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Identification of Hypoxia-Inducible Factor HIF-1A as Transcriptional Regulator of the A2B Adenosine Receptor during Acute Lung Injury

Tobias Eckle,* Emily M. Kewley,* Kelley S. Brodsky,* Eunyoung Tak,* Stephanie Bonney,* Merit Gobel,* Devon Anderson,* Louise E. Glover,† Ann K. Riegel,* Sean P. Colgan,† and Holger K. Eltzschig*

Although acute lung injury (ALI) contributes significantly to critical illness, resolution often occurs spontaneously through endogenous pathways. We recently found that mechanical ventilation increases levels of pulmonary adenosine, a signaling molecule known to attenuate lung inflammation. In this study, we hypothesized a contribution of transcriptionally controlled pathways to pulmonary adenosine receptor (ADOR) signaling during ALI. We gained initial insight from microarray analysis of pulmonary epithelia exposed to conditions of cyclic mechanical stretch, a mimic for ventilation-induced lung disease. Surprisingly, these studies revealed a selective induction of the ADORA2B. Using real-time RT-PCR and Western blotting, we confirmed an up to 9-fold induction of the ADORA2B following cyclic mechanical stretch (A549, Calu-3, or human primary alveolar epithelial cells). Studies using ADORA2B promoter constructs identified a prominent region within the ADORA2B promoter conveying stretch responsiveness. This region of the promoter contained a binding site for the transcription factor hypoxia-inducible factor (HIF)-1. Additional studies using site-directed mutagenesis or transcription factor binding assays demonstrated a functional role for HIF-1 in stretch-induced increases of ADORA2B expression. Moreover, studies of ventilator-induced lung injury revealed induction of the ADORA2B during ALI in vivo that was abolished following HIF inhibition or genetic deletion of Hif1a. Together, these studies implicate HIF in the transcriptional control of pulmonary adenosine signaling during ALI.

Acute lung injury (ALI) is a syndrome consisting of acute hypoxic respiratory failure with bilateral pulmonary infiltrates, not attributable to left heart failure (1, 2). Although there is currently no specific therapy available, management consists of aggressive treatment of the initiating cause, vigilant supportive care, and the prevention of nosocomial infections. Despite optimal management, mortality ranges between 35 and 60% (2). In fact, ~200,000 patients develop ALI annually in the U.S., leading to 75,000 deaths and accounting for up to 3.6 million hospital days (3). Among the hallmarks of ALI is a massive accumulation of inflammatory cells into different compartments of the lungs, in conjunction with cytokine release and inflammatory activation of recruited or resident cells. Other characteristics include a disruption of the alveolar–capillary barrier function, resulting in extensive pulmonary edema and attenuated gas exchange. In such settings, several factors contribute to pulmonary tissue hypoxia. First, ALI is frequently associated with obstruction of airflow into the distal airways, resulting in attenuated oxygen supply into atelectatic areas of the lungs. Secondly, pulmonary edema causing interstitial and intra-alveolar fluid accumulation results in attenuated gas exchange and increased alveolar–arterial oxygen gradient. Finally, mechanical ventilation and inflammatory cell accumulation and activation are associated with dramatic increases of metabolic requirements, including oxygen, thereby causing additional shifts in pulmonary oxygen supply and demand ratio and resulting in pulmonary hypoxia (4–7).

Despite the large impact of ALI on morbidity and mortality in critically ill patients (2), many episodes of ALI are self-limiting and resolve spontaneously through unknown mechanisms. For example, patients undergoing major thoracic surgery for lung cancer have an overall ALI incidence of <5%, open heart surgery with cardiopulmonary bypass <0.5%, or kidney transplantation of <0.2% (8). These clinical observations have inspired many studies of endogenous pathways that dampen acute increases in the capillary–alveolar barrier and lung inflammation in different models of ALI, including hypoxia- (9–14), chemical- (15, 16), or endotoxin-induced (5, 17, 18) forms of lung injury. Previous studies have identified the extracellular signaling molecule adenosine in endogenous lung protection during ALI (8, 19). Mice deficient in the production of extracellular adenosine experience enhanced lung inflammation or pulmonary edema during ALI induced by mechanical ventilation or endotoxin (8, 20). Extracellular adenosine can signal through four distinct
adenosine receptors (ADORs); ADORA1, ADORA2A, ADORA2B, and ADORA3 (21–23). ADORA2A and ADORA2B have specifically been implicated in dampening acute inflammation, including lung protection during ALI (5, 17, 24–28). Although previous studies have shown increased pulmonary adenosine levels and signaling during ALI (8, 17, 20, 27), regulatory mechanisms controlling pulmonary adenosine signaling are only poorly understood. In the current study, we hypothesized a contribution of transcriptionally regulated pathways in the endogenous control of pulmonary adenosine signaling events during ALI. To address this hypothesis, we combined in vitro studies of cyclic mechanical stretch of pulmonary epithelia with in vivo studies of ALI induced by mechanical ventilation. Serendipitously, these studies identified a role for hypoxia-inducible factor-1 (HIF-1) signaling pathways (6, 29) in the transcriptional control of pulmonary ADORA2B signaling during ALI.

Materials and Methods

Cell culture

Calu-3 human airway epithelial cells, human microvascular endothelial cell-1 (HMEC-1; used for promoter studies), or cultured pulmonary epithelial A549 cells were cultured as described previously (10, 30–32). Human primary alveolar epithelial cells (HPAEpiC) (ScienCell Research Laboratories) were cultured according to the supplier’s instructions.

In vitro stretch model

To study the consequences of cyclic mechanical stretch on ADOR transcription, we adopted a previously described in vitro model resembling mechanical ventilation by applying cyclic mechanical stretch (3). In short, Calu-3, A549, HMEC-1, or HPAEpiC was plated on BioFlex culture plates-collagen type I (BF-3001C; FlexCell International) and allowed to attach and grow to ∼80% confluence. All cells were cultured in 4 ml media: Calu-3 cells were grown in Advanced MEM (Life Technologies), A549 cells were grown in DME-12 (Life Technologies), both cell lines with 10% FBS and 0.02% L-glutamine, HMEC-1 were grown in MCD131 (Life Technologies) 131, 0.2% hydrocortisone, 0.02% epidermal growth factor, and 10% FBS, and HPAEpiC were grown in AEpiCM (ScienCell Research Laboratories). For promoter analyses, HMEC were transfected in Opti-Mem plus GlutaMAX (Life Technologies) media. Plates were then placed on a FlexCell FX-4000T Tension Plus System (FlexCell International) and stretched at percent stretch indicated, 30% maximum, 0.7% passive stretch for 24 h. RNA was extracted using TRIzol, and microarray technology was used to assess relative fluorescence in A1, A2A, A2B, and A3 adenosine receptors in stretch versus control conditions. Results are presented as mean ± SD (n = 3).

Renilla reporter vector for 24 h using Firegene 6 transfection reagent (Roche; in accordance with the manufacturer’s instructions). Cells were exposed to cyclic mechanical stretch at 30% for specified time points up to 24 h before being washed in ice-cold PBS and collected in 200 μl 1× passive lysis buffer (Promega). The Dual Luciferase Reporter Assay (Promega) was carried out according to the manufacturer’s instructions. All activity was normalized to constitutively expressed Renilla luciferase.

Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) was performed as described previously using an Ab against HIF-1α (9, 34). HIF-1 binding to ADORA promoter DNA in stretch was quantified by standard PCR using primers (forward, 5′-CAGGGT GTC GGC AAA CTT CC-3′; reverse, 5′-CTT GTT GGA TTT GG GGC A-3′) designed to amplify a 374-bp region of the A2B promoter. Chromatin incubated with IgG Ab was used to control for nonspecific binding of DNA.

Inhibition of HIF-1α in vitro and in vivo

Echinomycin (Sigma-Aldrich) was administered to Calu-3 cells at concentrations of 3, 30, and 300 nM, and cells were exposed to cyclic mechanical stretch at 30%. In vivo, 30 μg echinomycin was administered to experimental mice i.p. 1 h before induction of anesthesia and subsequent ventilation (35).

Marine mechanical ventilation

All animal protocols were in accordance with the guidelines of the National Institutes of Health for the use of laboratory animals and approved by the Institutional Animal Care and Use Committee of the University of Colorado. C57BL6/J mice (Charles River Laboratories) were matched according to sex, age, and weight. For Hif1α tissue-specific knockout (KO) in the alveolar epithelium, triple-transgenic mice (Hif1flox SP-Crtα Tet-O-Cre Hif1af/f SurfactantCre+) were induced by doxycyclin therapy over 5 d i.p. and orally as described (35). Ventilator-induced lung injury (VILI) was induced as described previously (36).

Mouse and human cell transcriptional analysis

Transcriptional studies of ADORs during murine ALI were carried out as described previously (27).

Human and mouse protein analysis

Western blotting studies for murine or human ADORA2B were carried out as described previously (11, 27). In studies for HIF-1A Western blotting, we used anti–HIF-1α [H1alpha67] Ab (ab1) (35).

FIGURE 1. Transcriptional responses of ADORs during cyclic mechanical stretch of pulmonary epithelial cells. Confluent Calu-3 cells were plated on collagen-coated BioFlex plates and underwent cyclic mechanical stretch for 24 h. RNA was extracted using TRIzol, and microarray technology was used to assess relative fluorescence in A1, A2A, A2B, and A3 adenosine receptors in stretch versus control conditions. Results are presented as mean ± SD (n = 3).
Statistical analysis
Data are presented as mean ± SD. Statistical analysis was performed using ANOVA and the Student t test (two-sided, \( p < 0.05 \)). A \( p \) value < 0.05 was considered statistically significant.

Results
Selective induction of the ADORA2B during cyclic mechanical stretch of pulmonary epithelia
Previous studies have identified extracellular adenosine signaling events in endogenous protection from pathologic lung inflammation during ALI (14, 17, 20, 27, 37). However, transcriptional mechanisms that control ADOR expression during ALI are only poorly understood. To gain insight into the transcriptional control of pulmonary adenosine signaling events during ALI, we used cyclic mechanical stretch exposure of pulmonary epithelia as a well-defined in vitro model (8). As such, we performed a screening experiment in which Calu-3 cells were exposed to cyclic mechanical stretch at 30% over 24 h and microarray analysis used to study the transcriptional response of ADORs (microarray data were accepted and published on Gene Expression Omnibus, National Center for Biotechnology information, accession number GSE27128: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE27128). Consistent with previous studies of pulmonary AR expression (38), we observed highest basal expression levels of the ADORA2B subtype in untreated Calu-3 cells. Moreover, ADORA2B transcript levels were selectively induced following stretch exposure (Fig. 1). Taken together, these studies reveal for the first time, to our knowledge, a selective induction of the ADORA2B during cyclic mechanical stretch. Furthermore, these findings closely correlate with other studies that have shown a selective induction of the ADORA2B during in vivo exposure of mice to ALI (17, 27).

ADORA2B induction is time and stretch dependent
Based on the above microarray studies showing selective induction of ADORA2B transcript, we next used different stretch conditions and examined ADORA2B transcript and protein levels. For this purpose, Calu-3 cells were exposed to 0, 10, 20, or 30% cyclical stretch and protein and RNA analyses used to observe changes in expression (Fig. 2A, 2B). We observed that 30% stretch exposure was associated with the most profound induction of ADORA2B transcript and protein levels. Therefore, we performed cyclic mechanical stretch of Calu-3 epithelia at 30% over different time periods and stretch intensity. Transcript levels were determined by real-time quantitative PCR. Data were calculated relative to β-actin and expressed as fold change relative to control. β-actin was also used to control for protein loading. (A) Calu-3 were exposed to 0, 10, 20, and 30% stretch. (B) Protein change was determined following 0, 10, 20, and 30% stretch. Induction of ADORA2B mRNA (C) and ADORA2B protein (D) in Calu-3 cells following cyclic mechanical stretch at 30% for 2, 4, 8, and 24 h versus control at 0 h. ADORA2B transcript (E) and representative Western blots (F) are shown following 30% stretch in HPAEpiC. Data are mean ± SD (\( n = 3 \)).
Identification of a functional HIF-1 binding site that regulates ADORA2B promoter activity during cyclic mechanical stretch

Having shown that ADORA2B transcript and protein levels are induced during cyclic mechanical stretch of pulmonary epithelia, we next went on to study transcriptional pathways that could represent a regulatory component for the ADORA2B during stretch conditions. Analysis of the human ADORA2B promoter region (33) revealed several transcription factor binding sites, including a hypoxia-responsive element (HRE; HIF-binding site; 5'–CGTTGG–3') at position –642 to –647 bp relative to the transcription start site in combination with an HIF ancillary site (HAS; 5'–CGGGAG–3' at –546 to –541) and binding sites for SP1 (–528 to –534 bp), E2F (–402 to –406 bp), and AP2 (–223 to –225 bp) (Fig. 3A). To address the functional relevance of these transcription factor binding sites, we transfected a full-length (1095 bp) and truncated promoter-reporter construct (477 bp) into HMEC-1 (Fig. 3B), exposed the cells to 30% cyclic mechanical stretch over 24 h, and analyzed promoter activity. These studies revealed robust increases of luciferase activity with stretch-inducibility located between 477 and 1095 bp of the ADORA2B promoter. In conjunction with previous studies showing that the ADORA2B is induced during ambient hypoxia (10, 11, 33), along with the fact that this defined regulatory region within the ADORA2B promoter contains a prominent HRE/HAS site, we next performed site-directed mutagenesis of the HRE. Mutation of the HRE core sequence from ACGTGG to AATCG (Fig. 3D) was associated with a significant attenuation of the stretch-inducibility of the ADORA2B promoter (Fig 3E). Taken together, these studies implicate a functional HRE within the ADORA2B that regulates promoter activity during cyclic mechanical stretch.

HIF-1α is responsible for the transcriptional induction of the ADORA2B during conditions of cyclic mechanical stretch

Having identified a functional HRE within the ADORA2B promoter, we next went on to examine the role of HIF transcription factors in this response. Based on previous studies showing expression and functional regulation of HIF-1α in the lung (35, 39), we next examined previously characterized pulmonary epithelial cell lines (A549/Calu-3) with stable repression of HIF-1α using a short hairpin RNA interference approach (30, 35). Stretch-inducibility of ADORA2B transcript (Fig. 4A) or protein level (Fig. 4B) remain robust in control-transfected cells (HIF-1α scrambled) exposed to different time periods of cyclic mechanical stretch. Strikingly, stretch-inducibility of the ADORA2B is almost completely abolished in Calu-3 cells with small interfering RNA (siRNA)-mediated HIF-1α repression (HIF-1α KO). Taken together, these studies provide the first evidence, to our knowledge, of a selective role of HIF-1 in the stretch-inducibility of the ADORA2B.

As a next step, we performed a transcription factor binding assay of the ADORA2B promoter during cyclic mechanical stretch conditions to test for direct HIF-1α binding to the ADORA2B promoter. For this purpose, we performed ChIP using an HIF-1α Ab for immunoprecipitation and a primer set amplifying the area within the ADORA2B promoter containing the HRE. These studies demonstrated the presence of a band at 374 bp only in cells that had been exposed to cyclic mechanical stretch and not in control conditions, indicating that HIF-1α is binding directly to the HRE within the ADORA2B promoter.

As a next step, we used a pharmacological inhibitor for HIF-1 activity during cyclic mechanical stretch in vitro. In these studies, we used echinomycin (35, 40) to study HIF-1 and ADORA2B responses. In fact, we observed dose-dependent inhibition of HIF-1 activity with increasing echinomycin concentrations (Fig. 5A).
Consistent with our previous studies indicating HIF-1α in the regulation of the ADORA2B during cyclic mechanical stretch, these findings were paralleled by abolished induction of the ADORA2B transcript (Fig. 5A) and protein levels (Fig. 5B) with pharmacological HIF inhibition. Taken together, these studies indicate that HIF-1 regulates ADORA2B induction during conditions of cyclic mechanical stretch in vitro.

HIF-1 is responsible for transcriptional induction of the ADORA2B during VILI in vivo

Having demonstrated a functional role of HIF-1α in the stretch-inducibility of the ADORA2B, we next examined the functional role of HIF-1 during transcriptional regulation of the ADORA2B during ALI in vivo. For this purpose, we used a previously described model for inducing ALI in mice by means of high-pressure mechanical ventilation (36). Previous studies in this model had shown that gene-targeted mice for the ADORA2B suffer from more severe pulmonary edema and lung inflammation (27). As a first step, we used echinomycin as a pharmacologic inhibitor of HIF activity in vivo. As shown in Fig. 6A, echinomycin treatment was associated with abolished HIF-1α activity in mice exposed to 3 h of high-pressure mechanical ventilation. As such, induction of the ADORA2B transcript (Fig. 6A) and ADORA2B protein (Fig. 6B) was abolished following pharmacologic inhibition of HIF-1.

To recapitulate these findings in a genetic model, we examined pulmonary expression of the ADORA2B in mice with conditional deletion of Hif-1α in lung epithelium during ALI. In fact, Hif-1α<sup>−/−</sup> SurfactantCre<sup>+</sup> mice showed attenuated HIF-1α stabilization during ALI in vivo (Fig. 7B), in conjunction with abolished induction of ADORA2B transcript (Fig. 7A) and protein (Fig. 7B) upon exposure to ALI. Together, these findings implicate HIF-1 in the transcriptional induction of the ADORA2B during ALI induced by mechanical ventilation in vivo.

Discussion

Previous studies have implicated adenosine signaling events in endogenous lung protection during ALI (5, 17, 20, 26, 27, 41). These findings are supported by increased extracellular adenosine production and signaling events during VILI (8, 27), endotoxin-induced lung injury (17, 20), or lung injury induced by chemical irritants such as bleomycin (15). At present, there is only very little known about transcriptional mechanisms that govern pul-

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**FIGURE 4.** Role of HIF-1α in stretch-induced ADORA2B expression in vitro. Use of epithelial cell lines (A549/Calu-3), one stably expressing HIF-1α siRNA (HIF-1 KO) and one expressing scrambled (Scr) siRNA. Cells were exposed to stretch (30%) for the indicated time course. Shown are transcript levels of ADORA2B (A) and ADORA2B protein expression (Western blot) (B), both normalized to β-actin. (C) ChIP assay analysis was performed with an Ab against HIF-1α to define and confirm the consensus binding sequence within the ADORA2B promoter, in control and stretched HMEC-1. Shown are the PCR amplification product at 374 bp and the fold change in PCR expression (D). Data are mean ± SD (n = 3). Ctrl, Control.
A2B ADENOSINE RECEPTOR AND HIF DURING ALI

Due to their large surface areas, mucosal organs such as the intestine or the lungs are particularly prone to hypoxia-elicited inflammation (13). Consistent with the present article, several studies have implicated HIF-1 in the transcriptional induction of molecular pathways that dampen hypoxia-elicited inflammation. For example, a recent study found that the neuronal guidance molecule netrin-1 is expressed by mucosal epithelia, and HIF-1A functions to dampen ALI is supported by pharmacological HIF inhibition with echinomycin (13).

In the present studies, we pursued the hypothesis that transcriptional mechanisms may regulate AR expression during cyclic mechanical stretch in vitro or during ALI in vivo. Initial screening studies took advantage of a microarray analysis of pulmonary epithelia exposed to cyclic mechanical stretch. These studies revealed a selective and robust induction of the ADORA2B. In fact, studies in different pulmonary epithelial cell lines confirmed time- and stretch-dose-dependent ADORA2B induction. Studies using promoter constructs, site-directed mutagenesis, loss- and gain-of-function, or transcription factor binding assays identified HIF-1 as the key regulator of ADORA2B induction during stretch conditions. Moreover, in vivo studies of ALI provided multiple levels of evidence for HIF-1 in the transcriptional induction of the ADORA2B during ventilator-induced ALI in vivo. Taken together, these findings implicate previously unknown signaling pathways in the regulation of pulmonary AR signaling during ALI.

The present studies implicate a functional role for HIF-1A in the regulation of adenosine-elicited lung protection during ALI, thereby indirectly implicating HIF1A in lung protection. The hypothesis that HIF-1A functions to dampen ALI is supported by a recent study in which the authors hypothesized that stretch conditions, such as those that occur during mechanical ventilation, result in transcriptional adaptation of alveolar epithelial cells, the innermost lining of the lungs. Indeed, they identified an unexpected involvement of the transcription factor hypoxia-inducible factor HIF-1A in lung protection. A genome-wide screen revealed a transcriptional response similar to hypoxia signaling. Surprisingly, they found that stabilization of HIF-1A during stretch conditions in vitro or during ventilator-induced ALI in vivo occurs under normoxic conditions. Extension of these findings identified a functional role for stretch-induced inhibition of succinate dehydrogenase in mediating normoxic HIF-1A stabilization, concomitant increases in glycolytic capacity, and improved tricarboxylic acid cycle function. Pharmacologic studies with HIF activator or inhibitor treatment implicated HIF-1A stabilization in attenuating pulmonary edema and lung inflammation during ALI in vivo. Systematic deletion of HIF-1A in the lungs, endothelia, myeloid cells, or pulmonary epithelia linked these findings to alveolar–epithelial HIF-1A. In vivo analysis of $[^{13}C]$glucose metabolites using liquid-chromatography–tandem mass spectrometry demonstrated that increases in glycolytic capacity, improvement of mitochondrial respiration, and concomitant attenuation of lung inflammation during ALI were specific for alveolar–epithelial expressed HIF-1A. These preclinical findings highlight the potential for pharmacological HIF-1A stabilization in the treatment of ALI (35). These findings are also consistent with other studies indicating that elevations of succinate levels can function as HIF activators via normoxic inhibition of prolyl hydroxylases (42–44).

Due to their large surface areas, mucosal organs such as the intestine or the lungs are particularly prone to hypoxia-elicited inflammation (13). Consistent with the present article, several studies have implicated HIF-1 in the transcriptional induction of molecular pathways that dampen hypoxia-elicited inflammation. For example, a recent study found that the neuronal guidance molecule netrin-1 is expressed by mucosal epithelia, and HIF-
CreER+ mice during ALI. Frozen, and lysed. ADORA2B transcript at 0 and 3 h ventilator (pressure-controlled ventilation) for 0 or 3 h at an inspiratory pressure of 45 mbar, control of a doxycyclin-inducible cre-mediated recombination system. One weight (as control). In these transgenic mice, Cre recombinase is under the control of a doxycyclin-inducible cre-mediated recombination system. One week following cre induction by doxycycline treatment, mice were ventilated (pressure-controlled ventilation) for 0 or 3 h at an inspiratory pressure of 45 mbar (control mice were ventilated at 15 mbar). Lungs were excised, flash frozen, and lysed. ADORA2B transcript at 0 and 3 h ventilation (A) and representative Western blot of HIF-1α and ADORA2B protein expression (B), membranes being reprobed for β-actin to control for protein loading, and ADORA2B transcript calculated relative to β-actin. Results are presented as mean ± SD, derived from n = 3 in each condition.

FIGURE 7. ADORA2B expression in whole-body-induced Hif-1α: CreER+ mice during ALI. Hif-1αf/f SPC-tTA Tet-O-Cre+ mice and littermate controls without the floxed HIF allele were matched in age, sex, and weight (as control). In these transgenic mice, Cre recombinase is under the control of a doxycyclin-inducible cre-mediated recombination system. One week following cre induction by doxycycline treatment, mice were ventilated (pressure-controlled ventilation) for 0 or 3 h at an inspiratory pressure of 45 mbar (control mice were ventilated at 15 mbar). Lungs were excised, flash frozen, and lysed. ADORA2B transcript at 0 and 3 h ventilation (A) and representative Western blot of HIF-1α and ADORA2B protein expression (B), membranes being reprobed for β-actin to control for protein loading, and ADORA2B transcript calculated relative to β-actin. Results are presented as mean ± SD, derived from n = 3 in each condition.

dependent induction of netrin-1 dampens hypoxia-induced mucosal inflammation in the intestine and the lungs (13). Such findings are consistent with previous studies that have demonstrated a transcriptional role for hypoxia in directly regulating the adenosine pathway. During mucosal hypoxia, HIF-1α-mediated changes in gene expression result in increased production of adenosine by the 5′-ecto-nucleotidase (CD73) and enhanced adenosine signaling events due to hypoxia induction of the ADORA2B, in conjunction with attenuated adenosine uptake (34) and metabolism (12). This pathway has been implicated in experimental studies of ALI induced by mechanical ventilation (8, 27) or polymicrobial lung infection (4, 5, 45). In fact, the latter study demonstrated that inhibition of hypoxia-induced enhancement of adenosine production and signaling by using high inspired oxygen concentrations is associated with higher mortality in the model used (5). The authors concluded that oxygenation with high inspired oxygen concentrations may inhibit the physiological tissue-protective mechanisms elicited by hypoxia signaling and may thereby exacerbate lung injury (5). Consistent with the role of HIF-1α in attenuating acute mucosal inflammation, several studies have implicated the ADORA2B in dampening tissue inflammation in acute models of injury, including VILI, polymicrobial sepsis, myocardial ischemia, or acute kidney injury (27, 41, 46, 47).

Presently, there are only a few studies that show mechanisms of how ADORA2B protein expression is regulated in response to environmental stimuli. As such, previous studies had identified transcriptionally regulated alterations of ADORA2B expression during hypoxia-elicited inflammation. These studies demonstrated selective induction of the ADORA2B following exposure of human vascular endothelia to ambient hypoxia. In contrast, transcript levels of other ARs were either repressed or unaltered (10). Subsequent studies identified a previously unrecognized binding site for HIF-1 within the promoter region of the ADORA2B (33). Additional studies investigating the promoter activity, functional chromatin binding, and HIF-1 loss-of-function studies demonstrated a critical role of HIF-1 in mediating hypoxia-associated induction of the ADORA2B (33). Other studies demonstrated HIF-1-dependent induction of the ADORA2B during myocardial ischemia (41). Similarly, a recent study identified a transcriptionally regulated pathway elicited by hypoxia involving HIF-2-dependent induction of the ADORA2A (48). Although these studies demonstrate transcriptionally regulated alterations of ADOR gene expression, a recent study failed to demonstrate alterations of ADORA2B promoter activity elicited by inflammatory mediators (17). In contrast, this study indicated that increases in ADORA2B following exposure to inflammatory stimuli involve alterations in mRNA stability.

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 (49–51). For example, levels of adenosine are increased in the lungs of asthmatics, and correlate with the degree of inflammatory insult (49). 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 (46). 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 (15). 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, and pharmacological studies suggest that attenuation of adenosine signaling through the ADORA2B may reverse the severe pulmonary phenotypes in ADA-deficient mice (49). To address these findings on a genetic level, a very elegant study examined the contribution of ADORA2B signaling in this model via a genetic approach by generating ADA/ADORA2B double-KO mice (16). The authors’ initial hypothesis was that genetic removal of the ADORA2B from ADA-deficient mice would lead to diminished pulmonary inflammation and damage. Unexpectedly, ADA/ADORA2B double-KO 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 ADORA2B signaling during acute stages of lung disease (16). Importantly, however, a recent study brings these findings together by pointing out the existence of distinct roles for the ADORA2B dependent on the time course of the disease (acute versus chronic stages of bleomycin-induced lung injury) (46).

Taken together, the present studies of ALI induced by cyclic mechanical stretch in pulmonary epithelia in vitro or by mechanical ventilation of mice in vivo indicate a critical role for the transcription factor HIF in the regulation of pulmonary adenosine signaling events.

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