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Alveolar Epithelial A2B Adenosine Receptors in Pulmonary Protection during Acute Lung Injury

Sandra Hoegl,*⁺ Kelley S. Brodsky,* Michael R. Blackburn,⁺ Harry Karmouty-Quintana,‡ Bernhard Zwissler,⁺ and Holger K. Eltzschig*⁺

Acute lung injury (ALI) is an acute inflammatory lung disease that causes morbidity and mortality in critically ill patients. However, there are many instances where ALI resolves spontaneously through endogenous pathways that help to control excessive lung inflammation. Previous studies have implicated the extracellular signaling molecule adenosine and signaling events through the A2B adenosine receptor in lung protection. In this context, we hypothesized that tissue-specific expression of the A2B adenosine receptor is responsible for the previously described attenuation of ALI. To address this hypothesis, we exposed mice with tissue-specific deletion of Adora2b to ALI, utilizing a two-hit model where intratracheal LPS treatment is followed by injurious mechanical ventilation. Interestingly, a head-to-head comparison of mice with deletion of Adora2b in the myeloid lineage (Adora2blox/loxP LysM Cre⁺), endothelial cells (Adora2blox/loxP VE-cadherin Cre⁺), or alveolar epithelial cells (Adora2blox/loxP SPC Cre⁺) revealed a selective increase in disease susceptibility in Adora2blox/loxP SPC Cre⁺ mice. More detailed analysis of Adora2blox/loxP SPC Cre⁺ mice confirmed elevated lung inflammation and attenuated alveolar fluid clearance. To directly deliver an A2B adenosine receptor–specific agonist to alveolar epithelial cells, we subsequently performed studies with inhaled BAY 60-6583. Indeed, aerosolized BAY 60-6583 treatment was associated with attenuated pulmonary edema, improved histologic lung injury, and dampened lung inflammation. Collectively, these findings suggest that alveolar epithelial A2B adenosine receptor signaling contributes to lung protection, and they implicate inhaled A2B adenosine receptor agonists in ALI treatment.

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Abbreviations used in this article: AFC, alveolar fluid clearance; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; HIF, hypoxia-inducible factor; i.e., intratrachealy; LPS + VILI, LPS + ventilator-induced lung injury; MPO, myeloperoxidase; PEEP, positive end-expiratory pressure; VILI, ventilator-induced lung injury.

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endothelial cells, and alveolar epithelia. Indeed, our studies specifically implicate alveolar epithelial A2B adenosine receptor signaling in lung protection during ALI.

Materials and Methods

Two-hit model of ALI

Patients frequently experience ALI via a septic event that subsequently requires mechanical ventilation. Therefore, we decided to use a two-hit model of ALI where an inflammatory event (intratracheal [i.t.] LPS treatment) is followed by injurious mechanical ventilation. Age- (8–12 wk old), weight-, and gender-matched mice were anesthetized with pentobarbital (70 mg/kg i.p.) before the procedure. LPS (Escherichia coli 011:B4, L4391; Sigma-Aldrich, St. Louis, MO; 3.75 μg/g body weight) or PBS (Life Technologies, Grand Island, NY) as vehicle were administered i.t. via a 22-gauge catheter. After 24 h, mice were anesthetized again, underwent tracheotomy, and were ventilated for 3 h in a pressure-controlled ventilation mode (Servo 900C, Siemens, Munich, Germany). In the LPS + ventilator-induced lung injury (LPS + VILI) group, animals were ventilated with an inspiratory pressure level of 35 cmH₂O, whereas control (sham) mice were ventilated with an inspiratory pressure level of 15 cmH₂O. Both groups were maintained at a positive end expiratory pressure (PEEP) of 3 cmH₂O and an FiO₂ of 1. To differentiate the effects of ventilator-induced lung injury and LPS treatment, we used animals that received PBS i.t. before ventilation with 35 cmH₂O (VILI) and animals that received LPS i.t. following low inspiratory pressure ventilation with 15 cmH₂O (LPS). At the end of the experiment, mice were killed by exsanguination under deep anesthesia. Bronchoalveolar lavage (BAL) fluid was obtained by lavaging the lungs three times with 1 ml PBS. After centrifugation at 300 × g for 5 min at 4 °C, cell-free BAL was immediately snap frozen for subsequent ELISA studies. Pulmonary tissue was flushed with 10 ml saline via the right ventricle, and either snap frozen in liquid nitrogen and stored at −80 °C or conserved in formalin for histologic analysis.

Mice

Wild-type (C57/B6), VE-cadherin Cre⁺ [B6.Cg-Tg(Cdh5-cre)7Mlia/J] (21), and LysM Cre⁺ [B6.129P2-Ly6C-cre/J] (22) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). SPC Cre⁺ mice were obtained from the National Institutes of Health protection of Institutional Review Board at the University of Colorado (Denver, CO) and were bred and maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals (DuoSet, R&D Systems, Minneapolis, MN) following the manufacturer’s instructions (28).

Isolation of murine alveolar epithelial cells

Mice were euthanized by pentobarbital overdose. Following tracheotomy, lungs were lavaged three times with 1 ml PBS and then perfused with PBS via the right ventricle. As previously described (29), dispase (BD Biosciences, Bedford, MA) was instilled into the lungs followed by a low-melting point agarose (IBI/Shelton Scientific, Shelton, CT) plug. Lungs were removed and incubated at 37 °C for 30 min. After tissue dissection using forceps, cells were progressively filtered (70 μm/40 μm, BD Falcon). Using the Miltenyi QuadroMACS separator and magnetic microbeads (Miltenyi Biotec), cells were first negatively selected for CD45 depletion and in a second step positively selected for EpCAM (eBioscience, San Diego, CA).

Measurement of BAL fluid albumin content, myeloperoxidase assay, and cytokine concentrations

Albumin content of BAL was measured with a mouse albumin ELISA quantitation set (Bethyl Laboratories, Montgomery, TX) according to the manufacturer’s instructions. Myeloperoxidase (MPO) is rapidly released by activated polymorphonuclear neutrophils, monocytes, and macrophages. MPO concentrations in the BAL were measured with a mouse MPO ELISA kit (HyQuich Biotech, Plymouth Meeting, PA) according to the manufacturer’s instructions (17, 30). CXCL1 and IL-6 concentrations in the BAL were measured by ELISA using specific Abs and standards (DuoSet, R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

Lang histology and lung injury scoring

Formalin-fixed lungs were dehydrated in ethanol gradients and embedded in paraffin, sectioned at 5 μm, and stained with H&E. Lung samples were analyzed blinded to group assignments and assessment of histological lung injury was performed by grading as follows (1–9) (31): infiltration or aggregation of inflammatory cells in air space or vessel wall: 1, only wall; 2, few cells (one to five cells) in air space; 3, intermediate; 4, severe (air space congested); interstitial congestion and hyaline membrane formation: 1, normal lung; 2, moderate (<25% of lung section); 3, intermediate (25–50% of lung section); 4, severe (>50% of lung section); hemorrhage: 0, absent; 1, present.

RNA isolation and real-time PCR

Total RNA was extracted from lung tissue or isolated cells by QIAzol reagent (Qiagen), followed by cDNA synthesis using the iScript cDNA synthesis kit (Qiagen) according to the manufacturer’s instructions (31). Quantitative RT-PCR (Qiagen) was performed to measure relative mRNA levels for various transcripts. Data were normalized to β-actin expression and are presented as fold change in mRNA expression relative to controls. The following murine Quantitect primer assays were used according to the manufacturer’s instructions (Qiagen): β-actin (QT01136772), TNP-α (QT00104006), IL-1β (QT01048355), IL-6 (QT00098875), and Adora2b (QT00257558).

Alveolar fluid clearance

Alveolar fluid clearance (AFC) was assessed using a previously described technique (32). In short, mice were pretreated and ventilated as described above, and AFC was measured at the end of the 3-h ventilation time. First,
300 μl iso-osmolar (320 mOsm) 0.9% NaCl solution containing 5% albumin (Sigma-Aldrich) and 0.1% FITC-labeled albumin was instilled via the endotracheal catheter into the alveolar space during 10 s, followed by injection of 100 μl air to clear the dead space and position the fluid in the alveolar space. After 15 min, the chest was opened to produce bilateral pneumothoraces to facilitate aspiration of the remaining alveolar fluid via the tracheal catheter. FITC-labeled albumin concentration was quantified against a standard curve of FITC-albumin using spectrofluorometry at an excitation wavelength of 485 nm and an emission wavelength of 528 nm. AFC was calculated using the following equation: AFC = (1 – C1/C15)/0.95 (33), where C is the FITC-albumin concentration of the instillate and C15 is the FITC-albumin concentration of the sample obtained after 15 min. Clearance is expressed as a percentage of total instilled volume (percentage/15 min).

**Statistical analysis**

All parametric data were compared by two-way ANOVA with a Bonferroni post hoc test or t test where appropriate. Comparison of nonparametric results between groups was performed using the Mann–Whitney U test. Values are expressed as mean and SD or as median (5th to 95th percentiles), as indicated. A p value <0.05 was considered statistically significant. For all statistical analysis GraphPad Prism 5.0 software was used.

**Results**

Mice with global deletion of the Adora2b adenosine receptor experience more severe ALI

To address tissue-specific functions of the A2B adenosine receptor during ALI, we first established the phenotype of mice with global deletion of the Adora2b in a two-hit lung injury model. In a clinical setting, sepsis is one of the initiating causes of ARDS in patients, requiring subsequent endotracheal intubation and mechanical ventilation (34). Therefore, we induced ALI by first exposing mice to LPS during 24 h, followed by injurious mechanical ventilation utilizing pressure-controlled ventilation at an inspiratory pressure level of 35 cmH2O during 3 h (LPS + VILI, Fig. 1A). We think that this two-hit model closely resembles the clinical scenario in which many patients develop ARDS. To demonstrate the effects of 3 h of ventilation with an inspiratory pressure level of 35 cmH2O without preinjury by LPS (VILI) and, alternately, the administration of LPS i.t. without subsequent injurious ventilation (LPS), we included appropriate control groups additionally.

**FIGURE 1.** Two-hit model of ALI in mice gene-targeted for the A2B adenosine receptor. (A) Experimental set-up: Adora2b−/− mice or control mice matched in age, gender, and weight (Adora2b+/+) were exposed to i.t. LPS treatment (from E. coli 0111:B4, 3.75 mg/kg body weight) 24 h prior to injurious mechanical ventilation (180 min of pressure-controlled mechanical ventilation, inspiratory pressure of 35 cmH2O, PEEP of 3 cmH2O, inspired oxygen concentration of 100%; LPS + VILI). Intratracheal PBS instillation and mechanical ventilation at low pressure was used in sham controls (180 min of pressure-controlled mechanical ventilation, inspiratory pressure of 15 cmH2O, PEEP of 3 cmH2O, inspired oxygen concentration of 100%). To differentiate the effects of ventilator-induced lung injury and LPS treatment, additional groups with animals only exposed to injurious ventilation (VILI, 180 min of pressure-controlled mechanical ventilation, inspiratory pressure of 35 cmH2O, PEEP of 3 cmH2O, inspired oxygen concentration of 100%) and, respectively, LPS i.t. only followed by mechanical ventilation at low pressure (inspiratory pressure of 15 cmH2O) were included. (B) Albumin concentration in the BAL fluid was determined by ELISA. Results are presented as mean and SD (n = 5). *p < 0.05. (C) Representative microscopic images of lungs (H&E; original magnification ×10) and (D) quantification of tissue damage by a histologic injury score. Results are displayed as median and range.
to a sham-treated control group (Fig. 1A). LPS exposure followed by injurious mechanical ventilation was associated with significant increases in pulmonary edema in wild-type mice, as assessed by measurements of the albumin content in the BAL (Fig. 1B), indicating that the two-hit model is efficient and useful for studying ALI. Animals in the one-hit groups (VILI, LPS) showed only small increases in albumin BAL content compared with sham animals. Importantly, and consistent with previous studies in LPS- or ventilation-induced injury alone (16, 17), we found that Adora2b−/− mice were more prone to lung injury. Indeed, they experienced more dramatic increases in albumin leakage (Fig. 1B) and histologic tissue injury (Fig. 1C, 1D) when exposed to LPS injury followed by subsequent mechanical ventilation at elevated pressure levels. The same trend is noticeable in the VILI- and LPS-only animals, although due to the less injurious effect of the one-hit models, the observation is less dramatic. Taken together, these findings indicate that global A2B adenosine receptor deficiency is associated with a more severe degree of ALI in a two-hit model of lung injury.

Deletion of Adora2b in the myeloid compartment does not alter susceptibility to ALI

Based on previous studies implicating Adora2b signaling on myeloid cells in mediating inflammatory outcomes during sepsis induced by cecal ligation and puncture (35, 36), we subsequently performed studies in mice with myeloid Adora2b deletion. This was achieved by crossing transgenic mice with a floxed Adora2b gene (Adora2bloxP/loxP) with mice expressing Cre recombinase in the myeloid compartment (LysM Cre+) (37). Adora2bloxP/loxP LysM Cre+ mice show similar A2B adenosine receptor transcript levels in the whole lung, whereas A2B adenosine receptor transcript levels are almost undetectable in isolated alveolar macrophages and neutrophils (22) (Fig. 2A). Exposure of Adora2bloxP/loxP LysM Cre+ mice to the above-described two-hit model of ALI revealed that there were no differences in albumin leakage (Fig. 2B) or histologic tissue injury (Fig. 2C, 2D) compared with controls (LysM Cre+ mice). Taken together, these findings indicate that Adora2b deletion on myeloid cells fails to alter the disease severity of ALI during LPS exposure and subsequent injurious mechanical ventilation.

Endothelial-specific Adora2b deletion fails to alter the outcome of ALI

Based on previous reports indicating that Adora2b is highly expressed on vascular endothelial cells and functions to dampen vascular injury and inflammation (38, 39), we subsequently generated mice with endothelial Adora2b deletion on the vasculature. We pursued this task by crossing transgenic mice with a floxed Adora2b gene with mice that express Cre recombinase predominantly in vascular endothelial cells (VE-cadherin Cre+) (21). Indeed, Adora2bloxP/loxP VE-cadherin Cre+ mice showed normal A2B adenosine receptor transcript levels in isolated alveolar epithelial cells, whereas expression of the A2B adenosine receptor transcript was essentially undetectable in isolated pulmonary endothelial cells (40) (Fig. 3A). Similar to the above studies in mice with deletion of Adora2b in the myeloid lineage, Adora2bloxP/loxP VE-cadherin Cre+ mice demonstrated no phenotype in our studies of ALI. As such, Adora2bloxP/loxP VE-cadherin Cre+ mice experienced a similar degree of albumin leakage and histologic tissue injury.

**FIGURE 2.** Genetic deletion of the Adora2b in the myeloid lineage during ALI. (A) A2B adenosine receptor transcript levels were determined in whole lungs, alveolar macrophages (AM), and isolated bone marrow neutrophils (PMN) from the lungs of Adora2bloxP/loxP LysM Cre+ mice or LysM Cre− controls; transcript levels were assessed by real-time RT-PCR relative to housekeeping gene β-actin (n = 4). (B–D) Adora2bloxP/loxP LysM Cre+ mice or corresponding Cre− control mice matched in age, weight, and gender were exposed to i.t. LPS treatment, followed after 24 h by injurious mechanical ventilation (180 min of pressure-controlled mechanical ventilation, inspiratory pressure of 35 cmH2O, PEEP of 3 cmH2O, inspired oxygen concentration of 100%). Intratracheal PBS instillation and mechanical ventilation at low pressure was used in sham controls (180 min of pressure-controlled mechanical ventilation, inspiratory pressure of 15 cmH2O, PEEP of 3 cmH2O, inspired oxygen concentration of 100%). Albumin concentration in the BAL fluid was determined by ELISA (mean and SD, n = 5). Representative microscopic images of lungs (H&E; original magnification ×10) and quantification of tissue damage by a histologic injury score are shown. Histology scores are displayed as median and range. *p < 0.05.
injury when exposed to LPS and subsequent injurious mechanical ventilation (Fig. 3B–D). Taken together, these studies highlight that deletion of Adora2b on vascular endothelial cells does not alter the outcome of ALI in this two-hit model.

**Tissue-specific deletion of Adora2b on alveolar epithelia is associated with more severe tissue injury during ALI**

Based on previous reports indicating that the A2B adenosine receptor is expressed on alveolar epithelial cells (41), we next examined mice with alveolar-specific Adora2b deletion during ALI. Alveolar epithelial cell–specific Adora2b deletion was achieved by crossing transgenic mice with a floxAdora2b gene with mice that constitutively express Cre recombinase in alveolar epithelial cells (Adora2bloxP/loxP SPC Cre+). Indeed, Adora2bloxP/loxP SPC Cre+ mice had normal expression levels of the Adora2b transcript in isolated pulmonary endothelial cells and alveolar macrophages, whereas A2B adenosine receptor transcript levels in isolated murine alveolar epithelial cells were essentially undetectable (42) (Fig. 4A). Consistent with a functional role for alveolar epithelial A2B adenosine receptor signaling in lung protection during ALI, we found that Adora2bloxP/loxP SPC Cre+ mice experienced more severe albumin leakage (Fig. 4B) and a more severe degree of tissue injury during ALI (Fig. 4C, 4D). Based on these findings, we undertook a more detailed analysis of additional disease parameters. Consistent with our findings on albumin leakage and histologic tissue injury, we observed that Adora2bloxP/loxP SPC Cre+ mice showed more severe pulmonary inflammation as compared with SPC Cre+ controls. As such, Adora2bloxP/loxP SPC Cre+ mice had more dramatic elevations of neutrophil influx, as assessed by measuring BAL MPO levels (Fig. 5A), as well as more profound elevations in pulmonary IL-1 (Fig. 5B), IL-6 (Fig. 5C), and TNF-α (Fig. 5D) transcript levels. Additionally, BAL protein concentrations of CXCL1 and IL-6 were significantly higher in Adora2bloxP/loxP SPC Cre+ mice compared with SPC Cre+ mice in the LPS + VILI groups. Finally, measurement of alveolar fluid clearance revealed that ALI-associated elevations of alveolar fluid clearance were abolished in Adora2bloxP/loxP SPC Cre+ mice (Fig. 5E). Taken together, these findings indicate that the detrimental effects of global Adora2b deletion during ALI are predominantly mediated through alveolar epithelial A2B adenosine receptor deficiency.

**Inhaled Adora2b agonist treatment provides lung protection during ALI**

Based on the above findings that indirectly implicate alveolar epithelial A2B adenosine receptor signaling in lung protection during ALI, we wondered whether this information could be useful in a therapeutic setting. Alveolar epithelial signaling events for the treatment of lung disease are particularly accessible to inhaled therapeutic effects, where high concentrations of a compound can be achieved on alveolar epithelial cells with significantly smaller systemic effects of a drug. Therefore, we set up a pharmacologic treatment approach utilizing an inhaled A2B adenosine receptor agonist (BAY 60-6583). Similar to the clinical situation where a septic patient will typically be started on treatments once the disease is established (e.g., when the patient requires mechanical ventilation), we initiated A2B adenosine receptor agonist treatment after 24 h of LPS injury concomitant with the onset of injurious

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**FIGURE 3.** Genetic deletion of Adora2b in endothelial cells during ALI. (A) A2B adenosine receptor transcript levels in isolated pulmonary endothelial cells (EC) or alveolar epithelial cells (AEC) from the lungs of Adora2bloxP/loxP VE-cadherin Cre+ mice or VE-cadherin Cre+ controls; transcript levels were assessed by real-time RT-PCR relative to housekeeping gene β-actin (n = 3–4). (B–D) Adora2bloxP/loxP VE-cadherin Cre+ or corresponding Cre+ control mice matched in age, weight, and gender were exposed to i.t. LPS treatment, followed after 24 h by injurious mechanical ventilation (180 min of pressure-controlled mechanical ventilation, inspiratory pressure of 35 cmH2O, PEEP of 3 cmH2O, inspired oxygen concentration of 100%). Intratracheal PBS instillation and mechanical ventilation at low pressure was used in sham controls (180 min of pressure-controlled mechanical ventilation, inspiratory pressure of 15 cmH2O, PEEP of 3 cmH2O, inspired oxygen concentration of 100%). Albumin concentration in the BAL fluid was determined by ELISA (mean and SD, n = 5). Representative microscopic images of lungs (H&E; original magnification ×10) and quantification of tissue damage by a histologic injury score are shown. Histology scores are displayed as median and range. *p < 0.05.
mechanical ventilation in the above two-hit model (Fig. 6A).
Consistent with the genetic findings in mice with alveolar epithelial Adora2b deletion, we observed that inhaled treatment with BAY 60-6583 was associated with attenuated pulmonary edema (Fig. 6B) and improved histologic tissue injury (Fig. 6C, 6D) during ALI. Similarly, inhaled BAY 60-6583 treatment was able to reduce lung inflammation (Fig. 6E–J) and to improve alveolar fluid clearance (Fig. 6K). Taken together, these experimental studies in mice suggest that inhaled A2B adenosine receptor agonists can be used for the treatment of ALI.

To complete the clinical picture and to get additional insight regarding the point of action of the A2B adenosine receptor agonist BAY 60-6583, we also treated animals after VILI or LPS only with BAY 60-6583 (Supplemental Fig. 1A). Not surprisingly, ventilation for 3 h with an inspiratory pressure of 35 cmH2O had only marginal influence on the measured inflammatory parameters, so we were not able to observe a protective effect of the given A2B adenosine receptor agonist (Supplemental Fig. 1B). Alternatively, consistent with previous studies (16), treatment with BAY 60-6583 was effective in animals given i.t. LPS only (Supplemental Fig. 1B). In contrast to Schingnitz et al. (16), we gave the A2B adenosine receptor agonist after LPS injury (not 30 min prior) by inhalation (not i.p.).

Discussion

ALI is one of the leading causes of morbidity and mortality of critical illness with extremely limited therapeutic options. In the present study, we pursued the hypothesis that tissue-specific adenosine signaling events through the A2B adenosine receptor contribute to lung protection and can thus be targeted for ALI treatment. To make progress on this front, we performed a head-to-head comparison of mice with genetic deletion of Adora2b in the myeloid lineage, vascular endothelial cells, or alveolar epithelial cells. Interestingly, we only observed a phenotype in mice with tissue-specific Adora2b deletion in alveolar epithelial cells, closely resembling the observed detrimental effects of global Adora2b deletion during ALI. Interestingly, the injurious effects of our two-hit model where an inflammatory event (i.t. LPS treatment) is followed by injurious mechanical ventilation seem to be supra-additive compared with the effects of injurious ventilation or LPS i.t. alone. Based on these findings indirectly implicating alveolar epithelial Adora2b signaling in lung protection, we subsequently performed studies to deliver an A2B adenosine receptor agonist (BAY 60-6583) via inhalation to alveolar A2B adenosine receptors. Indeed, these studies showed improvement of lung injury as well as alveolar fluid clearance during ALI. Taken together, these findings suggest that alveolar epithelial A2B adenosine receptor participates in an endogenous pathway for lung protection during ALI and implicate inhaled A2B adenosine receptor agonists for ALI treatment.

Our findings are consistent with previous studies indicating that A2B adenosine receptors are expressed in alveolar epithelial cells. For example, previous studies used a mouse model to examine pulmonary A2B adenosine receptor expression, where knockout of
the Adora2b receptors was combined with a β-galactosidase reporter gene under the control of the endogenous promoter (39, 41). In that study, the authors used this mouse model to determine site-specific expression of the A2B adenosine receptor in different tissue compartments of the murine lung. Consistent with our present studies, they described that the major site of Adora2b promoter activity was found to be in alveolar epithelial cells, which was identified by coexpression of prosurfactant protein C. In contrast, Adora2b promoter activity was relatively less in alveolar macrophages, bronchial epithelial cells, or in the vasculature (41). Together with the present studies, these findings highlight expression and function of alveolar epithelial A2B adenosine receptor in lung protection during ALI.

Other studies have suggested that A2B adenosine receptor expression is selectively induced in the lungs of mice exposed to injurious mechanical ventilation (17, 24). Interestingly, additional studies that were designed to examine the transcriptional pathway responsible for A2B adenosine receptor expression during injurious mechanical ventilation suggested that this pathway involves the transcription factor hypoxia-inducible factor (HIF)1A (43). HIF1A is a transcription factor that is typically stabilized during conditions of limited oxygen availability and induces gene products that are critical for hypoxia adaptation, such as erythropoietin (44–46). However, other stimuli than hypoxia have also been shown to stabilize HIF, including stress conditions during ALI (47). As such, a recent study performed a microarray analysis of pulmonary epithelia exposed to conditions of cyclic mechanical stretch, closely resembling injurious mechanical ventilation. That study found that stretch conditions are associated with the induction of known HIF target genes, subsequently indicating “normoxic” stabilization of HIF1A during mechanical stretch of pulmonary epithelial cells. Similarly, HIF1A was stabilized in mice exposed to injurious mechanical ventilation. Finally, tissue-specific deletion of HIF1A in alveolar epithelial cells of the lungs was associated with profound increases of lung injury during injurious mechanical ventilation (47). Taken together, these studies suggest the possibility that HIF1A-driven A2B adenosine receptor expression in alveolar epithelial cells could function in endogenous lung protection.

Although several studies have implicated A2B adenosine receptor signaling in lung protection during acute forms of lung diseases, A2B adenosine receptor signaling may become detrimental in chronic forms of lung diseases, such as pulmonary fibrosis (48). For example, mice with genetic deletion of the enzyme adenosine deaminase experience chronically elevated adenosine levels and concomitant lung inflammation (49). Pharmacologic studies with adenosine receptor antagonist treatment show that chronic lung disease in adenosine deaminase–deficient mice is at least in part mediated through A2B adenosine receptor signaling (50). A subsequent study describes highly distinct roles for the A2B adenosine receptor in acute versus chronic stages of bleomycin-induced lung injury (51). The goal of this study was to examine the contribution of A2B adenosine receptor signaling in models of bleomycin-induced lung injury that exhibit varying degrees of acute versus chronic injury phases. It is well known that i.t. bleomycin exposure results in substantial acute lung injury followed by progressive fibrosis. In this acute model of lung injury, genetic removal of the A2B adenosine receptor in mice resulted in enhanced loss of barrier function and increased pulmonary inflammation. As such, these results support an anti-inflammatory role for this receptor in acute lung injury. When the authors examined A2B adenosine receptor signaling in a chronic model by systemic exposure of mice to bleomycin, the effects of genetic Adora2b removal were associated with a substantial reduction in pulmonary fibrosis, supporting a profibrotic role for this receptor during chronic lung injury (51).

In addition to A2B adenosine receptor signaling, many studies have implicated the A2A adenosine receptor in attenuating lung inflammation. For example, an important study demonstrated that A2A adenosine receptors have a nonredundant role in pulmonary inflammation. As such, these results support an anti-inflammatory role for this receptor in acute lung injury. When the authors examined A2A adenosine receptor signaling in a chronic model by systemic exposure of mice to bleomycin, the effects of genetic Adora2b removal were associated with a substantial reduction in pulmonary fibrosis, supporting a profibrotic role for this receptor during chronic lung injury (51).
FIGURE 6. Inhalational treatment of ALI with the A2B adenosine receptor agonist BAY 60-6583. (A) Experimental set-up: C57/B6 mice matched in age, gender, and weight were exposed to ALI via i.t. LPS treatment followed after 24 h by injurious mechanical ventilation (180 min of pressure-controlled mechanical ventilation, inspiratory pressure of 35 cmH2O, PEEP of 3 cmH2O, inspired oxygen concentration of 100%). Mice in the LPS + VILI group received aerosolized BAY 60-6583 (1 mg/kg body weight) or vehicle during the mechanical ventilation period. After i.t. PBS instillation, the sham group was ventilated with a lower inspiratory pressure (15 cmH2O) and received aerosolized BAY 60-6583 or vehicle during mechanical ventilation. (B) At the end of the experiment BAL fluid was collected and albumin concentration was determined by ELISA. (C) Representative microscopic images of lungs and (D) quantification of tissue damage by a histologic injury score. (E) Neutrophil accumulation in the lung was quantified using MPO ELISA. Transcript expression levels in whole lung tissue for (F) IL-1, (G) IL-6, and (H) TNF-α were assessed by real-time RT-PCR relative to housekeeping gene β-actin. Pulmonary cytokine protein concentrations of CXCL1 (I) and IL-6 (J) were determined using ELISA. (K) Alveolar fluid clearance (expressed as percentage of instilled volume) was measured by instilling 300 µl iso-osmolar 0.9% NaCl solution with 5% BSA and 0.1% FITC-labeled albumin at the end of the experiment. Results are presented as mean and SD, except for histology scores, which are displayed as median and range (n = 5). *p < 0.05.
of proinflammatory cytokines, and death of mice deficient in the A2A adenosine receptor (19). Based on several reports indicating that the A2A adenosine receptor is highly expressed on inflammatory cells, it is conceivable that a combination of inflammatory cell–dependent A2A adenosine receptor signaling and alveolar epithelial A2B adenosine receptor signaling functions together to mediate the protective effects of endogenous adenosine signaling during ALI.

Taken together, the present studies reveal that alveolar epithelial–expressed A2B adenosine receptors contribute to lung protection during ALI. Experimental studies suggest that utilizing inhaled A2B adenosine receptor agonists can be used for ALI treatment, thus providing a high concentration of the compound on alveolar epithelial cells, with reduced systemic absorption and potential unwanted side effects.

Disclosures

The authors have no financial conflicts of interest.

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