosis after renal ischemia-reperfusion injury in mice. *J Am Soc Nephrol* 2004;15:102-11.
- [49] Lee HT, Xu H, Nasr SH, Schnermann J, Emala CW. A1 adenosine receptor knockout mice exhibit increased renal injury following ischemia and reperfusion. *Am J Physiol Renal Physiol* 2004;286:F298-306.

- [50] Sun CX, Young HW, Molina JG, Volmer JB, Schnermann J, Blackburn MR. A protective role for the A1 adenosine receptor in adenosine-dependent pulmonary injury. *J Clin Invest* 2005;115:35-43.
- [51] Tsutsui S, Schnermann J, Noorbakhsh F, Henry S, Yong VW, Winston BW, et al. A1 adenosine receptor upregulation and activation attenuates neuroinflammation and demyelination in a model of multiple sclerosis. *J Neurosci* 2004;24:1521-9.
- [52] Gallos G, Ruyle TD, Emala CW, Lee HT. A1 adenosine receptor knockout mice exhibit increased mortality, renal dysfunction, and hepatic injury in murine septic peritonitis. *Am J Physiol Renal Physiol* 2005;289:F369-76.
- [53] Joo JD, Kim M, Horst P, Kim J, D'Agati VD, Emala CW, Sr., et al. Acute and delayed renal protection against renal ischemia and reperfusion injury with A1 adenosine receptors. *Am J Physiol Renal Physiol* 2007;293:F1847-57.
- [54] Cunha RA, Ferre S, Vaugeois JM, Chen JF. Potential therapeutic interest of adenosine A2A receptors in psychiatric disorders. *Curr Pharm Des* 2008;14:1512-24.
- [55] Furlong TJ, Pierce KD, Selbie LA, Shine J. Molecular characterization of a human brain adenosine A2 receptor. *Brain Res Mol Brain Res* 1992;15:62-6.
- [56] Kim J, Wess J, van Rhee AM, Schoneberg T, Jacobson KA. Site-directed mutagenesis identifies residues involved in ligand recognition in the human A2a adenosine receptor. *J Biol Chem* 1995;270:13987-97.
- [57] Ijzerman AP, Von Frijtag Drabbe Kunzel JK, Kim J, Jiang Q, Jacobson KA. Site-directed mutagenesis of the human adenosine A2A receptor. Critical involvement of Glu13 in agonist recognition. *Eur J Pharmacol* 1996;310:269-72.
- [58] Barbhuiya H, McClain R, Ijzerman A, Rivkees SA. Site-directed mutagenesis of the human A1 adenosine receptor: influences of acidic and hydroxy residues in the first four transmembrane domains on ligand binding. *Mol Pharmacol* 1996;50:1635-42.
- [59] Gao ZG, Jiang Q, Jacobson KA, Ijzerman AP. Site-directed mutagenesis studies of human A(2A) adenosine receptors: involvement of glu(13) and his(278) in ligand binding and sodium modulation. *Biochem Pharmacol* 2000;60:661-8.
- [60] Jiang Q, Lee BX, Glashofer M, van Rhee AM, Jacobson KA. Mutagenesis reveals structure-activity parallels between human A2A adenosine receptors and biogenic amine G protein-coupled receptors. *J Med Chem* 1997;40:2588-95.
- [61] Kim J, Jiang Q, Glashofer M, Yehle S, Wess J, Jacobson KA. Glutamate residues in the second extracellular loop of the human A2a adenosine receptor are required for ligand recognition. *Mol Pharmacol* 1996;49:683-91.
- [62] Sitkovsky MV, Lukashev D, Apasov S, Kojima H, Koshiba M, Caldwell C, et al. Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. *Annu Rev Immunol* 2004;22:657-82.

- [63] Cronstein BN, Levin RI, Philips M, Hirschhorn R, Abramson SB, Weissmann G. Neutrophil adherence to endothelium is enhanced via adenosine A1 receptors and inhibited via adenosine A2 receptors. *J Immunol* 1992;148:2201-6.
- [64] Wollner A, Wollner S, Smith JB. Acting via A2 receptors, adenosine inhibits the upregulation of Mac-1 (Cd11b/CD18) expression on FMLP-stimulated neutrophils. *Am J Respir Cell Mol Biol* 1993;9:179-85.
- [65] Zalavary S, Bengtsson T. Adenosine inhibits actin dynamics in human neutrophils: evidence for the involvement of cAMP. *Eur J Cell Biol* 1998;75:128-39.
- [66] Thiel M, Chambers JD, Chouker A, Fischer S, Zourelidis C, Bardenheuer HJ, et al. Effect of adenosine on the expression of beta(2) integrins and L-selectin of human polymorphonuclear leukocytes in vitro. *J Leukoc Biol* 1996;59:671-82.
- [67] McPherson JA, Barringhaus KG, Bishop GG, Sanders JM, Rieger JM, Hesselbacher SE, et al. Adenosine A(2A) receptor stimulation reduces inflammation and neointimal growth in a murine carotid ligation model. *Arterioscler Thromb Vasc Biol* 2001;21:791-6.
- [68] Sullivan GW, Lee DD, Ross WG, DiVietro JA, Lappas CM, Lawrence MB, et al. Activation of A2A adenosine receptors inhibits expression of alpha 4/beta 1 integrin (very late antigen-4) on stimulated human neutrophils. *J Leukoc Biol* 2004;75:127-34.
- [69] Cassada DC, Tribble CG, Long SM, Laubach VE, Kaza AK, Linden J, et al. Adenosine A2A analogue ATL-146e reduces systemic tumor necrosis factor-alpha and spinal cord capillary platelet-endothelial cell adhesion molecule-1 expression after spinal cord ischemia. *J Vasc Surg* 2002;35:994-8.
- [70] Pinhal-Enfield G, Ramanathan M, Hasko G, Vogel SN, Salzman AL, Boons GJ, et al. An angiogenic switch in macrophages involving synergy between Toll-like receptors 2, 4, 7, and 9 and adenosine A(2A) receptors. *Am J Pathol* 2003;163:711-21.
- [71] Lappas CM, Rieger JM, Linden J. A2A adenosine receptor induction inhibits IFN-gamma production in murine CD4+ T cells. *J Immunol* 2005;174:1073-80.
- [72] Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *J Exp Med* 2007;204:1257-65.
- [73] Day YJ, Marshall MA, Huang L, McDuffie MJ, Okusa MD, Linden J. Protection from ischemic liver injury by activation of A2A adenosine receptors during reperfusion: inhibition of chemokine induction. *Am J Physiol Gastrointest Liver Physiol* 2004;286:G285-93.
- [74] Yang Z, Day YJ, Toufektsian MC, Xu Y, Ramos SI, Marshall MA, et al. Myocardial infarct-sparing effect of adenosine A2A receptor activation is due to its action on CD4+ T lymphocytes. *Circulation* 2006;114:2056-64.

- [75] Reutershan J, Cagnina RE, Chang D, Linden J, Ley K. Therapeutic anti-inflammatory effects of myeloid cell adenosine receptor A2a stimulation in lipopolysaccharide-induced lung injury. *J Immunol* 2007;179:1254-63.
- [76] Li Y, Oskouian RJ, Day YJ, Rieger JM, Liu L, Kern JA, et al. Mouse spinal cord compression injury is reduced by either activation of the adenosine A2A receptor on bone marrow-derived cells or deletion of the A2A receptor on non-bone marrow-derived cells. *Neuroscience* 2006;141:2029-39.
- [77] Odashima M, Bamias G, Rivera-Nieves J, Linden J, Nast CC, Moskaluk CA, et al. Activation of A2A adenosine receptor attenuates intestinal inflammation in animal models of inflammatory bowel disease. *Gastroenterology* 2005;129:26-33.
- [78] Bonneau O, Wyss D, Ferretti S, Blaydon C, Stevenson CS, Trifilieff A. Effect of adenosine A2A receptor activation in murine models of respiratory disorders. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L1036-43.
- [79] Nadeem A, Fan M, Ansari HR, Ledent C, Jamal Mustafa S. Enhanced airway reactivity and inflammation in A2A adenosine receptor-deficient allergic mice. *Am J Physiol Lung Cell Mol Physiol* 2007;292:L1335-44.
- [80] Schulte G, Fredholm BB. Signalling from adenosine receptors to mitogen-activated protein kinases. *Cell Signal* 2003;15:813-27.
- [81] Linden J, Thai T, Figler H, Jin X, Robeva AS. Characterization of human A(2B) adenosine receptors: radioligand binding, western blotting, and coupling to G(q) in human embryonic kidney 293 cells and HMC-1 mast cells. *Mol Pharmacol* 1999;56:705-13.
- [82] Feoktistov I, Biaggioni I. Adenosine A2B receptors. *Pharmacol Rev* 1997;49:381-402.
- [83] Schiedel AC, Hinz S, Thimm D, Sherbiny F, Borrmann T, Maass A, et al. The four cysteine residues in the second extracellular loop of the human adenosine A2B receptor: role in ligand binding and receptor function. *Biochem Pharmacol* 2011;82:389-99.
- [84] Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, et al. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev* 2001;22:153-83.
- [85] Feoktistov I, Goldstein AE, Biaggioni I. Role of p38 mitogen-activated protein kinase and extracellular signal-regulated protein kinase kinase in adenosine A2B receptor-mediated interleukin-8 production in human mast cells. *Mol Pharmacol* 1999;55:726-34.
- [86] Donoso MV, Lopez R, Miranda R, Briones R, Huidobro-Toro JP. A2B adenosine receptor mediates human chorionic vasoconstriction and signals through arachidonic acid cascade. *Am J Physiol Heart Circ Physiol* 2005;288:H2439-49.
- [87] Muller CE, Jacobson KA. Recent developments in adenosine receptor ligands and their potential as novel drugs. *Biochim Biophys Acta* 2011;1808:1290-308.

- [88] Yan L, Burbiel JC, Maass A, Muller CE. Adenosine receptor agonists: from basic medicinal chemistry to clinical development. *Expert Opin Emerg Drugs* 2003;8:537-76.
- [89] Baraldi PG, Tabrizi MA, Fruttarolo F, Romagnoli R, Preti D. Recent improvements in the development of A(2B) adenosine receptor agonists. *Purinergic Signal* 2008;4:287-303.
- [90] Borrmann T, Hinz S, Bertarelli DC, Li W, Florin NC, Scheiff AB, et al. 1-alkyl-8-(piperazine-1-sulfonyl)phenylxanthines: development and characterization of adenosine A2B receptor antagonists and a new radioligand with subnanomolar affinity and subtype specificity. *J Med Chem* 2009;52:3994-4006.
- [91] Karnik SS, Sakmar TP, Chen HB, Khorana HG. Cysteine residues 110 and 187 are essential for the formation of correct structure in bovine rhodopsin. *Proc Natl Acad Sci U S A* 1988;85:8459-63.
- [92] Ai LS, Liao F. Mutating the four extracellular cysteines in the chemokine receptor CCR6 reveals their differing roles in receptor trafficking, ligand binding, and signaling. *Biochemistry* 2002;41:8332-41.
- [93] Seibt BF, Schiedel AC, Thimm D, Hinz S, Sherbiny FF, Muller CE. The second extracellular loop of GPCRs determines subtype-selectivity and controls efficacy as evidenced by loop exchange study at A2 adenosine receptors. *Biochem Pharmacol* 2013;85:1317-29.
- [94] Thimm D, Schiedel AC, Sherbiny FF, Hinz S, Hochheiser K, Bertarelli DC, et al. Ligand-specific binding and activation of the human adenosine A(2B) receptor. *Biochemistry* 2013;52:726-40.
- [95] Eckle T, Faigle M, Grenz A, Laucher S, Thompson LF, Eltzschig HK. A2B adenosine receptor dampens hypoxia-induced vascular leak. *Blood* 2008;111:2024-35.
- [96] Sun CX, Zhong H, Mohsenin A, Morschl E, Chunn JL, Molina JG, et al. Role of A2B adenosine receptor signaling in adenosine-dependent pulmonary inflammation and injury. *J Clin Invest* 2006;116:2173-82.
- [97] Ryzhov S, Goldstein AE, Matafonov A, Zeng D, Biaggioni I, Feoktistov I. Adenosine-activated mast cells induce IgE synthesis by B lymphocytes: an A2B-mediated process involving Th2 cytokines IL-4 and IL-13 with implications for asthma. *J Immunol* 2004;172:7726-33.
- [98] Wei W, Du C, Lv J, Zhao G, Li Z, Wu Z, et al. Blocking A2B adenosine receptor alleviates pathogenesis of experimental autoimmune encephalomyelitis via inhibition of IL-6 production and Th17 differentiation. *J Immunol* 2013;190:138-46.
- [99] Jin X, Shepherd RK, Duling BR, Linden J. Inosine binds to A3 adenosine receptors and stimulates mast cell degranulation. *J Clin Invest* 1997;100:2849-57.

- [100] Fredholm BB, Irenius E, Kull B, Schulte G. Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells. *Biochem Pharmacol* 2001;61:443-8.
- [101] da Rocha Lapa F, de Oliveira AP, Accetturi BG, de Oliveira Martins I, Domingos HV, de Almeida Cabrini D, et al. Anti-inflammatory effects of inosine in allergic lung inflammation in mice: evidence for the participation of adenosine A(2A) and A (3) receptors. *Purinergic Signal* 2013.
- [102] Zhou QY, Li C, Olah ME, Johnson RA, Stiles GL, Civelli O. Molecular cloning and characterization of an adenosine receptor: the A3 adenosine receptor. *Proc Natl Acad Sci U S A* 1992;89:7432-6.
- [103] Abbracchio MP, Brambilla R, Ceruti S, Kim HO, von Lubitz DK, Jacobson KA, et al. G protein-dependent activation of phospholipase C by adenosine A3 receptors in rat brain. *Mol Pharmacol* 1995;48:1038-45.
- [104] Gessi S, Merighi S, Varani K, Leung E, Mac Lennan S, Borea PA. The A3 adenosine receptor: an enigmatic player in cell biology. *Pharmacol Ther* 2008;117:123-40.
- [105] Merighi S, Benini A, Mirandola P, Gessi S, Varani K, Leung E, et al. A3 adenosine receptor activation inhibits cell proliferation via phosphatidylinositol 3-kinase/Akt-dependent inhibition of the extracellular signal-regulated kinase 1/2 phosphorylation in A375 human melanoma cells. *J Biol Chem* 2005;280:19516-26.
- [106] Merighi S, Benini A, Mirandola P, Gessi S, Varani K, Simioni C, et al. 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* 2007;72:395-406.
- [107] Tilley SL, Wagoner VA, Salvatore CA, Jacobson MA, Koller BH. Adenosine and inosine increase cutaneous vasopermeability by activating A(3) receptors on mast cells. *J Clin Invest* 2000;105:361-7.
- [108] Polosa R. Adenosine-receptor subtypes: their relevance to adenosine-mediated responses in asthma and chronic obstructive pulmonary disease. *Eur Respir J* 2002;20:488-96.
- [109] Spicuzza L, Di Maria G, Polosa R. Adenosine in the airways: implications and applications. *Eur J Pharmacol* 2006;533:77-88.
- [110] Paoletta S, Tosh DK, Finley A, Gizewski ET, Moss SM, Gao ZG, et al. Rational Design of Sulfonated A3 Adenosine Receptor-Selective Nucleosides as Pharmacological Tools to Study Chronic Neuropathic Pain. *J Med Chem* 2013.
- [111] Bar-Yehuda S, Madi L, Barak D, Mittelman M, Ardon E, Ochaion A, et al. Agonists to the A3 adenosine receptor induce G-CSF production via NF-kappaB activation: a new class of myeloprotective agents. *Exp Hematol* 2002;30:1390-8.

- [112] Palmer TM, Stiles GL. Identification of threonine residues controlling the agonist-dependent phosphorylation and desensitization of the rat A(3) adenosine receptor. *Mol Pharmacol* 2000;57:539-45.
- [113] Ferguson G, Watterson KR, Palmer TM. Subtype-specific kinetics of inhibitory adenosine receptor internalization are determined by sensitivity to phosphorylation by G protein-coupled receptor kinases. *Mol Pharmacol* 2000;57:546-52.
- [114] Moro S, Spalluto G, Jacobson KA. Techniques: Recent developments in computer-aided engineering of GPCR ligands using the human adenosine A3 receptor as an example. *Trends Pharmacol Sci* 2005;26:44-51.
- [115] Gao ZG, Chen A, Barak D, Kim SK, Muller CE, Jacobson KA. Identification by site-directed mutagenesis of residues involved in ligand recognition and activation of the human A3 adenosine receptor. *J Biol Chem* 2002;277:19056-63.
- [116] Jacobson KA, Gao ZG, Chen A, Barak D, Kim SA, Lee K, et al. Neoreceptor concept based on molecular complementarity in GPCRs: a mutant adenosine A(3) receptor with selectively enhanced affinity for amine-modified nucleosides. *J Med Chem* 2001;44:4125-36.
- [117] Hasko G, Linden J, Cronstein B, Pacher P. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nat Rev Drug Discov* 2008;7:759-70.
- [118] Sherwood ER, Toliver-Kinsky T. Mechanisms of the inflammatory response. *Best Pract Res Clin Anaesthesiol* 2004;18:385-405.
- [119] Hasko G, Cronstein B. Regulation of inflammation by adenosine. *Front Immunol* 2013;4:85.
- [120] Cronstein BN, Kramer SB, Weissmann G, Hirschhorn R. A new physiological function for adenosine: regulation of superoxide anion production. *Trans Assoc Am Physicians* 1983;96:384-91.
- [121] Cronstein BN, Rosenstein ED, Kramer SB, Weissmann G, Hirschhorn R. Adenosine; a physiologic modulator of superoxide anion generation by human neutrophils. Adenosine acts via an A2 receptor on human neutrophils. *J Immunol* 1985;135:1366-71.
- [122] Meltzer S, Goldberg B, Lad P, Easton J. Superoxide generation and its modulation by adenosine in the neutrophils of subjects with asthma. *J Allergy Clin Immunol* 1989;83:960-6.
- [123] Cronstein BN, Daguma L, Nichols D, Hutchison AJ, Williams M. The adenosine/neutrophil paradox resolved: human neutrophils possess both A1 and A2 receptors that promote chemotaxis and inhibit O₂ generation, respectively. *J Clin Invest* 1990;85:1150-7.

- [124] Thiel M, Chouker A. Acting via A2 receptors, adenosine inhibits the production of tumor necrosis factor- α of endotoxin-stimulated human polymorphonuclear leukocytes. *J Lab Clin Med* 1995;126:275-82.
- [125] Krump E, Lemay G, Borgeat P. Adenosine A2 receptor-induced inhibition of leukotriene B4 synthesis in whole blood ex vivo. *Br J Pharmacol* 1996;117:1639-44.
- [126] Fredholm BB, Zhang Y, van der Ploeg I. Adenosine A2A receptors mediate the inhibitory effect of adenosine on formyl-Met-Leu-Phe-stimulated respiratory burst in neutrophil leukocytes. *Naunyn Schmiedebergs Arch Pharmacol* 1996;354:262-7.
- [127] Bouma MG, Jeunhomme TM, Boyle DL, Dentener MA, Voitenok NN, van den Wildenberg FA, et al. Adenosine inhibits neutrophil degranulation in activated human whole blood: involvement of adenosine A2 and A3 receptors. *J Immunol* 1997;158:5400-8.
- [128] McColl SR, St-Onge M, Dussault AA, Laflamme C, Bouchard L, Boulanger J, et al. Immunomodulatory impact of the A2A adenosine receptor on the profile of chemokines produced by neutrophils. *FASEB J* 2006;20:187-9.
- [129] Gessi S, Varani K, Merighi S, Cattabriga E, Iannotta V, Leung E, et al. A(3) adenosine receptors in human neutrophils and promyelocytic HL60 cells: a pharmacological and biochemical study. *Mol Pharmacol* 2002;61:415-24.
- [130] Chen Y, Corriden R, Inoue Y, Yip L, Hashiguchi N, Zinkernagel A, et al. ATP release guides neutrophil chemotaxis via P2Y2 and A3 receptors. *Science* 2006;314:1792-5.
- [131] Fishman P, Bar-Yehuda S, Farbstein T, Barer F, Ohana G. Adenosine acts as a chemoprotective agent by stimulating G-CSF production: a role for A1 and A3 adenosine receptors. *J Cell Physiol* 2000;183:393-8.
- [132] Pozzi LA, Maciaszek JW, Rock KL. Both dendritic cells and macrophages can stimulate naive CD8 T cells in vivo to proliferate, develop effector function, and differentiate into memory cells. *J Immunol* 2005;175:2071-81.
- [133] Bouma MG, Stad RK, van den Wildenberg FA, Buurman WA. Differential regulatory effects of adenosine on cytokine release by activated human monocytes. *J Immunol* 1994;153:4159-68.
- [134] Kreckler LM, Wan TC, Ge ZD, Auchampach JA. Adenosine inhibits tumor necrosis factor- α release from mouse peritoneal macrophages via A2A and A2B but not the A3 adenosine receptor. *J Pharmacol Exp Ther* 2006;317:172-80.
- [135] Csoka B, Nemeth ZH, Virag L, Gergely P, Leibovich SJ, Pacher P, et al. A2A adenosine receptors and C/EBP β are crucially required for IL-10 production by macrophages exposed to *Escherichia coli*. *Blood* 2007;110:2685-95.

- [136] Hasko G, Kuhel DG, Nemeth ZH, Mabley JG, Stachlewitz RF, Virag L, et al. Inosine inhibits inflammatory cytokine production by a posttranscriptional mechanism and protects against endotoxin-induced shock. *J Immunol* 2000;164:1013-9.
- [137] Hasko G, Nemeth ZH, Vizi ES, Salzman AL, Szabo C. An agonist of adenosine A3 receptors decreases interleukin-12 and interferon-gamma production and prevents lethality in endotoxemic mice. *Eur J Pharmacol* 1998;358:261-8.
- [138] Link AA, Kino T, Worth JA, McGuire JL, Crane ML, Chrousos GP, et al. Ligand-activation of the adenosine A2a receptors inhibits IL-12 production by human monocytes. *J Immunol* 2000;164:436-42.
- [139] Ramackers BP, Riksen NP, Rongen GA, van der Hoeven JG, Smits P, Pickkers P. The effect of adenosine receptor agonists on cytokine release by human mononuclear cells depends on the specific Toll-like receptor subtype used for stimulation. *Cytokine* 2006;35:95-9.
- [140] Ferrante CJ, Pinhal-Enfield G, Elson G, Cronstein BN, Hasko G, Outram S, et al. The Adenosine-Dependent Angiogenic Switch of Macrophages to an M2-Like Phenotype is Independent of Interleukin-4 Receptor Alpha (IL-4Ralpha) Signaling. *Inflammation* 2013;36:921-31.
- [141] Panther E, Corinti S, Idzko M, Herouy Y, Napp M, la Sala A, et al. Adenosine affects expression of membrane molecules, cytokine and chemokine release, and the T-cell stimulatory capacity of human dendritic cells. *Blood* 2003;101:3985-90.
- [142] Bradding P, Holgate ST. Immunopathology and human mast cell cytokines. *Crit Rev Oncol Hematol* 1999;31:119-33.
- [143] Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol* 2006;117:1277-84.
- [144] Bradding P. Asthma: eosinophil disease, mast cell disease, or both? *Allergy Asthma Clin Immunol* 2008;4:84-90.
- [145] Feoktistov I, Biaggioni I. Adenosine A2b receptors evoke interleukin-8 secretion in human mast cells. An enprofylline-sensitive mechanism with implications for asthma. *J Clin Invest* 1995;96:1979-86.
- [146] Salvatore CA, Tilley SL, Latour AM, Fletcher DS, Koller BH, Jacobson MA. Disruption of the A(3) adenosine receptor gene in mice and its effect on stimulated inflammatory cells. *J Biol Chem* 2000;275:4429-34.
- [147] Zhong H, Shlykov SG, Molina JG, Sanborn BM, Jacobson MA, Tilley SL, et al. Activation of murine lung mast cells by the adenosine A3 receptor. *J Immunol* 2003;171:338-45.

- [148] Ryzhov S, Zaynagetdinov R, Goldstein AE, Novitskiy SV, Dikov MM, Blackburn MR, et al. Effect of A2B adenosine receptor gene ablation on proinflammatory adenosine signaling in mast cells. *J Immunol* 2008;180:7212-20.
- [149] Blackburn MR, Vance CO, Morschl E, Wilson CN. Adenosine receptors and inflammation. *Handb Exp Pharmacol* 2009;215-69.
- [150] Cushley MJ, Tattersfield AE, Holgate ST. Inhaled adenosine and guanosine on airway resistance in normal and asthmatic subjects. *Br J Clin Pharmacol* 1983;15:161-5.
- [151] Livingston M, Heaney LG, Ennis M. Adenosine, inflammation and asthma--a review. *Inflamm Res* 2004;53:171-8.
- [152] Yip KH, Lau HY, Wise H. Reciprocal modulation of anti-IgE induced histamine release from human mast cells by A(1) and A(2B) adenosine receptors. *Br J Pharmacol* 2011;164:807-19.
- [153] Alfieri A, Parisi A, Maione F, Grassia G, Morello S, Ialenti A, et al. Hyperresponsiveness to adenosine in sensitized Wistar rats over-expressing A1 receptor. *Eur J Pharmacol* 2012;695:120-5.
- [154] Hoffmann K, Xifro RA, Hartweg JL, Spitzlei P, Meis K, Molderings GJ, et al. Inhibitory effects of benzodiazepines on the adenosine A(2B) receptor mediated secretion of interleukin-8 in human mast cells. *Eur J Pharmacol* 2013;700:152-8.
- [155] Suzuki H, Takei M, Nakahata T, Fukamachi H. Inhibitory effect of adenosine on degranulation of human cultured mast cells upon cross-linking of Fc epsilon RI. *Biochem Biophys Res Commun* 1998;242:697-702.
- [156] Driver AG, Kukoly CA, Ali S, Mustafa SJ. Adenosine in bronchoalveolar lavage fluid in asthma. *Am Rev Respir Dis* 1993;148:91-7.
- [157] Kumar V, Sharma A. Adenosine: an endogenous modulator of innate immune system with therapeutic potential. *Eur J Pharmacol* 2009;616:7-15.
- [158] Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell* 2008;133:775-87.
- [159] Ohta A, Kini R, Subramanian M, Madasu M, Sitkovsky M. The development and immunosuppressive functions of CD4(+) CD25(+) FoxP3(+) regulatory T cells are under influence of the adenosine-A2A adenosine receptor pathway. *Front Immunol* 2012;3:190.
- [160] Sitkovsky M, Lukashev D, Deaglio S, Dwyer K, Robson SC, Ohta A. Adenosine A2A receptor antagonists: blockade of adenosinergic effects and T regulatory cells. *Br J Pharmacol* 2008;153 Suppl 1:S457-64.
- [161] Polosa R, Blackburn MR. Adenosine receptors as targets for therapeutic intervention in asthma and chronic obstructive pulmonary disease. *Trends Pharmacol Sci* 2009;30:528-35.

- [162] Oosterhoff Y, de Jong JW, Jansen MA, Koeter GH, Postma DS. Airway responsiveness to adenosine 5'-monophosphate in chronic obstructive pulmonary disease is determined by smoking. *Am Rev Respir Dis* 1993;147:553-8.
- [163] Caruso M, Varani K, Tringali G, Polosa R. Adenosine and adenosine receptors: their contribution to airway inflammation and therapeutic potential in asthma. *Curr Med Chem* 2009;16:3875-85.
- [164] Mortaz E, Folkerts G, Nijkamp FP, Henricks PA. ATP and the pathogenesis of COPD. *Eur J Pharmacol* 2010;638:1-4.
- [165] Fozard JR, Ellis KM, Villela Dantas MF, Tigani B, Mazzoni L. Effects of CGS 21680, a selective adenosine A2A receptor agonist, on allergic airways inflammation in the rat. *Eur J Pharmacol* 2002;438:183-8.
- [166] El-Hashim AZ, Abduo HT, Rachid OM, Luqmani YA, Al Ayadhy BY, Alkhaledi GM. Intranasal administration of NECA can induce both anti-inflammatory and pro-inflammatory effects in BALB/c mice: evidence for A 2A receptor sub-type mediation of NECA-induced anti-inflammatory effects. *Pulm Pharmacol Ther* 2009;22:243-52.
- [167] Impellizzeri D, Di Paola R, Esposito E, Mazzon E, Paterniti I, Melani A, et al. CGS 21680, an agonist of the adenosine (A2A) receptor, decreases acute lung inflammation. *Eur J Pharmacol* 2011;668:305-16.
- [168] Mabley JG, Pacher P, Murthy KG, Williams W, Southan GJ, Salzman AL, et al. The novel inosine analogue INO-2002 exerts an anti-inflammatory effect in a murine model of acute lung injury. *Shock* 2009;32:258-62.
- [169] Hua X, Chason KD, Fredholm BB, Deshpande DA, Penn RB, Tilley SL. Adenosine induces airway hyperresponsiveness through activation of A3 receptors on mast cells. *J Allergy Clin Immunol* 2008;122:107-13, 13 e1-7.
- [170] Zaynagetdinov R, Ryzhov S, Goldstein AE, Yin H, Novitskiy SV, Goleniewska K, et al. Attenuation of chronic pulmonary inflammation in A2B adenosine receptor knockout mice. *Am J Respir Cell Mol Biol* 2010;42:564-71.
- [171] Cicala C, Ialenti A. Adenosine signaling in airways: Toward a promising antiasthmatic approach. *Eur J Pharmacol* 2013.
- [172] Roman J, Rivera HN, Roser-Page S, Sitaraman SV, Ritzenthaler JD. Adenosine induces fibronectin expression in lung epithelial cells: implications for airway remodeling. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L317-25.
- [173] Young HW, Molina JG, Dimina D, Zhong H, Jacobson M, Chan LN, et al. A3 adenosine receptor signaling contributes to airway inflammation and mucus production in adenosine deaminase-deficient mice. *J Immunol* 2004;173:1380-9.
- [174] Rudich N, Ravid K, Sagi-Eisenberg R. Mast cell adenosine receptors function: a focus on the a3 adenosine receptor and inflammation. *Front Immunol* 2012;3:134.

- [175] Gomez G, Zhao W, Schwartz LB. Disparity in FcεpsilonRI-induced degranulation of primary human lung and skin mast cells exposed to adenosine. *J Clin Immunol* 2011;31:479-87.
- [176] Ryzhov S, Goldstein AE, Biaggioni I, Feoktistov I. Cross-talk between G(s)- and G(q)-coupled pathways in regulation of interleukin-4 by A(2B) adenosine receptors in human mast cells. *Mol Pharmacol* 2006;70:727-35.
- [177] Feoktistov I, Polosa R, Holgate ST, Biaggioni I. Adenosine A2B receptors: a novel therapeutic target in asthma? *Trends Pharmacol Sci* 1998;19:148-53.
- [178] Polosa R, Holgate ST. Adenosine receptors as promising therapeutic targets for drug development in chronic airway inflammation. *Curr Drug Targets* 2006;7:699-706.
- [179] Ali S, Mustafa SJ, Metzger WJ. Adenosine-induced bronchoconstriction and contraction of airway smooth muscle from allergic rabbits with late-phase airway obstruction: evidence for an inducible adenosine A1 receptor. *J Pharmacol Exp Ther* 1994;268:1328-34.
- [180] Nyce JW, Metzger WJ. DNA antisense therapy for asthma in an animal model. *Nature* 1997;385:721-5.
- [181] Ngamsri KC, Wagner R, Vollmer I, Stark S, Reutershan J. Adenosine receptor A1 regulates polymorphonuclear cell trafficking and microvascular permeability in lipopolysaccharide-induced lung injury. *J Immunol* 2010;185:4374-84.
- [182] Burnstock G, Knight GE, Greig AV. Purinergic signaling in healthy and diseased skin. *J Invest Dermatol* 2012;132:526-46.
- [183] Nestle FO, Di Meglio P, Qin JZ, Nickoloff BJ. Skin immune sentinels in health and disease. *Nat Rev Immunol* 2009;9:679-91.
- [184] Ring S, Oliver SJ, Cronstein BN, Enk AH, Mahnke K. CD4+CD25+ regulatory T cells suppress contact hypersensitivity reactions through a CD39, adenosine-dependent mechanism. *J Allergy Clin Immunol* 2009;123:1287-96 e2.
- [185] Ring S, Enk AH, Mahnke K. Regulatory T cells from IL-10-deficient mice fail to suppress contact hypersensitivity reactions due to lack of adenosine production. *J Invest Dermatol* 2010;131:1494-502.
- [186] Oliver SJ, Mathew S, Wilder TF, Cronstein BN. Restraint stress fails to modulate cutaneous hypersensitivity responses in mice lacking the adenosine A1 receptor. *Purinergic Signal* 2011;7:47-56.
- [187] Peirce SM, Skalak TC, Rieger JM, Macdonald TL, Linden J. Selective A(2A) adenosine receptor activation reduces skin pressure ulcer formation and inflammation. *Am J Physiol Heart Circ Physiol* 2001;281:H67-74.

- [188] Montesinos MC, Desai A, Chen JF, Yee H, Schwarzschild MA, Fink JS, et al. Adenosine promotes wound healing and mediates angiogenesis in response to tissue injury via occupancy of A(2A) receptors. *Am J Pathol* 2002;160:2009-18.
- [189] Desai A, Victor-Vega C, Gadangi S, Montesinos MC, Chu CC, Cronstein BN. Adenosine A2A receptor stimulation increases angiogenesis by down-regulating production of the antiangiogenic matrix protein thrombospondin 1. *Mol Pharmacol* 2005;67:1406-13.
- [190] Victor-Vega C, Desai A, Montesinos MC, Cronstein BN. Adenosine A2A receptor agonists promote more rapid wound healing than recombinant human platelet-derived growth factor (Becaplermin gel). *Inflammation* 2002;26:19-24.
- [191] Montesinos MC, Gadangi P, Longaker M, Sung J, Levine J, Nilsen D, et al. Wound healing is accelerated by agonists of adenosine A2 (G alpha s-linked) receptors. *J Exp Med* 1997;186:1615-20.
- [192] Fernandez P, Trzaska S, Wilder T, Chiriboga L, Blackburn MR, Cronstein BN, et al. Pharmacological blockade of A2A receptors prevents dermal fibrosis in a model of elevated tissue adenosine. *Am J Pathol* 2008;172:1675-82.
- [193] Attur M, Krasnokutsky-Samuels S, Samuels J, Abramson SB. Prognostic biomarkers in osteoarthritis. *Curr Opin Rheumatol* 2013;25:136-44.
- [194] Radin EL, Burr DB, Caterson B, Fyhrie D, Brown TD, Boyd RD. Mechanical determinants of osteoarthritis. *Semin Arthritis Rheum* 1991;21:12-21.
- [195] Ishiguro N, Ito T, Ito H, Iwata H, Jugessur H, Ionescu M, et al. Relationship of matrix metalloproteinases and their inhibitors to cartilage proteoglycan and collagen turnover: analyses of synovial fluid from patients with osteoarthritis. *Arthritis Rheum* 1999;42:129-36.
- [196] Lohmander LS, Ionescu M, Jugessur H, Poole AR. Changes in joint cartilage aggrecan after knee injury and in osteoarthritis. *Arthritis Rheum* 1999;42:534-44.
- [197] Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. *Lancet* 2010;376:1094-108.
- [198] Feldmann M, Brennan FM, Maini RN. Rheumatoid arthritis. *Cell* 1996;85:307-10.
- [199] van der Linden MP, van der Woude D, Ioan-Facsinay A, Levarht EW, Stoeken-Rijsbergen G, Huizinga TW, et al. Value of anti-modified citrullinated vimentin and third-generation anti-cyclic citrullinated peptide compared with second-generation anti-cyclic citrullinated peptide and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis. *Arthritis Rheum* 2009;60:2232-41.
- [200] Boyle DL, Sajjadi FG, Firestein GS. Inhibition of synoviocyte collagenase gene expression by adenosine receptor stimulation. *Arthritis Rheum* 1996;39:923-30.

- [201] Tesch AM, MacDonald MH, Kollias-Baker C, Benton HP. Chondrocytes respond to adenosine via A(2)receptors and activity is potentiated by an adenosine deaminase inhibitor and a phosphodiesterase inhibitor. *Osteoarthritis Cartilage* 2002;10:34-43.
- [202] Picher M, Graff RD, Lee GM. Extracellular nucleotide metabolism and signaling in the pathophysiology of articular cartilage. *Arthritis Rheum* 2003;48:2722-36.
- [203] Tesch AM, MacDonald MH, Kollias-Baker C, Benton HP. Endogenously produced adenosine regulates articular cartilage matrix homeostasis: enzymatic depletion of adenosine stimulates matrix degradation. *Osteoarthritis Cartilage* 2004;12:349-59.
- [204] Pettersson T, Klockars M, Weber TH, von Essen R. Adenosine deaminase activity in joint effusions. *Scand J Rheumatol* 1988;17:365-9.
- [205] Ungerer JP, Oosthuizen HM, Bissbort SH, Vermaak WJ. Serum adenosine deaminase: isoenzymes and diagnostic application. *Clin Chem* 1992;38:1322-6.
- [206] Iwaki-Egawa S, Watanabe Y, Matsuno H. Correlations between matrix metalloproteinase-9 and adenosine deaminase isozymes in synovial fluid from patients with rheumatoid arthritis. *J Rheumatol* 2001;28:485-9.
- [207] Forrest CM, Harman G, McMillan RB, Stoy N, Stone TW, Darlington LG. Modulation of cytokine release by purine receptors in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2005;23:89-92.
- [208] Bar-Yehuda S, Rath-Wolfson L, Del Valle L, Ochaion A, Cohen S, Patoka R, et al. Induction of an antiinflammatory effect and prevention of cartilage damage in rat knee osteoarthritis by CF101 treatment. *Arthritis Rheum* 2009;60:3061-71.
- [209] Laubach VE, French BA, Okusa MD. Targeting of adenosine receptors in ischemia-reperfusion injury. *Expert Opin Ther Targets* 2011;15:103-18.
- [210] Becker LB. New concepts in reactive oxygen species and cardiovascular reperfusion physiology. *Cardiovasc Res* 2004;61:461-70.
- [211] Li C, Jackson RM. Reactive species mechanisms of cellular hypoxia-reoxygenation injury. *Am J Physiol Cell Physiol* 2002;282:C227-41.
- [212] Kaczorowski DJ, Tsung A, Billiar TR. Innate immune mechanisms in ischemia/reperfusion. *Front Biosci (Elite Ed)* 2009;1:91-8.
- [213] Eltzschig HK, Carmeliet P. Hypoxia and inflammation. *N Engl J Med* 2011;364:656-65.
- [214] Schingnitz U, Hartmann K, Macmanus CF, Eckle T, Zug S, Colgan SP, et al. Signaling through the A2B adenosine receptor dampens endotoxin-induced acute lung injury. *J Immunol* 2010;184:5271-9.
- [215] Sharma AK, Linden J, Kron IL, Laubach VE. Protection from pulmonary ischemia-reperfusion injury by adenosine A2A receptor activation. *Respir Res* 2009;10:58.

- [216] Grenz A, Osswald H, Eckle T, Yang D, Zhang H, Tran ZV, et al. The reno-vascular A2B adenosine receptor protects the kidney from ischemia. *PLoS Med* 2008;5:e137.
- [217] Eckle T, Krahn T, Grenz A, Kohler D, Mittelbronn M, Ledent C, et al. Cardioprotection by ecto-5'-nucleotidase (CD73) and A2B adenosine receptors. *Circulation* 2007;115:1581-90.
- [218] Eltzschig HK. Adenosine: an old drug newly discovered. *Anesthesiology* 2009;111:904-15.
- [219] Chunn JL, Molina JG, Mi T, Xia Y, Kellems RE, Blackburn MR. Adenosine-dependent pulmonary fibrosis in adenosine deaminase-deficient mice. *J Immunol* 2005;175:1937-46.
- [220] Peng Z, Borea PA, Varani K, Wilder T, Yee H, Chiriboga L, et al. Adenosine signaling contributes to ethanol-induced fatty liver in mice. *J Clin Invest* 2009;119:582-94.
- [221] Chan ES, Fernandez P, Merchant AA, Montesinos MC, Trzaska S, Desai A, et al. Adenosine A2A receptors in diffuse dermal fibrosis: pathogenic role in human dermal fibroblasts and in a murine model of scleroderma. *Arthritis Rheum* 2006;54:2632-42.
- [222] Dai Y, Zhang W, Wen J, Zhang Y, Kellems RE, Xia Y. A2B adenosine receptor-mediated induction of IL-6 promotes CKD. *J Am Soc Nephrol* 2011;22:890-901.
- [223] Zhao Y, LaPar DJ, Steidle J, Emaminia A, Kron IL, Ailawadi G, et al. Adenosine signaling via the adenosine 2B receptor is involved in bronchiolitis obliterans development. *J Heart Lung Transplant* 2010;29:1405-14.
- [224] Hart ML, Grenz A, Gorzolla IC, Schittenhelm J, Dalton JH, Eltzschig HK. Hypoxia-inducible factor-1alpha-dependent protection from intestinal ischemia/reperfusion injury involves ecto-5'-nucleotidase (CD73) and the A2B adenosine receptor. *J Immunol* 2011;186:4367-74.
- [225] Loffler M, Morote-Garcia JC, Eltzschig SA, Coe IR, Eltzschig HK. Physiological roles of vascular nucleoside transporters. *Arterioscler Thromb Vasc Biol* 2007;27:1004-13.
- [226] Grenz A, Bauerle JD, Dalton JH, Ridyard D, Badulak A, Tak E, et al. Equilibrative nucleoside transporter 1 (ENT1) regulates postischemic blood flow during acute kidney injury in mice. *J Clin Invest* 2012;122:693-710.
- [227] Eckle T, Grenz A, Laucher S, Eltzschig HK. A2B adenosine receptor signaling attenuates acute lung injury by enhancing alveolar fluid clearance in mice. *J Clin Invest* 2008;118:3301-15.
- [228] Goldhaber SZ, Pohost GM, Kloner RA, Andrews E, Newell JB, Ingwall JS. Inosine: a protective agent in an organ culture model of myocardial ischemia. *Circ Res* 1982;51:181-8.

- [229] Matsumoto K, Graf R, Rosner G, Taguchi J, Heiss WD. Elevation of neuroactive substances in the cortex of cats during prolonged focal ischemia. *J Cereb Blood Flow Metab* 1993;13:586-94.
- [230] Chen P, Goldberg DE, Kolb B, Lanser M, Benowitz LI. Inosine induces axonal rewiring and improves behavioral outcome after stroke. *Proc Natl Acad Sci U S A* 2002;99:9031-6.
- [231] Shen H, Chen GJ, Harvey BK, Bickford PC, Wang Y. Inosine reduces ischemic brain injury in rats. *Stroke* 2005;36:654-9.
- [232] Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002;347:417-29.
- [233] Lakatos PL. Recent trends in the epidemiology of inflammatory bowel diseases: up or down? *World J Gastroenterol* 2006;12:6102-8.
- [234] Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest* 2007;117:514-21.
- [235] Oz HS, Chen TS, McClain CJ, de Villiers WJ. Antioxidants as novel therapy in a murine model of colitis. *J Nutr Biochem* 2005;16:297-304.
- [236] Martin GR, Wallace JL. Gastrointestinal inflammation: a central component of mucosal defense and repair. *Exp Biol Med (Maywood)* 2006;231:130-7.
- [237] Antonioli L, Fornai M, Colucci R, Ghisu N, Da Settimo F, Natale G, et al. Inhibition of adenosine deaminase attenuates inflammation in experimental colitis. *J Pharmacol Exp Ther* 2007;322:435-42.
- [238] Siegmund B, Rieder F, Albrich S, Wolf K, Bidlingmaier C, Firestein GS, et al. Adenosine kinase inhibitor GP515 improves experimental colitis in mice. *J Pharmacol Exp Ther* 2001;296:99-105.
- [239] Cavalcante IC, Castro MV, Barreto AR, Sullivan GW, Vale M, Almeida PR, et al. Effect of novel A2A adenosine receptor agonist ATL 313 on *Clostridium difficile* toxin A-induced murine ileal enteritis. *Infect Immun* 2006;74:2606-12.
- [240] Keegan BM, Noseworthy JH. Multiple sclerosis. *Annu Rev Med* 2002;53:285-302.
- [241] Runia TF, van Pelt-Gravesteijn ED, Hintzen RQ. Recent gains in clinical multiple sclerosis research. *CNS Neurol Disord Drug Targets* 2012;11:497-505.
- [242] Wucherpfennig KW. Mechanisms for the induction of autoimmunity by infectious agents. *J Clin Invest* 2001;108:1097-104.
- [243] Bagasra O, Michaels FH, Zheng YM, Bobroski LE, Spitsin SV, Fu ZF, et al. Activation of the inducible form of nitric oxide synthase in the brains of patients with multiple sclerosis. *Proc Natl Acad Sci U S A* 1995;92:12041-5.

- [244] Cross AH, Manning PT, Keeling RM, Schmidt RE, Misko TP. Peroxynitrite formation within the central nervous system in active multiple sclerosis. *J Neuroimmunol* 1998;88:45-56.
- [245] Beckmann JS, Ye YZ, Anderson PG, Chen J, Accavitti MA, Tarpey MM, et al. Extensive nitration of protein tyrosines in human atherosclerosis detected by immunohistochemistry. *Biol Chem Hoppe Seyler* 1994;375:81-8.
- [246] Whiteman M, Halliwell B. Protection against peroxynitrite-dependent tyrosine nitration and alpha 1-antiproteinase inactivation by ascorbic acid. A comparison with other biological antioxidants. *Free Radic Res* 1996;25:275-83.
- [247] Spitsin S, Hooper DC, Leist T, Streletz LJ, Mikheeva T, Koprowski H. Inactivation of peroxynitrite in multiple sclerosis patients after oral administration of inosine may suggest possible approaches to therapy of the disease. *Mult Scler* 2001;7:313-9.
- [248] Mills JH, Thompson LF, Mueller C, Waickman AT, Jalkanen S, Niemela J, et al. CD73 is required for efficient entry of lymphocytes into the central nervous system during experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* 2008;105:9325-30.
- [249] Mills JH, Kim DG, Krenz A, Chen JF, Bynoe MS. A2A adenosine receptor signaling in lymphocytes and the central nervous system regulates inflammation during experimental autoimmune encephalomyelitis. *J Immunol* 2012;188:5713-22.
- [250] Brown DA, Sawchenko PE. Time course and distribution of inflammatory and neurodegenerative events suggest structural bases for the pathogenesis of experimental autoimmune encephalomyelitis. *J Comp Neurol* 2007;502:236-60.
- [251] Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S, et al. C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. *Nat Immunol* 2009;10:514-23.
- [252] Rane K, Segerdahl M, Goigny M, Sollevi A. Intrathecal adenosine administration: a phase 1 clinical safety study in healthy volunteers, with additional evaluation of its influence on sensory thresholds and experimental pain. *Anesthesiology* 1998;89:1108-15; discussion 9A.
- [253] Goblyos A, Ijzerman AP. Allosteric modulation of adenosine receptors. *Purinergic Signal* 2009;5:51-61.
- [254] Childers SR, Li X, Xiao R, Eisenach JC. Allosteric modulation of adenosine A1 receptor coupling to G-proteins in brain. *J Neurochem* 2005;93:715-23.
- [255] Nadeem A, Obiefuna PC, Wilson CN, Mustafa SJ. Adenosine A1 receptor antagonist versus montelukast on airway reactivity and inflammation. *Eur J Pharmacol* 2006;551:116-24.

- [256] Awad AS, Huang L, Ye H, Duong ET, Bolton WK, Linden J, et al. Adenosine A2A receptor activation attenuates inflammation and injury in diabetic nephropathy. *Am J Physiol Renal Physiol* 2006;290:F828-37.
- [257] Udelson JE, Heller GV, Wackers FJ, Chai A, Hinchman D, Coleman PS, et al. Randomized, controlled dose-ranging study of the selective adenosine A2A receptor agonist binodenoson for pharmacological stress as an adjunct to myocardial perfusion imaging. *Circulation* 2004;109:457-64.
- [258] Cerqueira MD. Advances in pharmacologic agents in imaging: new A2A receptor agonists. *Curr Cardiol Rep* 2006;8:119-22.
- [259] Press NJ, Fozard JR. Progress towards novel adenosine receptor therapeutics gleaned from the recent patent literature. *Expert Opin Ther Pat* 2010;20:987-1005.
- [260] Zylka MJ. Pain-relieving prospects for adenosine receptors and ectonucleotidases. *Trends Mol Med* 2011;17:188-96.
- [261] Azam F, Ibn-Rajab IA, Alruiad AA. Adenosine A2A receptor antagonists as novel anti-Parkinsonian agents: a review of structure-activity relationships. *Pharmazie* 2009;64:771-95.
- [262] Morelli M, Carta AR, Jenner P. Adenosine A2A receptors and Parkinson's disease. *Handb Exp Pharmacol* 2009;589-615.
- [263] Gillespie RJ, Bamford SJ, Botting R, Comer M, Denny S, Gaur S, et al. Antagonists of the human A(2A) adenosine receptor. 4. Design, synthesis, and preclinical evaluation of 7-aryltriazolo[4,5-d]pyrimidines. *J Med Chem* 2009;52:33-47.
- [264] Wan TC, Ge ZD, Tampo A, Mio Y, Bienengraeber MW, Tracey WR, et al. The A3 adenosine receptor agonist CP-532,903 [N6-(2,5-dichlorobenzyl)-3'-aminoadenosine-5'-N-methylcarboxamide] protects against myocardial ischemia/reperfusion injury via the sarcolemmal ATP-sensitive potassium channel. *J Pharmacol Exp Ther* 2008;324:234-43.
- [265] Gao ZG, Ye K, Goblyos A, Ijzerman AP, Jacobson KA. Flexible modulation of agonist efficacy at the human A3 adenosine receptor by the imidazoquinoline allosteric enhancer LUF6000. *BMC Pharmacol* 2008;8:20.