Role of Extracellular Adenosine in Acute Lung Injury

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Acute lung injury (ALI) is a lung disease characterized by pulmonary edema and severe hypoxia. The past decade hosted a search for endogenous mechanisms controlling lung inflammation and pulmonary edema during ALI. As such, recent evidence indicates extracellular adenosine in orchestrating the resolution of pulmonary edema and inflammation during ALI.

Acute lung injury (ALI) is a lung disease characterized by acute onset of hypoxemic respiratory failure with bilateral pulmonary edema in the absence of left heart failure (96). It is important to rule out left heart failure as a mechanism, because the treatment for pulmonary edema caused by congestive heart failure would be very different. Based on the degree of hypoxia as indicated by the gradient of arterial oxygen partial pressure (PaO_2) to the fraction of inspired oxygen (FiO_2), ALI is defined by a PaO_2/FiO_2 gradient below 300 mmHg. In its more severe form with a PaO_2/FiO_2 gradient below 200 mmHg, the term acute respiratory distress syndrome (ARDS) is used. From an etiological perspective, ALI can be caused by a diverse spectrum of mechanisms, including disorders directly involving the lungs such as pneumonia, aspiration of gastric content, or lung contusion. However, in many instances, ALI develops following an indirect injury to the lungs, for example, during sepsis or following severe trauma (96). Moreover, the transfusion of blood products is an important cause for ALI [transfusion-related ALI (TRALI)]. In fact, TRALI has been the leading cause of transfusion-related deaths in the USA (85). Despite optimal management consisting of aggressive treatment of the initiating cause, vigilant supportive care, and the prevention of nosocomial infections, mortality ranges between 35 and 60% (96). In fact, ~200,000 patients develop ALI annually in the USA, leading to 75,000 deaths and accounting for up to 3.6 million hospital days (78). The pathogenesis of ALI is characterized by the influx of a protein-rich edema fluid into the interstitial and intra-alveolar spaces as a consequence of increased permeability of the capillary-alveolar barrier. Molecular details of how capillary-alveolar leakage is caused and maintained during ALI are largely unknown, and studies on identifying its molecular or genetic basis (42) as well as linking molecular mechanisms with mechanical ventilation are currently areas of intense investigation (18, 65, 92). Particularly, studies that try to identify endogenous pathways to promote the resolution of pulmonary inflammation and injury hold the promise to identify novel therapeutic approaches to ALI, which are urgently needed (60, 80–82).

Consistent with this concept, many episodes of ALI are self-limiting and resolve spontaneously through unknown mechanisms. For example, patients undergoing major thoracic surgery for lung cancer suffer from ALI in <5% (61), open heart surgery with cardiopulmonary bypass <0.5% (66), or kidney transplantation <0.2% (84). Based on these clinical observations, studies are aimed to identify innate adaptive pathways that could dampen acute increases in the capillary-alveolar barrier, and attenuate or resolve lung inflammation. Some of these studies pointed toward the signaling molecule adenosine in endogenous lung protection during ALI. In the extracellular compartment, adenosine is produced from precursor nucleotides (ATP, ADP, and AMP) via enzymatic phosphohydrolysis. Adenosine can activate any of four G-protein-coupled adenosine receptors (ARs, A1AR, A2AAR, A2BAR, A3AR) and has been implicated as endogenous distress signal to balance inflammatory responses (33, 34, 45–47, 49) and to attenuate alveolar-capillary leakage (19, 20). In fact, recent studies suggest extracellular adenosine receptors, particularly the A2A and A2BAR, as a potential therapeutic target for the treatment of ALI.

Identification of Pulmonary-Epithelial-Derived Adenosine as Inhibitor of Capillary-Alveolar Leakage

Clinical observations indicate that ALI is a self-limiting disease with the tendency to resolve spontaneously in many instances. As such, a recent study cast a wide net to identify endogenous mediators that counterbalance increases in capillary-alveolar leakage during ALI. Because of lung failure and systemic hypoxia, most patients who experience ALI are intubated and require mechanical ventilation. To model mechanical ventilation, the authors of this study exposed pulmonary epithelial cells to cyclic mechanical stretch in vitro and studied the accumulation of mediators within the supernatant (FIGURE 1) (19). Following resolution via high-performance liquid chromatography (HPLC), the authors exposed individual fractions of the supernatant to an in vitro model of endothelial

barrier function. Indeed, the authors identified a single fraction of the supernatant from stretch-induced injury that diminished endothelial leakage. Subsequent analysis of size, stability, UV spectroscopy, and HPLC retention time demonstrated that the biologically active compound within that fraction was adenosine (19). Indeed, previous studies had indicated that extracellular adenosine can function as a signaling molecule that attenuates leakage through endothelial or epithelial barriers in the setting of acute inflammation or hypoxia (26, 29, 59, 70, 86, 87, 91–93). To confirm these in vitro findings from stretch-exposed pulmonary epithelial cells under in vivo conditions of ALI, the authors went on to utilize mechanical ventilation to induce lung injury (18). They exposed mice to mechanical ventilation at high inspiratory pressure levels over several hours and measured total pulmonary adenosine levels or adenosine concentrations within the bronchoalveolar fluid. Consistent with their initial in vitro studies, they found that adenosine levels were elevated in the lungs of mice or in the bronchoalveolar fluid following the induction of ALI (19). Taken together, these studies identified extracellular adenosine as a barrier protective factor within the supernatant of pulmonary epithelial cells exposed to cyclic mechanical stretch in vitro or within the bronchoalveolar fluid of lungs exposed to ALI (19).

Extracellular Nucleotide Release

In the extracellular space, adenosine mainly stems from phosphohydrolysis of precursor nucleotides, specifically ATP and ADP. Such nucleotides are released from multiple cell types (69). Studies have shown that, during acute injury (e.g., by hypoxia or inflammation), extracellular nucleotide levels are dramatically increased (22, 39, 40, 55). These increases in extracellular nucleotide levels are mainly due to release of ATP or ADP from the intracellular to the extracellular space (25, 26). One has to keep in mind that intracellular ATP concentrations are relatively high, ranging from 2 to 6 mM (69). As such, leakage of intracellular ATP stores from injured cells during ALI represents an important source for elevating extracellular ATP levels. For example, exposure of pulmonary endo- or epithelial cells to oxidative stress or to hyperoxia results in a rapid release of ATP (2, 3, 36). Previous studies have also demonstrated regulated mechanisms of cellular ATP release (25, 26, 28, 31). As such, platelets can release nucleotides (particularly in the form of ADP) into the extracellular space on activation by ADP or collagen (37, 97). Moreover, PMNs that accumulate in pulmonary tissues during ALI represent an additional source for extracellular nucleotides. In fact, a recent study showed a critical role of PMN-dependent ATP release in directed movement of PMNs. Such purinergic chemotaxis appears to involve ATP release on the leading edge of the PMN (10, 63). Studies on the mechanism of PMN-dependent ATP release have revealed an important role of the pore-forming hemichannel connexin 43 (Cx43) (25). Although PMN-dependent ATP release occurs on activation with inflammatory stimuli, pharmacological inhibition of Cx43-dependent transport with specific connexin-mimetic peptides completely abolished the
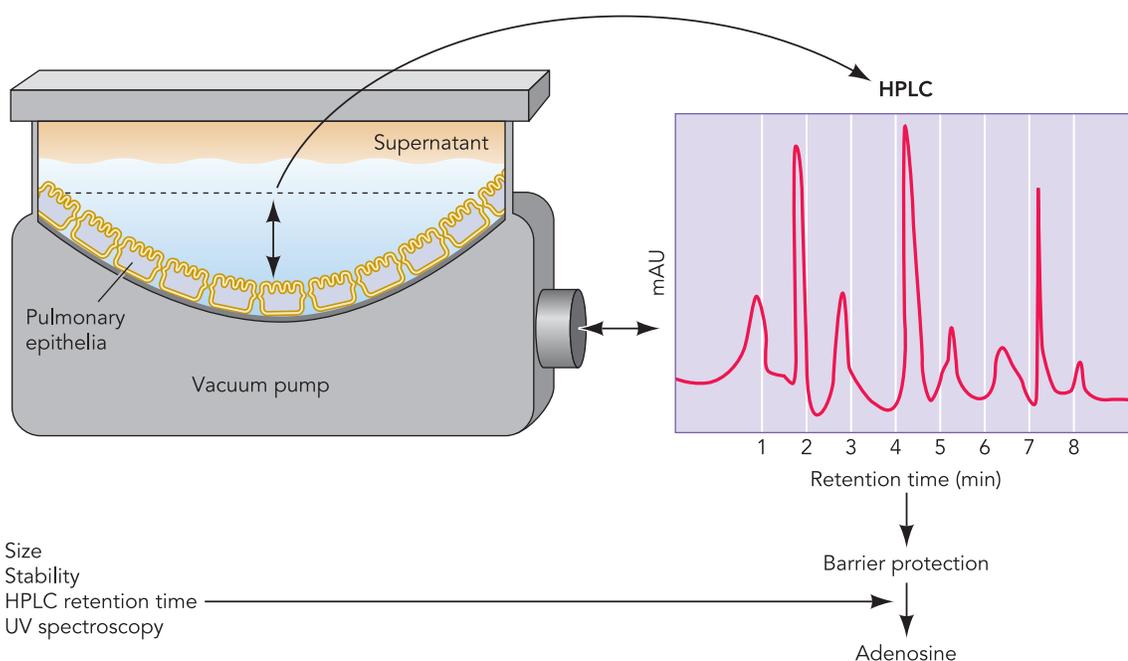

FIGURE 1.
Identification of adenosine as barrier-protective factor in the supernatant of pulmonary epithelial cells during stretch-injury
A recent study identified accumulation of extracellular adenosine within the supernatant of stretched pulmonary epithelial cells as barrier protective mediator (19). For this purpose, the authors exposed Calu-3 human bronchial epithelial cells to cyclic mechanical stretch over 48 h. Supernatants were harvested, fractionated by HPLC, and tested for bioactivity for endothelial barrier protection. A single fraction was found to be biologically active, and subsequent studies based on molecular size, stability, HPLC retention time, and UV spectroscopy determined adenosine as the biologically active compound.

rapid and robust ATP-release from stimulated PMN. These pharmacological studies were confirmed in genetic models, where neutrophils isolated from mice with induced gene-deletion of Cx43 had attenuated ATP-release on inflammatory activation (25). Taken together, such studies indicate the likelihood that extracellular levels of nucleotides, particularly ATP and ADP, are elevated during ALI.

Extracellular Nucleotide Signaling During Lung Injury

There are two principal pathways that extracellular nucleotides can be involved in. First, they can activate extracellular nucleotide receptors (so-called P2 receptors), or they can be rapidly hydrolyzed to extracellular AMP and adenosine. Although there is only very little known about the role of AMP as a signaling molecule, ATP or ADP signaling has been implemented in lung disease. As such, ATP or ADP can signal through G-protein-coupled receptors (P2Y receptors) or through ligand-gated ion channels (P2X receptors). Although their role in ALI has yet to be better defined, some studies suggest a detrimental role in lung inflammation and asthma. As such, a very elegant study by Idzko et al. showed that extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells (53). The authors showed that allergen challenge causes acute accumulation of ATP in the airways of asthmatic subjects and mice with experimentally induced asthma. All the cardinal features of

FIGURE 2. **Pulmonary ecto-apyrase (CD39) in extracellular adenosine generation**
During conditions of acute lung injury (ALI), extracellular nucleotides in the form of ATP (and also ADP; not shown in the figure) are released by different cell types. The ecto-apyrase (CD39), which is expressed on pulmonary epithelial cells, vascular endothelia, or immune cells, rapidly converts extracellular ATP to AMP. This transcriptionally controlled metabolic pathway represents the first step for the extracellular generation of adenosine during ALI.

asthma were abrogated when lung ATP levels were locally neutralized using apyrase or when mice were treated with broad-spectrum P2-receptor antagonists. As such, this study shows that ATP signaling has a key role in allergen-driven lung inflammation (53).

Enzymatic Nucleotide Phosphohydrolysis Via CD39 and CD73

Extracellular nucleotides in the form of ATP or ADP are rapidly hydrolyzed in the extracellular space to adenosine. In fact, extracellular adenosine generation from precursor nucleotides occurs in a two-step enzymatic reaction. First, extracellular ATP and ADP are converted to AMP (FIGURE 2). This reaction is catalyzed by the ecto-nucleotide-diphosphohydrolase (E-NTPDase) 1, also known as ecto-apyrase or CD39 (76). Research work from the laboratory of Simon C. Robson indicates that CD39 is expressed ubiquitously on endothelia, epithelia, or inflammatory cells (4, 14, 25–27, 30, 43) and rapidly catalyzes the generation of AMP from ATP or ADP. The second step of extracellular adenosine generation is catalyzed by the ecto-5'-nucleotidase, also known as CD73, which is a GPI anchored ecto-enzyme catalyzing the conversion of extracellular AMP into adenosine (FIGURE 3) (12). Research work from the laboratories of Linda Thompson and Sean Colgan demonstrates that the ecto-5'-nucleotidase is expressed ubiquitously with high enzymatic activity in pulmonary tissues (93). Localized on the extracellular surface of endothelial or epithelial cells, CD73 rapidly converts AMP to adenosine (22, 39, 91, 93). During acute injury (e.g., hypoxia), extracellular adenosine production via CD39 and CD73 are increased (19). For example, recent studies on the influence of hypoxia or ischemia on CD39 or CD73 transcript, protein, and enzymatic function found that both enzymes are rapidly induced by limited oxygen availability (22, 26, 27, 39–41, 46, 49, 55, 91). Although the transcriptional pathways of CD39-dependent induction during hypoxia involves the transcription factor SP1 (27), transcriptional mechanisms of hypoxia-dependent induction of CD73 are coordinated by hypoxia-inducible factor (HIF)-1 (91). As such, epithelia or endothelia preexposed to hypoxia show dramatic increases in their capacity to hydrolyze extracellular ATP to adenosine (26, 91). Such studies also highlight the role of hypoxia-inducible factor in attenuating mucosal inflammation during inflammation or limited oxygen availability (13, 21, 24, 44, 54, 57, 67, 68, 75, 77, 91).

SiRNA approaches, or studies in gene-targeted mice for CD39 or CD73 confirmed, their importance for the increased phosphohydrolysis during hypoxia (22, 26, 27, 39–41, 46, 49, 55, 91). As such, several studies utilized an in vivo model of ambient hypoxia with exposure of mice to 8% oxygen over 4 h (24, 67, 68).
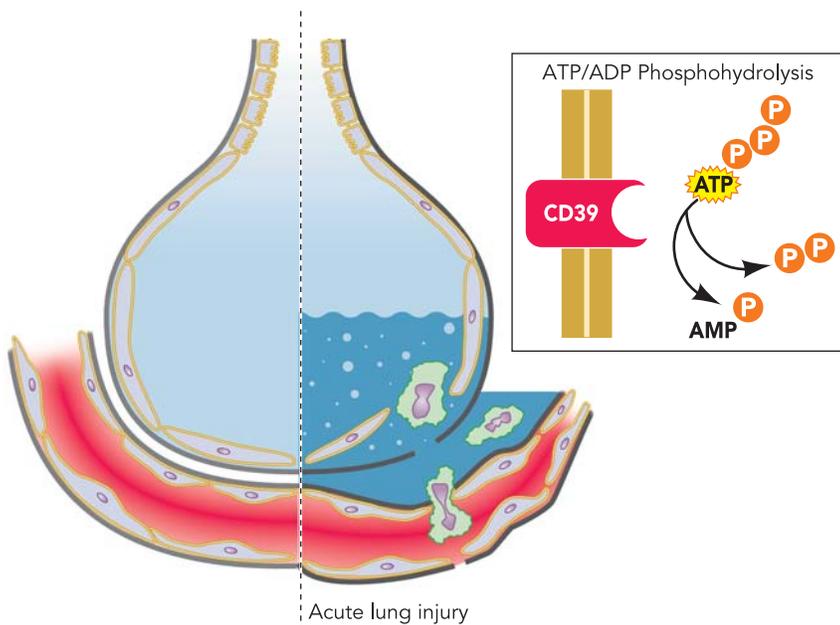
Although hypoxia elicited increases in vascular leakage, inflammatory cell accumulation, and pulmonary edema in littermate controls, these responses were dramatically enhanced in $cd39^{-/-}$ or $cd73^{-/-}$ mice (26, 93), thereby confirming an in vivo role of CD39- and CD73-dependent nucleotide phosphohydrolysis and adenosine signaling in protecting the alveolar-capillary barrier function and balancing pulmonary inflammation during hypoxia (26, 29).

Other studies examined the role of CD39 and CD73 in models of ALI. Utilizing mechanical ventilation to model ALI, the authors found induction of pulmonary CD39 and CD73 levels (19). Moreover, these studies observed pressure- and time-dependent increases in pulmonary edema and inflammation in $cd39^{-/-}$ mice during ALI compared with control animals. Similarly, pharmacological inhibition or targeted gene deletion of cd73 was associated with increased symptom severity of ventilator-induced ALI. Reconstitution of $cd39^{-/-}$ or $cd73^{-/-}$ mice with soluble apyrase (converts ATP/ADP to AMP) or 5'-nucleotidase (converts AMP to adenosine), respectively, reversed such increases. In addition, ALI was significantly attenuated and survival improved after intraperitoneal treatment of wild-type mice with soluble apyrase or 5'-nucleotidase.

Similar to these studies in ALI, a very elegant study from the research team of Michael Blackburn found that adenosine levels are elevated in the lungs of mice injured by the drug bleomycin (95). In addition, increased activity of ecto-5'-nucleotidase (CD73) was found in the lungs in conjunction with adenosine elevations. To determine the contribution of CD73 to the generation of adenosine in the lung, $cd73^{-/-}$ mice were subjected to bleomycin challenges, which represents a more chronic type of lung injury compared with studies utilizing hypoxia, mechanical ventilation, or LPS inhalation to induce lung injury (17–20, 74, 94). Results demonstrated that $cd73^{-/-}$ mice challenged with bleomycin no longer accumulated adenosine in their lungs, suggesting that the primary means of adenosine production following bleomycin injury resulted from the release and subsequent dephosphorylation of adenine nucleotides. $Cd73^{-/-}$ mice challenged with bleomycin exhibited enhanced pulmonary inflammation and fibrosis as well as exaggerated expression of proinflammatory and profibrotic mediators in the lung. Intranasal instillations of exogenous nucleotidase restored the ability of lungs of $cd73^{-/-}$ mice to accumulate adenosine following bleomycin challenge. Furthermore, these treatments were associated with a decrease in pulmonary inflammation and fibrosis. Wild-type mice challenged with bleomycin and supplemented with exogenous nucleotidase also exhibited reduced inflammation. These findings indicate CD73-dependent adenosine production as anti-inflammatory pathways in bleomycin-induced lung injury, a murine model for pulmonary fibrosis and chronic forms of lung disease (95).

Other studies examined the role of CD39 and CD73 in mediating pulmonary neutrophil (PMN) transmigration during lipopolysaccharide (LPS)-induced lung injury. Similar to ALI induced by mechanical ventila-

> *"Taken together, these data reveal an important role for CD39- and CD73-dependent generation of extracellular adenosine during ventilator-, LPS-, or bleomycin-induced forms of lung injury."*

tion, pulmonary CD39 and CD73 transcript levels were elevated following LPS exposure in vivo. Moreover, LPS-induced accumulation of PMN into the lungs was enhanced in gene-targeted mice for $cd39$ or $cd73$, particularly into the interstitial and intra-alveolar compartment. Such increases in PMN trafficking were accompanied by corresponding changes in alveolar-capillary leakage. Similarly, inhibition of extracellular nucleotide phosphohydrolysis with the nonspecific ecto-nucleoside-triphosphate-diphosphohydrolases inhibitor POM-1 confirmed increased pulmonary PMN accumulation in wild-type, but not in gene-targeted mice for $cd39$ or $cd73$. Taken together, these data reveal an important role for CD39- and CD73-dependent generation of extracellular adenosine during ventilator-, LPS-, or bleomycin-induced forms of lung injury. In addition, these studies suggest
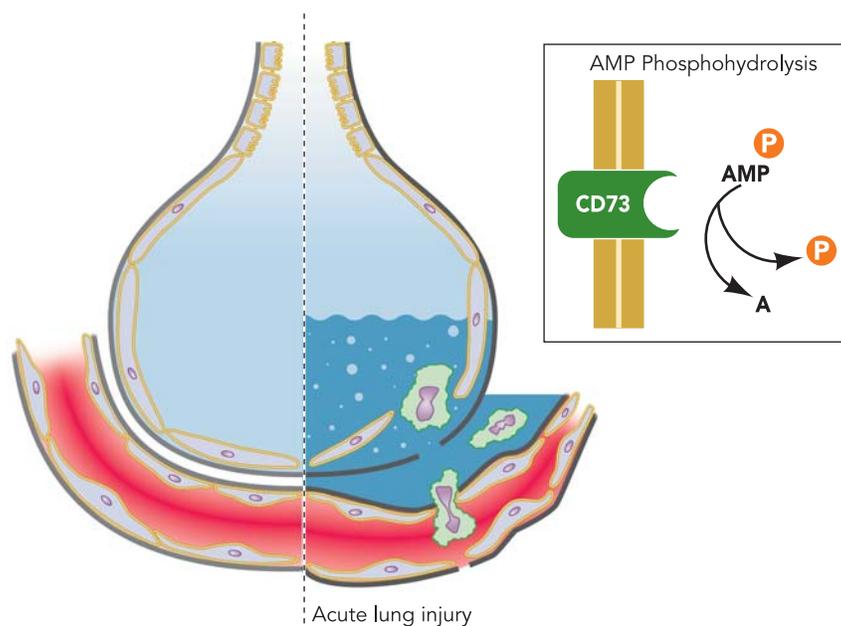
FIGURE 3. **Ecto-5'-nucleotidase (CD73) in extracellular adenosine production of the lungs**
The ecto-5'-nucleotidase (CD73) is a GPI-anchored enzyme localized exclusively on the extracellular surface and rapidly converts extracellular AMP to adenosine. The lungs express high levels of CD73, including pulmonary epithelia, vascular endothelia, or immune cells. As such, CD73-dependent adenosine generation represents the pacemaker in extracellular adenosine production during acute lung injury.

their soluble compounds (e.g., soluble nucleotidase or apyrase) as a therapeutic strategy for improving the capillary alveolar barrier function, attenuating inflammatory cell trafficking into the lungs, or dampening fibrotic changes (19, 74, 95).

Adenosine Signaling During ALI

In the extracellular space, adenosine mainly serves as signaling molecule that can activate four adenosine receptors (ARs) (33). Four different receptors have been described (A1AR, A2AAR, A2BAR, and A3AR; FIGURE 4) (22, 58, 79, 90). Although ARs result in different biological functions, extracellular adenosine signaling effects depend on the expression of individual ARs on the extracellular surface (17, 20, 22, 26, 38, 45, 56, 77). ARs contain seven transmembrane-spanning domains and are coupled to intracellular GTP-binding proteins, utilizing intracellular cyclic AMP (cAMP) as second messenger (23, 50, 51). As such, AR signaling occurs through changes in adenylyl cyclase activity, resulting in subsequent alteration of intracellular cAMP levels as second messenger (33). In general terms, the A1AR and the A3AR are thought to attenuate cAMP. In contrast, the A2AAR and the A2BAR are associated with cAMP elevations (23, 33, 50, 51, 62). In addition to alerting cAMP levels, adenosine signaling has also been implemented in modulating intracellular calcium levels (33, 50, 51).

Previous studies compared expressional levels of ARs in the lungs of mice (64). These studies demonstrated the highest expressional levels of the A2BAR, followed by the A2AAR (64). Other studies found increased levels of A2BAR expression with large tidal volume ventilation (15) or following the induction of ALI by mechanical ventilation (18). Studies using an A2BAR reporter mouse model revealed high expression of the A2BAR in murine type II alveolar epithelial cells (8). In addition, other studies using the same A2BAR reporter model identified a vascular A2BAR expression pattern (9, 38, 98, 99).

A2A Adenosine Receptors

Although there is only little known about the role of the A1AR or the A3AR in lung injury, several studies point toward a protective role of A2A or A2BAR signaling during ALI. One of the first studies to provide genetic in vivo evidence for adenosine-dependent protection from excessive inflammation in different models utilized gene-targeted mice for the A2AAR (70). Subthreshold doses of an inflammatory stimulus that caused minimal tissue damage in wild-type mice were sufficient to induce extensive tissue damage, more prolonged and higher levels of pro-inflammatory cytokines, and death in $A2AAR^{-/-}$ mice. Similar observations were made in studies of three different models of inflammation and liver damage as well as during bacterial endotoxin-induced septic shock (70). These studies indicate that the A2AAR plays a critical part of the physiological negative feedback mechanism for limitation and termination of tissue-specific and systemic inflammatory responses (70). Other studies confirmed a role for the A2AAR receptor in lung protection during ALI. Several studies identified a role of A2AAR signaling predominantly via activation of myeloid A2AARs in different models of lung protection, including LPS-induced lung injury (73), or in models of lung injury induced by pulmonary ischemia reperfusion injury (83) or lung transplantation (35). This is also consistent with studies from the laboratory of Joel Linden demonstrating myeloid A2AARs in attenuating myocardial ischemia reperfusion injury (101).

A very elegant study by Thiel et al., in a team led by Michail Sitkovsky, tested the hypothesis that oxygenation weakens a tissue-protecting mechanism triggered by hypoxia. This study is based on the observation that hypoxia triggers signaling pathways mediated by the A2AAR that attenuate lung inflammation and tissue damage (92). This hypoxia-driven pathway protects the lungs from the toxic effects of overactive immune cells such as neutrophils (92). Using a mouse model of ALI induced by bacterial infection, Thiel et al. exposed one group of mice to 100% oxygen, mimicking therapeutic oxygenation, and left another group at normal ambient levels (21% oxygen) (92). Five times more mice died after receiving 100% oxygen than died breathing normal oxygen levels. Mice given 60% oxygen—which is considered clinically safe—got worse but did not die. Hypoxia protects
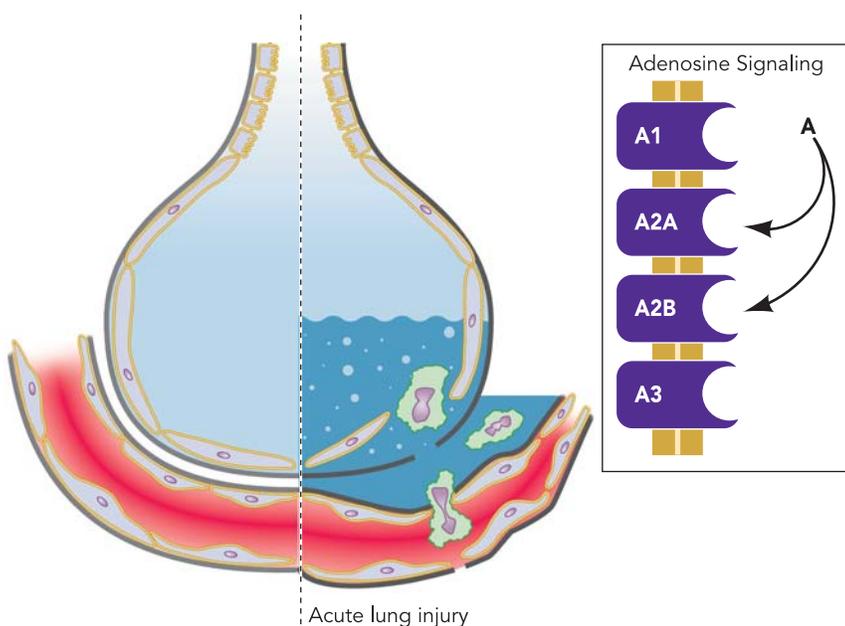

FIGURE 4. **Extracellular adenosine signaling during acute lung injury**
Adenosine (A) signaling during acute lung injury can involve any of four adenosine receptors: the A1, A2A, A2B, and A3 receptors. Based on transcriptional profiling studies, highest expression levels were found for the A2A and the A2B adenosine receptor. In addition, expression of both receptors can be increased under conditions of hypoxia, cyclic mechanical stretch, or acute inflammation. As such, several studies have provided evidence for attenuation of lung inflammation or pulmonary edema via A2A or A2B adenosine signaling.

against lung damage, the authors conclude, by working through the A2AR signaling pathway to control inflammation. Above-normal oxygen levels interrupt this anti-inflammatory pathway, paving the way for further lung injury (52a). Consistent with this work, a very recent study demonstrated a specific transcriptional pathway for HIF-2α in the control of the A2AAR during hypoxia (1). Taken together, such studies indicate that high levels of inspired oxygen, as may be required to provide sufficient tissue oxygenation in patients suffering from ALI, may weaken the local tissue hypoxia-driven and AR-mediated anti-inflammatory mechanism and thereby further exacerbate lung injury (23, 92).

A2B Adenosine Receptors

Other studies found an important contribution of signaling via the A2BAR in lung protection from ALI. First evidence comes from studies of hypoxia-induced vascular leakage, lung inflammation, and pulmonary edema (17). Here, the authors exposed gene-targeted mice for each individual AR to ambient hypoxia (4% oxygen over 4 h) and assessed pulmonary edema and vascular leakage. The authors found a selective phenotype in gene-targeted mice for the *A2BAR*. Moreover, treatment with a highly specific A2BAR agonist (BAY 60-6583) (22) attenuated hypoxia-induced alveolar-capillary leakage. Further studies examined the contribution of endogenous adenosine signaling to attenuation of VILI or endotoxin-induced lung injury (20). Initial profiling studies using gene-targeted mice for the *A1*, *A2A*, *A2B*, or *A3AR* revealed that genetic deletion of the *A2BAR* was specifically associated with reduced survival time and increased pulmonary albumin leakage (5.3 ± 0.15-fold) during ALI. Studies in wild-type mice showed that treatment with A2BAR-selective antagonist PSB1115 resulted in enhanced pulmonary inflammation, edema, and attenuated gas-exchange, whereas treatment with the A2BAR-agonist BAY 60-6583 (22) attenuated VILI. Studies in bone marrow chimeric *A2BAR* mice demonstrated pulmonary A2BARs in VILI-induced albumin leakage and edema, whereas increases in pulmonary inflammation were, at least in part, bone marrow mediated. Measurement of alveolar fluid clearance indicated that A2BAR signaling enhanced amiloride-sensitive fluid transport via elevation of pulmonary cAMP levels, similar to beta-adrenergic agonist stimulation, suggesting that A2BAR-agonist treatment protects by drying out the lungs during VILI (20). Taken together, such studies demonstrate that extracellular adenosine production via CD39 and CD73, in conjunction with A2A or A2BAR signaling, represents an endogenous pathway to protect the lungs from pulmonary edema and excessive inflammation (19, 20, 23).

Chronic Elevation of Extracellular Adenosine Levels

In contrast to the beneficial effects of increased adenosine production and signaling during ALI, there is some evidence suggesting a potentially detrimental role of chronically elevated adenosine levels (5, 11, 88, 89). For example, levels of adenosine are increased in the lungs of asthmatics (16) and correlate with the degree of inflammatory insult (52). At present, it is not entirely clear whether such elevations of adenosine are part of a protective pathway to dampen lung inflammation or play a provocative role of adenosine in asthma or chronic obstructive pulmonary disease (32). For example, mice incapable of extracellular adenosine generation (*cd73⁻/⁻* mice) exhibit a more severe phenotype in bleomycin-induced lung injury, indicating a protective role of extracellular adenosine signaling in this chronic model of lung disease (95).

In contrast, adenosine-deaminase (ADA)-deficient mice develop signs of chronic lung inflammation in association with dramatically elevated pulmonary adenosine levels. In fact, ADA-deficient mice die within weeks after birth from severe respiratory distress (7), and pharmacological studies suggest that attenuation of adenosine signaling through the A2BAR may reverse the severe pulmonary phenotypes in ADA-deficient mice (7, 89). To address these findings on a genetic level, the research team of Michael Blackburn recently examined the contribution of A2BAR signaling in this model by utilizing a genetic approach by generating ADA/A2BAR double-knockout mice (104). The authors' initial hypothesis was that genetic removal of the A2BAR from ADA-deficient mice would lead to diminished pulmonary inflammation and damage. Unexpectedly, ADA/A2BAR double-knockout mice exhibited enhanced pulmonary inflammation and airway destruction. Marked loss of pulmonary barrier function and excessive airway neutrophilia are thought to contribute to the enhanced tissue damage observed. These findings support an important protective role for A2BAR signaling during acute stages of lung disease (104).

Summary and Future Challenges

This review highlights the role of extracellular adenosine production and signaling as a transcriptionally controlled metabolic pathways in endogenous lung protection from acute injury. Many of these studies indicate that targeting extracellular adenosine production and signaling may represent a novel therapeutic approach for the treatment of lung inflammation and pulmonary edema during ALI. From a clinical perspective, new approaches for treating patients suffering from ALI are urgently needed for several reasons. First, ALI is among the leading causes for morbidity and mortality of critically ill patients (78). In

addition, treatment approaches for ALI are extremely limited and focus on elimination of potential causes, in conjunction with supportive therapy (96). In fact, no specific therapy for attenuating pulmonary edema and lung inflammation during ALI has been established as an effective clinical form of therapy (102, 103).

It is important to point out that the studies described in the present review were carried out in animal models, in fact, most of them in mice. Therefore, it will be a critical challenge for the future to translate these findings from mice to humans. In addition, potentially unwanted side effects of pharmacological elevations of extracellular adenosine levels or specific AR agonists have to be addressed. For example, such unwanted side effects could include alterations in blood pressure, heart rate, or sleep-awake cycle (100), fatty liver disease (71), or chronic forms of lung disease (89) or involve platelet function, thromboregulation, or bleeding (30, 48, 72). ■

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