Review
Bench-to-bedside review: Adenosine receptors – promising targets in acute lung injury?

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Abstract

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are life-threatening disorders that have substantial adverse effects on outcomes in critically ill patients. ALI/ARDS develops in response to pulmonary or extrapulmonary injury and is characterized by increased leakage from the pulmonary microvasculature and excessive infiltration of polymorphonuclear cells into the lung. Currently, no therapeutic strategies are available to control these fundamental pathophysiological processes in human ALI/ARDS. In a variety of animal models and experimental settings, the purine nucleoside adenosine has been demonstrated to regulate both endothelial barrier integrity and polymorphonuclear cell trafficking in the lung. Adenosine exerts its effects through four G-protein-coupled receptors (A_1, A_{2A}, A_{2B}, and A_3) that are expressed on leukocytes and nonhematopoietic cells, including endothelial and epithelial cells. Each type of adenosine receptor (AR) is characterized by a unique pharmacological and physiological profile. The development of selective AR agonists and antagonists, as well as the generation of gene-deficient mice, has contributed to a growing understanding of the cellular and molecular processes that are critically involved in the development of ALI/ARDS. Adenosine-dependent pathways are involved in both protective and proinflammatory effects, highlighting the need for a detailed characterization of the distinct pathways. This review summarizes current experimental observations on the role of adenosine signaling in the development of acute lung injury and illustrates that adenosine and ARs are promising targets that may be exploited in the development of innovative therapeutic strategies.

Introduction

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are life-threatening syndromes that are characterized by severe hypoxemia, decreased lung compliance, and diffuse bilateral infiltrates without evidence of left atrial hypertension. ALI and ARDS develop during the course of direct lung injury such as pneumonia, acid aspiration,

ischemia/reperfusion after lung transplantation, or direct traumatic damage. Alternatively, they may develop secondary to systemic inflammatory diseases such as sepsis, extrapulmonary trauma, transfusion, or cardiopulmonary resuscitation [1]. Despite recent advances in our understanding of the pathophysiology, therapeutic options are limited and focus on treating the underlying disease and preventing secondary lung damage by mechanical ventilation with low tidal volumes [2]. Nevertheless, mortality remains high at 40%, and the incidence of ALI/ARDS of approximately 80/100,000 person-years in the USA [3] underlines the importance of these entities to critical care medicine and public health in general.

The early phase of ALI/ARDS is characterized by an excessive inflammatory response that results in disruption of the endothelial barrier. As a consequence, a protein-rich lung edema develops and impairs pulmonary function [1]. The pulmonary endothelium is also critically involved in the recruitment and transmigration of polymorphonuclear cells (PMNs) into the lung [4]. PMNs are the leukocytes that predominantly mediate the initial phase of ALI. Numerous experimental and clinical observations have established a key role for PMNs in the pathogenesis of ALI in animals and patients. For instance, PMN depletion attenuates experimental lung damage, pulmonary function in neutropenic patients with lung injury can deteriorate as neutropenia resolves [5], and persistent pulmonary neutrophilia in ARDS is associated with poor outcomes [6].

Adenosine, an ancient endogenous molecule, has been shown to be a potent modulator of endothelial permeability [7], PMN migration, and PMN activation [8]. Adenosine

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ADA = adenosine deaminase; ALI = acute lung injury; AR = adenosine receptor; ARDS = acute respiratory distress syndrome; BAL = bronchoalveolar lavage; CXCL = C-X-C chemokine ligand; I/R = ischemia/reperfusion; LPS = lipopolysaccharide; PMN = polymorphonuclear cell; VILI = ventilator-induced lung injury.

signals through specific adenosine receptors (ARs) that are expressed on a variety of cells, including leukocytes and nonhematopoietic cells. Each of the four known ARs exhibits a distinct pharmacological and physiological profile [9]. A growing understanding of this multifaceted adenosine signaling complex has paved the way for new approaches to interfere with the inflammatory cascade during ALI. This review summarizes current experimental findings regarding the roles played by adenosine and ARs in pulmonary inflammation. Adenosine-dependent pathways in ALI are highlighted and the potential of ARs as therapeutic targets is critically discussed.

The physiological role of adenosine and adenosine receptors

Adenosine is a purine nucleoside with a short *in vivo* half-life of 1.5 seconds [10]. It is generated by intracellular hydrolysis from adenine nucleotides or *S*-adenosyl homocysteine. To exert its messenger function, adenosine is subsequently released into the extracellular space either by specific nucleoside transporters [11] or nonspecifically, upon damage to the cell membrane. In addition, adenine nucleotides can be extracellularly hydrolyzed to adenosine. Hydrolysis is mediated by the two enzymes ectoapyrase (CD39) and ecto-5'-nucleotidase (CD73; Figure 1) [12]. Adenosine is constitutively present in the extracellular space at a concentration of 1 µmol/l in resting tissues [13] and can increase up to 100-fold in response to oxidative stress or ischemia [14]. Its systemic bioavailabilty is limited because of its rapid reuptake, degradation by adenosine deaminase to inosine, or rephosphorylation by adenosine kinase.

Apart from its central role in energy metabolism, adenosine-dependent pathways are also involved in a variety of other physiological and pathophysiological events. Acknowledgement of the potent negative dromotropic and coronary vasodilatory capacity has led to the use of adenosine to treat supraventricular tachycardias. In the peripheral and central nervous systems, adenosine is involved in a variety of processes, including cerebral blood flow, pain transmission, basal ganglia function, and seizure activity [15].

Adenosine exerts its effects through specific cell-surface receptors that are present on a wide variety of cells, including leukocytes and endothelial cells. Specific antagonism of adenosine-dependent effects by methylxanthines, caffeine, and theophylline originally led to the discovery of these receptors. Four subtypes of ARs have been described: A_1, A_{2A}, A_{2B}, and A_3. All ARs are members of the superfamily of G-protein-coupled receptors [9]. Each AR exhibits a distinct pattern of tissue distribution, intracellular signaling, and pharmacokinetic characteristics (Table 1). Each receptor subtype shows high interspecies homology among mammals [9]. The availability of selective agonists and antagonists [16,17] and gene-deficient mice for each receptor has led to a growing understanding of the biologic functions of ARs [18].

Role of adenosine in inflammation

Acknowledgment of adenosine as a potential mediator of inflammatory responses has emerged from experimental studies that showed that adenosine exhibited protective effects in various models of ischemia/reperfusion (I/R) injury [17]. I/R injury is characterized by excessive invasion of activated PMNs into tissues in which blood flow has been restored after a period of ischemia. Clinical homologs of I/R include reperfusion after organ transplantation or myocardial infarction. Presence and extent of I/R injury substantially affect the outcome and the success of therapeutic strategies that aim to re-establish blood flow in ischemic organs. Pharmacological blockade of PMN infiltration into reperfused tissues mitigates the extent of I/R injury [19]. Both PMN activation and trafficking are critically regulated by adenosine. This includes the generation of superoxide, adhesion and migration, as well as secretion of cytokines and growth factors [20]. PMNs express all four ARs [8], enabling these central cells of the innate immune system to integrate various adenosine-dependent signaling pathways.

A canine model of myocardial I/R injury exemplifies the broad immunoregulatory capacity of the adenosine-dependent signaling complex [21]. Treatment with an A_{2A} agonist reduced PMN accumulation in the postischemic tissue and reduced infarct size. The same agonist attenuated superoxide generation and adherence to endothelial cells. In addition to PMNs, platelets, macrophages, mast cells, and T cells contribute to adenosine-dependent effects, although their impact on pulmonary inflammatory responses is less obvious [17].

Adenosine in pulmonary inflammation

Involvement of adenosine in PMN-dependent inflammatory responses has evoked growing interest in studying the role played by adenosine and AR in ALI. Adenosine is constitutively released into the distal airways of C57Bl6 mice [22]. In addition, pulmonary expression of all four ARs has been demonstrated in the lungs of mice [23] and humans [24].

Chronically elevated levels of adenosine may occur as a result of defective metabolism. Gene-deficient mice that fail to express adenosine deaminase (ADA$^{-/-}$ mice) succumb to severe pulmonary inflammation at the age of 3 weeks. This inflammation is characterized by an infiltration with eosinophils [25]. In addition, elevated adenosine levels induce pulmonary fibrosis in these mice [26], which is a typical complication in the delayed phase of ARDS. The phenotype observed with ADA$^{-/-}$ mice provides compelling evidence that activation of the adenosine signaling complex aggravates chronic airway inflammatory diseases such as asthma and chronic obstructive pulmonary disease [27,28]. In contrast, protective effects of adenosine have been demonstrated by others [29], emphasizing the complexity of adenosine signaling. Although adenosine has been identified as a pivotal mediator in chronic pulmonary inflammation, its effects in ALI have only recently become the focus of experimental research.

Figure 1
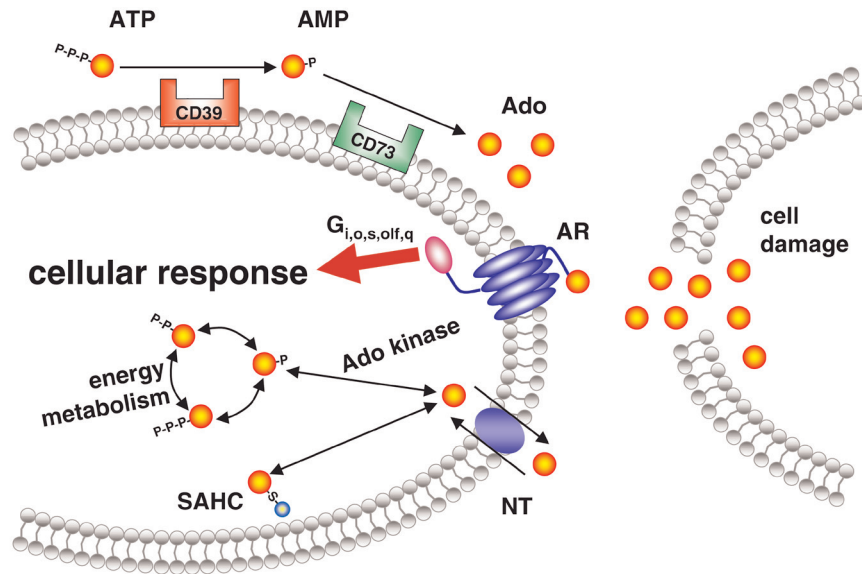
Sources of extracellular adenosine. Intracellular hydrolysis of adenine nucleotides or *S*-adenosylhomocysteine (SAHC) yields adenosine that is released via specific nucleoside transporters (NT) or nonspecifically upon cell membrane damage. In the extracellular space, adenine nucleotides are hydrolyzed by ectoapyrase (CD39) and ecto-5'-nucleotidase (CD73). Adenosine binds to specific G-protein-coupled receptors, namely the adenosine receptors (AR), which initiate various cellular responses.

Table 1

Relevant functional parameters of adenosine receptors

	A_1	A_{2A}	A_{2B}	A_3
Coupled G proteins	G_i, G_o	G_s, G_{olf}, $G_{15/16}$	G_s, G_q	G_i, G_q
Intracellular messengers	↓ cAMP ↑ IP_3/DAG ↑ Arachidonate ↑ PEtOH	↑ cAMP ↑ IP_3	↑ cAMP ↑ IP_3/DAG	↓ cAMP ↑ IP_3/DAG
Selected biologic functions	Bradycardia Antinociception Ischemic preconditioning ↓ Lipolysis, ↓ Glomerular filtration	↓ Platelet aggregation Vasodilation Protection against ischemic damage	Relaxation of vascular smooth muscle cells Stimulation of mast cell mediator release	↑ Mediator release from mast cells (mice) Preconditioning
Potency[a] (EC_{50} [μmol/l])	0.31	0.73	23.5	0.29

[a]Potency as reflected by intracellular cAMP levels. Adapted from [8,9,18,55]. DAG, diacylglycerol; ED_{50}, 50% effective dose; IP_3, inositoltriphosphate; PetOH, phosphatidylethanol.

Numerous studies have established a critical role for adenosine in the regulation of pulmonary microvascular permeability, a process that is profoundly disturbed in ALI. The majority of ALI patients show impaired alveolar fluid clearance, with a low maximal clearance being a predictor for decreased mortality [30]. Alveolar fluid clearance is reduced by activation of murine A_1, but it is increased by A_{2A} and A_3 activation [22]. In a model of lipopolysaccharide (LPS)-induced pulmonary inflammation, administration of 2-chloro-adenosine, an unselective AR agonist, leads to markedly decreased fluid accumulation in the lung of guinea pigs [31]. In pigs, administration of adenosine partly prevented the LPS-induced increase in microvascular permeability, as assessed by extravascular lung water [32]. Protective effects on pulmonary microvascular leakage have also been demonstrated in the model of fat emulsion induced ALI [33], implicating adenosine as a key molecule in the regulation of endothelial integrity and pulmonary fluid balance.

Mechanical ventilation, which is required to achieve adequate gas exchange in ARDS patients, can promote lung inflammation, especially when high tidal volumes are applied (ventilator-induced lung injury [VILI]) [2]. However, the underlying mechanisms are unclear. Mechanical ventilation of rats leads to increased levels of AMP and adenosine in bronchoalveolar lavage (BAL) fluid. This increase can be attenuated by lung-protective ventilation with low tidal volumes and application of positive end-expiratory pressure [34]. A similar increase in adenosine levels in BAL fluid has been demonstrated in mechanically ventilated mice. This increase is paralleled by induction of the rate-limiting enzymes for extracellular adenosine generation CD39 and CD73 on pulmonary endothelium and epithelium. Treatment of these mice with an A_{2B} antagonist increased albumin leakage into the BAL fluid and decreased survival. ALI was enhanced in CD39$^{-/-}$ and CD73$^{-/-}$ mice, as indicated by impaired oxygenation and increased protein content of the BAL fluid, increased PMN infiltration, and increased lung water content [35]. These findings suggest that upregulation of adenosine production in response to mechanical ventilation mediates protection from ALI. Interestingly, pulmonary PMN infiltration after hypoxia, a potent inflammatory stimulus in the lung, was also increased in CD39$^{-/-}$ and CD73$^{-/-}$ mice [36].

In humans, the role played by adenosine in acute pulmonary inflammation has not been studied systematically. Cultured human bronchial epithelial cells secrete adenosine when they are subjected to repeated mechanical stretching - a model that mimics cyclic opening and closing of distal airway structures during mechanical ventilation [35]. Shear stress on endothelial cells is also accompanied by a rise in extracellular ATP, soluble ATPase, and 5'-nucleotidase activity [37]. In addition, secreted adenosine reduces paracellular endothelial permeability *in vitro* [35], suggesting that adenosine serves as a physiological feedback mediator to protect the lung against mechanical stress. Whether insufficient production of adenosine promotes VILI in some patients has not been studied, but this might be one pathway that underlies the protective effects of ventilation with low tidal volumes.

One ligand - four receptors
A_1 - bad cop?
The AR subtype A_1 is expressed at low levels in the lung, whereas higher levels are found in the central nervous system and various other peripheral tissues [24]. In ADA$^{-/-}$ mice A_1 expression was identified on endothelial cells, airway epithelial cells and alveolar epithelial cells, and it was most pronounced on alveolar macrophages [29]. Activation of A_1 results in decreased adenylate cyclase activity via pertussis-toxin sensitive G_i and G_o proteins. A_1 also signals through phospholipase C via $G_{\beta\gamma}$ subunits, pertussis-toxin sensitive K^+ channels, and K_{ATP} channels [9,18,38]. Aside from its numerous effects on the central nervous system, A_1 signaling plays a pivotal role in manifesting I/R injury in various tissues [38,39].

Functional blocking of A_1 with a selective antagonist reduced I/R injury in the feline lung [40]. The same antagonist was examined in endotoxin-induced lung injury in cats. In that study, intralobar arterial infusion of endotoxin caused dose-dependent pulmonary infiltration with PMNs and alveolar edema. Application of the A_1 antagonist before and after the maximum dose of endotoxin abolished alveolar injury almost completely [41]. The significance of A_1 signaling in pulmonary fluid homeostasis has been demonstrated in a variety of other experimental settings. In mice, activation of A_1 but not of A_{2A} and A_3 reduced alveolar fluid clearance [22] and may have led to alveolar fluid accumulation. Consistent with these data, pretreatment with an A_1 antagonist blocked the increase in pulmonary capillary filtration in a canine model of ALI [42]. However, others have found contrary results. In rabbits, A_1 activation reduced LPS-induced pulmonary edema formation. A_2 activation had a similar effect but it also reduced pulmonary vasoconstriction [43].

The predominant proinflammatory role of A_1, as shown in different models of ALI, is counter to its protective function in chronically inflamed airways. Deletion of A_1 in ADA$^{-/-}$ mice resulted in markedly enhanced pulmonary inflammation, suggesting an anti-inflammatory role of A_1 in chronically inflamed airways [29].

In humans, the impact of A_1 signaling in ALI is not known. Interestingly, LPS has been shown to be a ligand for A_1 on human pulmonary endothelial cells. LPS displaces a selective A_1 antagonist from the receptor in a dose-dependent and competitive manner. Binding of LPS to A_1 leads to increased secretion of interleukin-6 and thromboxane A_2, which can be significantly reduced by administration of a selective A_1 antagonist [44]. The influence of A_1 signaling on pulmonary fluid homeostasis that has been observed in a variety of animal models remains to be elucidated in humans.

A_{2A} - good cop?
A_{2A} consists of 410 amino acids with an extended carboxyl-terminus, making A_{2A} the largest of all ARs. The highest expression of A_{2A} is found in the central nervous system, spleen, thymus, leukocytes, and platelets, whereas intermediate levels are found in the lung [9,18,38]. In macrophages, A_{2A} expression is increased more than 100-fold upon LPS exposure [45]. Activation of A_{2A} results in an increased adenylate cyclase activity by signaling via cholera-toxin sensitive G_s proteins. A_{2A} coupling to G_{olf} and $G_{15/16}$ has also been demonstrated, leading to increased adenylate cyclase activity and inositol triphosphate increase, respectively. Main cellular responses include coronary vasodilation and inhibition of platelet aggregation [9,18,38]. A_{2A} activation suppresses activation of immune cells, including T cells, monocytes, macrophages, PMNs, and dendritic cells [46]. Investigations in $A_{2A}^{-/-}$ mice have revealed its nonredundant role in downregulating acute inflammatory processes [47]. Activation of A_{2A} mediates protection from I/R injury in various tissues, including lung [48].

In a model of hemorrhage-induced ALI, pretreatment with an A_{2A} agonist attenuated increases in both PMN sequestration into the lung and microvascular permeability. Also clinically relevant is the fact that treatment after induction of ALI had similar effects, albeit to a lesser extent [49]. A critical role in maintaining pulmonary fluid homeostasis has been established in numerous experimental settings. Alveolar fluid clearance in mice was increased upon A_{2A} activation [22], and in a canine model of ALI pretreatment with an A_{2A} agonist or adenosine reduced capillary filtration induced by phorbol myristate acetate [42]. In accordance with these findings, treatment with an A_2 agonist completely abolished lung edema formation after LPS administration in the rabbit lung [43]. In a murine model of combined pneumonia and hyperoxia induced ALI, organ damage was attenuated and survival was prolonged after treatment with an A_{2A} agonist [50].

In a study that provided evidence of a profound anti-inflammatory effect of an A_{2A} antagonist in allergic lung inflammation [51], the authors included experiments with LPS-induced lung inflammation. Treatment with the antagonist did not have an effect on BAL fluid counts of PMNs, macrophages, or lymphocytes. Furthermore, levels of C-X-C chemokine ligand (CXCL)1 and CXCL2/3 were not significantly altered, but there was a trend toward lower levels after treatment. Levels of elastase in BAL fluid (a marker of PMN activation) were significantly reduced. Wild-type mice challenged with nebulized LPS exhibited significant accumulation of PMNs in all three compartments of the lung (pulmonary microvasculature, interstitium, and alveolar space). Pretreatment with a specific A_{2A} agonist reduced transepithelial migration of PMNs into the BAL fluid. This effect was abrogated in $A_{2A}^{-/-}$ mice and in chimeric mice that expressed A_{2A} only on non-hematopoietic cells, suggesting that A_{2A} activation on hematopoietic cells mediates protection from pulmonary inflammation. In addition, the same A_{2A} agonist reduced LPS-induced microvascular permeability and secretion of interleukin-6, tumor necrosis factor-α, CXCL1, and CXCL2/3 into the BAL fluid [52]. This highlights a predominant role for A_{2A} in mediating lung protection in various models of pulmonary inflammation.

At present, there are no data available supporting a role for A_{2A} in human ALI. However, the fact that specific A_{2A} agonists are currently being tested in clinical trials for other diseases makes this receptor an attractive target for therapeutic advancement in human ALI/ARDS. As with other immunomodulatory strategies, possible adverse effects that are associated with the biologic function of A_{2A}, such as inhibition of platelet aggregation or vasodilation, require attention. The development of agents that allow topical application to the lung via aerosol may be one concept to overcome these adverse effects.

A_{2B} - sometimes good, sometimes bad?
A_{2B} exhibits a broad expression pattern, including central nervous system, intestine, and lung. High levels of expression are found in the vasculature and on macrophages [53]. Its affinity for adenosine is the lowest of all four ARs [54,55]. In contrast to the other ARs, A_{2B} activation requires adenosine concentrations that are not reached under physiological conditions. A_{2B} is coupled to both G_s and G_q proteins, activating adenylate cyclase and phospholipase C, respectively. Consistent with an endothelial expression of A_{2B}, its activation induces vasodilation [9,18,38].

In A_{2B} gene deficient mice, baseline plasma levels of proinflammatory cytokines were greater than in wild-type mice. Upon LPS stimulation, the inflammatory response in $A_{2B}^{-/-}$ mice was substantially enhanced when compared with wild-type mice, implicating A_{2B} as a negative regulator of relevant cytokines. In addition, leukocytes from $A_{2B}^{-/-}$ mice exhibited increased adherence to the vessel wall, suggesting an important role for A_{2B} in inhibiting leukocyte trafficking to inflamed tissue [53].

A_{2B} is critical for the protective effects of adenosine in murine VILI. Treatment of mechanically ventilated mice with an A_{2B} antagonist increased pulmonary microvascular permeability and decreased survival [35]. A_{2B} was upregulated in response to a 'lung-destructive' ventilation strategy in rats, whereas its expression level remained unchanged with lung-protective ventilation [34]. Together with elevated adenosine levels during mechanical ventilation, A_{2B} appears to provide endogenous protection from VILI that might be overwhelmed by lung-hostile ventilation.

In contrast to these findings, treatment of ADA$^{-/-}$ mice with A_{2B} antagonists led to attenuation in pulmonary inflammation [56]. In addition, the authors were able to demonstrate similar proinflammatory properties of A_{2B} activation in murine bleomycin-induced lung injury [56]. These findings underline the complexity of adenosine signaling. A_{2B} - with its low affinity - is certainly a key player in the differential response to varying extracellular adenosine levels, but information on A_{2B} function in human pulmonary disease is lacking.

A_3 - good and bad?
A_3 is ubiquitously expressed. The highest levels of human A_3 are detected in lung and liver. Signaling requires pertussis-toxin sensitive G_i protein, leading to a reduction in adenylate cyclase activity. Activation of A_3 also leads to stimulation of phospholipase C via G_q proteins [9,18,38].

The biologic functions of A_3 in inflammatory disease are ambiguous. In I/R injury there is evidence for both protective [57,58] and exacerbating effects [59]. Pulmonary inflammation was attenuated by pharmacological blockade or genetic removal of A_3, suggesting a proinflammatory effect of A_3. Eosinophils and, although less pronounced, all other leukocytes - including PMNs - were reduced in the BAL fluid after treatment with an A_3 antagonist [60].

Figure 2
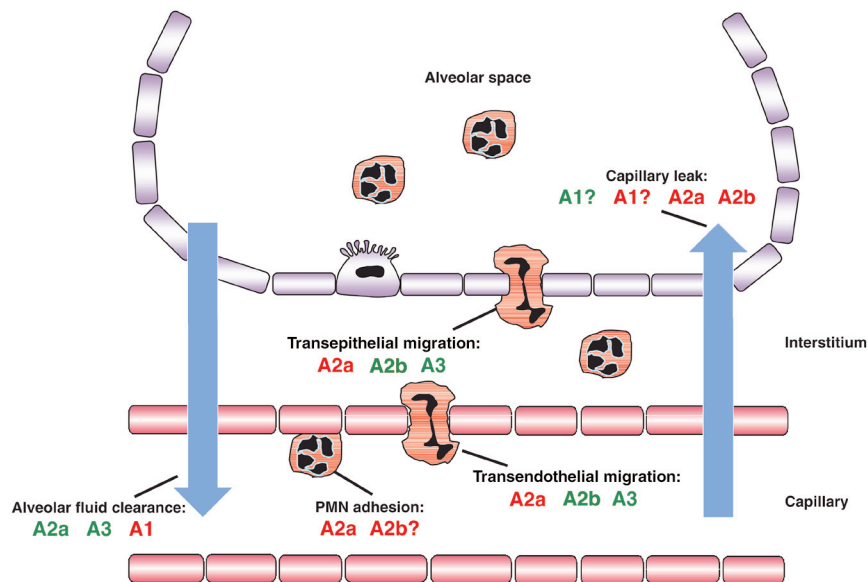
AR-dependent pathways involved in PMN trafficking and fluid homeostasis during acute pulmonary inflammation. Presented is a three compartment model (capillary, interstitium, and alveolar space). Red indicates inhibitory effects, and green indicates stimulatory effects. AR, adenosine receptor; PMN, polymorphonuclear cell.

PMN chemotaxis critically depends on A_3 signaling. PMNs that migrate toward an fMLP (N-formyl-Met-Leu-Phe) gradient exhibit an accumulation of A_3 molecules on the leading edge, the location where they bind adenosine generated through extracellular hydrolysis of ATP [61]. Because PMN migration is pivotal in ALI, A_3 appears to be an interesting target, but current knowledge on A_3 function in ALI is limited. A recent study investigated the role of A_3 in a murine model of sepsis [62]. In that study $A_3^{-/-}$ mice exhibited reduced pulmonary damage, and PMN migration into the lung was decreased. Leukocytes of these mice also failed to accumulate adequately in the intra-abdominal compartment, but survival of $A_3^{-/-}$ mice was prolonged. The authors attributed prolonged survival to the reduced secondary lung damage that had been observed in $A_3^{-/-}$ mice [62]. Data on A_3 function in human pulmonary disease are not available. However, degranulation of human PMNs has been demonstrated to be under the control of A_3 [63,64], which suggests that A_3 might well be involved in pulmonary inflammation.

Clinical implications

Selective agonist and antagonists for all four ARs are available [39]. In addition, gene-deficient mice provide an excellent tool for further elucidation of adenosine-mediated immunomodulation and validation of therapeutic approaches to ALI. However, major limitations should be considered before attempting to recapitulate experimental findings in humans. Numerous animal models have been established to study the pathophysiology of acute pulmonary inflammation.

These models mimic several aspects of the human disease, including neutrophil infiltration, microvascular permeability, or release of chemotactic cytokines. However, clinical parameters such as pulmonary gas exchange and radiological alterations are not monitored in most experimental studies. In addition, significant interspecies differences in AR amino acid sequences may be of functional significance. For example, activation of human A_{2B} induces mast cell activation [65], whereas in mice this effect is mediated by A_3 [66].

The expression pattern of AR on different cell types has not been investigated systematically. PMNs appear to be the predominant cell type that contributes to AR-dependent pulmonary inflammation. In addition, pulmonary endothelial cells are involved [52] and may be an attractive target for pharmacologic compounds that are administered topically (for instance, by inhalation). The relevance of other AR-expressing cells such as mast cells remains to be demonstrated.

Adenosine has profound influences not only on the immune system but also on hemostasis, hemodynamics, and the central nervous system. Thus, interference with this signaling complex may be associated with serious adverse effects. In ALI topical application of an aerolized agent with low systemic bioavailability might be a promising strategy.

Some agents that interfere with adenosine signaling are currently at various stages of clinical trials. Although these trials are aimed at diseases other than ALI - such as heart

failure, hypertension, renal failure, Parkinson's disease, pain, and asthma [67] - they will probably provide a first insight into the anti-inflammatory capacity and adverse effects of these agents in the clinical setting.

Conclusions

Adenosine and ARs are involved in various immunoregulatory processes, including pulmonary inflammation (Figure 2). ARs exert both proinflammatory and anti-inflammatory effects, depending on the type of receptor and the experimental setting. Profound protective effects of adenosine have been shown in systems representative of VILI and other models of primary and secondary ALI. A_{2A} and, to a lesser extent, A_{2B} appear to be major mediators of protective effects during ALI. The marked interference of adenosine signaling in pulmonary fluid homeostasis and PMN trafficking supports a key role in the pathophysiologic cascade of ALI. The availability of pharmacologic agents that selectively target ARs encourages further experimental and clinical investigation. Detailed characterization of adenosine-dependent pathways in ALI will help to develop innovative strategies for the treatment of ALI/ARDS.

Competing interests

The authors declare that they have no competing interests.

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