Partially adenosine deaminase-deficient mice develop pulmonary fibrosis in association with adenosine elevations

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Chunn, Janci L., Amir Mohsenin, Hays W. J. Young, Chun G. Lee, Jack A. Elias, Rodney E. Kellem, and Michael R. Blackburn. Partially adenosine deaminase-deficient mice develop pulmonary fibrosis in association with adenosine elevations. Am J Physiol Lung Cell Mol Physiol 290: L579–L587, 2006. First published October 28, 2005; doi:10.1152/ajplung.00258.2005.—Adenosine, a signaling nucleoside, exhibits tissue-protective and tissue-destructive effects. Adenosine levels in tissues are controlled in part by the enzyme adenosine deaminase (ADA). ADA-deficient mice accumulate adenosine levels in multiple tissues, including the lung, where adenosine contributes to the development of pulmonary inflammation and chronic airway remodeling. The present study describes the development of pulmonary fibrosis in mice that have been genetically engineered to possess partial ADA enzyme activity and, thus, accumulate adenosine over a prolonged period of time. These partially ADA-deficient mice live for up to 5 mo and die from apparent respiratory distress. Detailed investigations of the lung histopathology of partially ADA-deficient mice revealed progressive pulmonary fibrosis marked by an increase in the number of pulmonary myofibroblasts and an increase in collagen deposition. In addition, in regions of the distal airways that did not exhibit fibrosis, an increase in the number of large foamy macrophages and a substantial enlargement of the alveolar air spaces suggest emphysemic changes. Furthermore, important proinflammatory and profibrotic signaling pathways, including IL-13 and transforming growth factor-β1, were activated. Increases in tissue fibrosis were also seen in the liver and kidneys of these mice. These changes occurred in association with pronounced elevations of lung adenosine concentrations and alterations in lung adenosine receptor levels, supporting the hypothesis that elevation of endogenous adenosine is a proinflammatory and profibrotic signal in this model.

PULMONARY FIBROSIS IS A COMPONENT OF various interstitial pneumonias (38), which are characterized by varying degrees of inflammation, aberrant fibroblast proliferation, and extracellular matrix deposition. These disorders result in distortion of pulmonary architecture, which compromises pulmonary function (24, 29, 34, 38). The pulmonary fibrosis associated with desquamative interstitial pneumonias is typically associated with an inflammatory component and responds well to steroid therapy (38). In contrast, the pulmonary fibrosis characteristic of interstitial pneumonias (idiopathic pulmonary fibrosis) lacks a prominent inflammatory component, and immunosuppressant therapy is less effective (27). The various signaling pathways that mediate pulmonary inflammation and fibrosis in these disorders have not been completely elucidated. However, emerging evidence suggests that pulmonary fibrosis results from abnormal repair responses in the damaged lung (33). Thus understanding the contribution of factors generated during tissue injury to the regulation of inflammation and fibrosis could provide novel therapeutic approaches for fibrotic lung diseases.

Adenosine, a potent cellular signaling molecule, is produced in excess during cellular stress and damage as a result of ATP catabolism. Therefore, adenosine levels are elevated in the lungs of patients with chronic lung disease (10, 18), where significant airway inflammation and damage are often found (11). Once produced, adenosine can engage specific G-protein-coupled receptors on the surface of cells. Four adenosine receptors (ARs) have been identified: A₁AR, A₂AAR, A₂BAR, and A₃AR (13). Expression patterns of these receptors in tissues and cells are diverse, and their engagement can activate multiple cellular responses, including modulation of inflammatory cell function (16, 23), mast cell degranulation (25, 39), bronchoconstriction (8, 28), apoptosis (37), cell proliferation (12), and differentiation of pulmonary fibroblasts into myofibroblasts (44). Therefore, excess adenosine production after lung injury may access profibrotic pathways and, hence, contribute to the development and/or maintenance of pulmonary fibrosis.

Consistent with this notion, recent studies in mice have demonstrated a correlation between elevations in lung adenosine levels and development of pulmonary fibrosis. Mice overexpressing the pluripotent T helper type 2 (Th2) cytokine IL-13 exhibit severe pulmonary inflammation and airway remodeling, which included extensive pulmonary fibrosis (45). The pulmonary fibrosis in this model was associated with increases in lung adenosine levels, and the use of adenosine deaminase (ADA) enzyme therapy to reduce adenosine levels reduced pulmonary fibrosis (3). In addition, partial pharmacological replacement of ADA in ADA-deficient mice led to development of pulmonary fibrosis in association with elevations of adenosine (6). These findings suggest that adenosine may directly regulate pathways that contribute to the development and progression of pulmonary fibrosis.

Here we describe the development of pulmonary inflammation and fibrosis in a novel model of partial ADA deficiency. Mice completely deficient in ADA die from lung disease within a few weeks of birth (2); however, significant pulmonary fibrosis does not develop. An otherwise ADA-deficient...
line of mice that express an ADA minigene in the gastrointestinal tract was developed (7). These partially ADA-deficient mice live for up to 5 mo and die from apparent respiratory distress. Detailed investigations of the lungs of partially ADA-deficient mice in this study reveal severe pulmonary fibrosis, as indicated by the increased presence of pulmonary myofibroblasts and increased collagen deposition. Pulmonary inflammation, characterized by predominantly macrophages, and alveolar destruction were also seen. The levels of Th2 cytokines as well as transforming growth factor (TGF)-β1 were elevated. These changes occurred in association with pronounced elevation of lung adenosine concentrations, supporting the hypothesis that elevation of endogenous adenosine can serve as a proinflammatory and profibrotic signal.

**MATERIALS AND METHODS**

**Mice.** Partially ADA-deficient mice were generated and genotyped as described elsewhere (7). Mice homozygous for the null Ada allele and containing an ADA minitransgene were designated ADA-deficient (ADA^−/−), whereas mice heterozygous for the null
Ada allele and containing an ADA minitransgene were designated ADA control (ADA\(^{+/+}\)) mice (4). All mice were on a 129sv/C57BL/6J mixed background, and all phenotype comparisons were performed among littermates. All experiments performed on mice in this study were approved by the University of Texas-Houston Health Science Center Animal Care and Use Committee. Mice were housed in ventilated cages equipped with microisolator lids and maintained under strict containment protocols. No evidence of bacterial, parasitic, or fungal infection was found, and serology on cage littermates was negative for 12 of the most common murine viruses.

Fig. 3. BAL fluid cellular differentials and lung cellularity in partially ADA\(^{-/-}\) mice. A: cellular differentials in BAL fluid of 15-wk-old ADA\(^{+/+}\) and ADA\(^{-/-}\) mice. Cells were collected, counted, cytocentrin onto slides, and stained with Diff Quick for determination of cellular differentials. Values are means ± SE; n = 6. *P < 0.05 vs. ADA\(^{+/+}\) (by Student’s t-test). B and C: macrophages in lung sections from 15-wk-old ADA\(^{+/+}\) and ADA\(^{-/-}\) mice, respectively, incubated in F4/80 antiserum and studied by immunohistochemistry. Scale bars 50 \(\mu\)m.

Fig. 4. Alveolar space enlargement and protease expression in partially ADA\(^{-/-}\) mice. Lungs from 15-wk-old ADA\(^{+/+}\) (A) and partially ADA\(^{-/-}\) (B) mice were collected and processed for hematoxylin-and-eosin staining. Images are representative of 5 mice from each group. Scale bars 100 \(\mu\)m. C: air space size calculated using Image-Pro Plus. Values are means ± SE; n = 6 for ADA\(^{+/+}\) and n = 5 for ADA\(^{-/-}\). D: matrix metalloproteinase (MMP)-12 and tissue inhibitor of metalloproteinase (TIMP)-1 transcript levels in whole lung RNA extracts from 15-wk-old mice determined by quantitative RT-PCR. Values are means ± SE; n = 4. *P < 0.05 vs. ADA\(^{+/+}\) (by Student’s t-test).
Fig. 5. Cytokine production in lungs of partially ADA−/− mice. BAL fluid was collected from lungs of 15-wk-old mice, and cytokine levels of IL-4 (A), IL-5 (B), TNF-α (C), and IFN-γ (D) were measured using ELISA. Values are means ± SE; n = 6–10. *P < 0.05 vs. ADA−. E: IL-13 transcript levels in whole lung RNA extracts from 15-wk-old mice determined by quantitative RT-PCR. Values are means ± SE; n = 5. *P < 0.05 vs. ADA− (by Student’s t-test).

**Immunohistochemistry.** Immunohistochemistry was performed on 5-μm sections cut from formalin-fixed, paraffin-embedded lungs. Sections were rehydrated through a graded series of ethanol to water, and endogenous peroxidases were quenched with 3% H2O2. For α-smooth muscle actin, antigen retrieval was performed (DAKO, Carpentaria, CA), and endogenous avidin and biotin were blocked with the biotin blocking system (DAKO). The slides were processed with the Mouse-on-Mouse kit and the ABC Elite streptavidin reagents (Vector Laboratories, Burlingame, CA) and incubated with a 1:500 dilution of α-smooth muscle actin monoclonal antibody (Sigma, St. Louis, MO; monoclonal clone 1A-4) overnight at 4°C. For F4/80, trypsin antigen retrieval was performed according to the manufacturer’s instructions (Carezyme I, Biocare Medical, Concord, CA) followed by avidin-biotin blocking (DAKO). Sections were incubated with serum-free protein block (DAKO) for 1 h and then with rat anti-mouse F4/80 antigen primary antibody diluted 1:10 (Serotec, Oxford, UK) for 1 h at 37°C. Biotinylated rabbit anti-mouse IgG (Vector Laboratories) was used as the secondary antibody for 30 min at room temperature followed by the streptavidin-biotin system (ABC Elite kit) for 30 min. All sections were developed with 3,3’-diaminobenzidine and counterstained with methyl green.

**Masson’s trichrome staining.** Mice were anesthetized, and the lungs were perfused with 5–10 ml of PBS and then infused with 0.5 ml of fixative (4% paraformaldehyde in PBS) and fixed overnight at 4°C. For analysis of kidney and liver, tissues were removed and fixed overnight at 4°C (4% paraformaldehyde in PBS) and embedded in paraffin, and 5-μm sections were collected on slides and stained with Masson’s trichrome (Sigma) according to the manufacturer’s instructions.

**Collagen quantification.** Soluble collagen levels were quantified in bronchial alveolar lavage (BAL) fluid using the Sircol collagen assay (Biocolor, Belfast, N. Ireland) according to the manufacturer’s instructions.

**Cellular differentials.** Mice were anesthetized and tracheally intubated with a blunt 21-gauge needle. Lungs were lavaged with 1–2 ml of PBS, and the recovered BAL fluid was processed for the determination of cytokine levels. Briefly, total cell counts were performed on initial lavaged aliquots, and cellular differentiation (300 cells/sample) was determined from cells cytospun onto slides and stained with Diff-Quick (Dade Behring, Newark, DE).

**Alveolar air space measurement.** The size of alveolar spaces was determined in pressure-infused lungs by measurement of mean chord lengths on hematoxylin-and-eosin-stained lung sections. Representative images from throughout the distal airways of all lobes were digitized, and bronchial airways, blood vessels, and alveolar air spaces occluded by inflammation and fibrosis were blocked out. Next, a grid consisting of 53 black lines at 10.5-μm intervals was overlaid on the image. This line grid was subtracted from the lung images using Image-Pro Plus (Media Cybernetics) image analysis software, and the resultant lines were measured and averaged to give the mean chord length of the nonoccluded alveolar air spaces. The final mean chord lengths represent averages from 10 nonoverlapping images of each lung specimen. All quantitative studies were performed by a technician who was blinded to animal genotype.

**RNA isolation and quantitative RT-PCR.** Mice were anesthetized, and the lungs were rapidly removed and frozen in liquid nitrogen. RNA was isolated from frozen lung tissue using TRizol reagent (Life Technologies, Grand Island, NY). RNA samples were then treated with DNase and subjected to quantitative real-time RT-PCR. The primers, probes, and procedures for real-time RT-PCR for α1-procollagen, IL-13, matrix metalloproteinase-12, tissue inhibitor of metalloproteinase-1, and the ARs have been described previously (6, 7, 36). Reactions were carried out on a rapid thermal cycler system (Smart Cycler, Cepheid, Sunnyvale, CA). Specific transcript levels were determined using Smart Cycler analysis software by comparison with a standard curve generated from the PCR amplification of template dilutions.

**Quantification of cytokines.** Mice were anesthetized and tracheally intubated with a blunt 21-gauge needle. Lungs were lavaged with 1–2 ml of PBS, and the recovered BAL fluid was processed for the determination of cytokine levels using OptEIA mouse ELISA sets (Pharmingen, San Diego, CA).

**TGF-β1 immunohistochemistry and bioactivity.** For TGF-β1 localization, lungs were fixed overnight at 4°C (4% paraformaldehyde in PBS) and embedded in paraffin, and 5-μm sections were collected on microscope slides and processed for TGF-β1 immunohistochemistry as previously described (22). Briefly, sections were rehydrated, and endogenous peroxidase was blocked by quenching with H2O2 and methanol. Antigen was unmasked using target unmasking solution following the manufacturer’s instructions (DAKO). Blocking and
immunohistochemistry were conducted according the manufacturer’s instructions using a rabbit IgG Vectastain Elite ABC kit (Vector Laboratories). The slides were incubated overnight at 4°C with a 1:200 dilution of polyclonal rabbit anti-TGF-β antibody (Santa Cruz Biotechnology, Santa Cruz, CA) or antibody preabsorbed with the peptide used to generate the antibody as a control. The slides were incubated with 3,3’-diaminobenzidine and counterstained with hematoxylin.

Mink lung epithelial cells permanently transfected with a construct containing the TGF-β-responsive human plasminogen activator inhibitor-1 promoter fused to a luciferase reporter gene were used to measure the bioactivity of TGF-β in BAL fluid (22). The cells were seeded into 12-well tissue culture plates in DMEM supplemented with 10% FCS, incubated in triplicate with BAL fluid in the presence or absence of neutralizing antibodies specific for TGF-β1, TGF-β2, or TGF-β3 (R & D Systems, Minneapolis, MN) for 16 h, and then measured using the luciferase assay system (Promega, Madison, WI) according to the manufacturer’s instructions. TGF-β1 bioactivity was defined as the difference in the luciferase activity of identical cells incubated in the absence and presence of anti-TGF-β1.

Adenosine determinations. Mice were anesthetized, and the lungs were rapidly removed and frozen in liquid nitrogen. Adenine nucleosides were extracted from frozen lungs using 0.4 N perchloric acid as described previously (2, 20), and adenosine was separated and identified using reverse-phase HPLC.

RESULTS

Increased myofibroblasts and collagen deposition in lungs of partially ADA−/− mice. Mice completely deficient in ADA develop severe pulmonary inflammation and die at ~3 wk of age (2). In contrast, ADA−/− mice that express an ADA minigene in the gastrointestinal tract (partially ADA−/− mice) exhibit progressive pulmonary inflammation and alterations in airway structure and live for 4–5 mo (7). By 10 wk of age, partially ADA−/− mice exhibit focal regions of apparent fibrosis throughout the distal airways. The degree of fibrosis in the lungs was progressive and was extensive by 15 wk of age (data not shown). To confirm fibrosis in this model, the presence of myofibroblasts was examined using α-smooth muscle actin immunohistochemistry. Few non-vascular-associated α-smooth muscle actin-positive cells were seen in the lungs of 15-wk-old ADA+ mice (Fig. 1A), whereas an abundance of α-smooth muscle actin-positive cells was observed throughout the distal airways.
of 15-wk-old partially ADA−/− mice (Fig. 1B). These findings demonstrate an increase in myofibroblasts in the airways of partially ADA−/− mice.

Increased collagen production and deposition are characteristic of pulmonary fibrosis (24). Masson’s trichrome stain was used to examine the presence of collagen in the airways of partially ADA−/− mice. There was little evidence of collagen deposition in the airways of ADA+ mice (Fig. 2, A and C), whereas collagen deposition was extensive in the airways of 15-wk-old partially ADA−/− mice (Fig. 2, B and D). Similarly, α-procollagen transcripts (Fig. 2E) and total collagen content (Fig. 2F) were significantly increased in the lungs of partially ADA−/− mice. These findings demonstrate increased collagen production and deposition in the lungs of partially ADA−/− mice.

Pulmonary inflammation in partially ADA−/− mice. To determine the status of pulmonary inflammation in partially ADA−/− mice, cellular differentiation was determined in BAL fluid from 15-wk-old mice. The number of macrophages, lymphocytes, eosinophils, and neutrophils was significantly increased in BAL fluid from partially ADA−/− mice (Fig. 3A). The most prominent increases were in macrophages. In lung sections stained with a macrophage-specific antibody, these cells were abundant in alveolar spaces throughout the distal airways (Fig. 3C). These findings demonstrate substantial pulmonary inflammation associated with the pulmonary fibrosis in this model.

Alveolar space enlargement in partially ADA−/− mice. Histological examination of lungs from 15-wk-old partially ADA−/− mice showed enlargement of nonfibrotic regions of the distal air spaces (Fig. 4, A and B). Quantitative analysis of alveolar space size confirmed these observations, demonstrating a significant increase in mean chord length in the lungs of partially ADA−/− mice (Fig. 4C). These findings suggest that alveolar destruction is occurring in the lungs of these mice. To pursue this matter further, the levels of proteases and protease inhibitors were measured in whole lung RNA extracts. Increased levels of RNA transcripts for matrix metalloproteinase-12 and tissue inhibitor of metalloproteinase-1 were found in the lungs of partially ADA−/− mice (Fig. 4D). Collectively, these findings demonstrate alveolar space enlargement in conjunction with elevated expression of proteolytic enzymes in partially ADA−/− mice.

Cytokine production in lungs of partially ADA−/− mice. To determine the status of cytokine production in the lungs of partially ADA−/− mice, the levels of numerous cytokines were examined. Levels of IL-4, IL-5, and TNF-α protein were increased, whereas levels of IFN-γ were substantially decreased, in BAL fluid of 15-wk-old partially ADA−/− mice (Fig. 5). In addition, levels of IL-13 transcripts were increased in whole lung RNA extracts from partially ADA−/− mice (Fig. 5E). These data demonstrate increased production of proinflammatory and profibrotic cytokines in the lungs of partially ADA−/− mice.

Increased TGF-β1 in lungs of partially ADA−/− mice. TGF-β1 has been implicated in the pathogenesis of pulmonary fibrosis in humans and animal models (34). Examination of TGF-β1 by immunohistochemistry revealed a pronounced increase in TGF-β1 protein in the airways of partially ADA−/− mice (Fig. 6, B and C). TGF-β1 was expressed predominantly in hyperplastic epithelium lining in remodeled alveolar air spaces (Fig. 6C). The presence of active TGF-β1 was confirmed using a TGF-β1 bioassay, which revealed a threefold increase in TGF-β1 bioactivity in the BAL fluid of 15-wk-old partially ADA−/− mice (Fig. 6E). These findings demonstrate elevated TGF-β1 levels in the lungs of partially ADA−/− mice exhibiting pulmonary fibrosis.

Adenosine and AR levels in lungs of partially ADA−/− mice. Lung adenosine levels were analyzed to confirm that increases in pulmonary inflammation and fibrosis were associated with elevations of endogenous adenosine. Adenosine was detected at low levels in nucleoside extracts from the lungs of 15-wk-old ADA+ mice, while substantial increases were seen in the lungs of ADA−/− mice at this stage (Fig. 7A). Investigation of transcript levels for the ARs revealed significant elevations in A1AR and A2BAR in the lungs of partially ADA−/− mice (Fig. 7B). These findings demonstrate elevated endogenous adenosine levels and altered AR patterns in the lungs of ADA−/− mice exhibiting an advanced fibrotic phenotype.

Increased collagen deposition in liver and kidney of partially ADA−/− mice. Other organ systems were examined to determine whether fibrosis in partially ADA−/− mice was limited to the lungs. In various tissues of 15-wk-old mice stained with Masson’s trichrome, increased collagen deposition was noted in the liver (Fig. 8B) and in glomeruli of the kidneys (Fig. 8D). The increased fibrosis in multiple organs of partially ADA−/− mice suggests that elevated adenosine levels may affect general fibrotic pathways throughout the animal.
DISCUSSION

Results from this study demonstrate that mice genetically engineered to possess low levels of ADA expression develop features of pulmonary and tissue fibrosis in association with elevated adenosine levels. These findings validate previous studies from our laboratory that used a pharmacological approach to regulate adenosine levels in completely ADA<sup>/−/−</sup> mice (6). Whereas both studies implicate adenosine as a profibrotic signal in the lung, several significant findings of the present genetic study were not appreciated in the previous pharmacological model: 1) In addition to pronounced distal airway fibrosis, nonfibrotic regions of ADA<sup>/−/−</sup> lungs exhibit alveolar space enlargement and macrophage accumulation. 2) There is a bias toward production of Th2 cytokines, including IL-4, IL-5, and IL-13, and a decrease in IFN-γ. 3) In addition to pulmonary fibrosis, fibrotic changes in the liver and kidney suggest that adenosine has profibrotic effects on other organs. Taken together, these findings suggest that chronic elevations of adenosine can activate pathways that lead to pulmonary and tissue fibrosis. Because adenosine is generated in response to tissue injury, elevations of this nucleoside may provide an important mechanism for the exacerbation of normal tissue repair processes in fibrotic disorders.

Inflammation and repair are normal processes that follow tissue injury. However, when injury is excessive or repetitive, repair processes result in tissue fibrosis. Fibrosis is characterized by the accumulation of myofibroblasts, which express α-smooth muscle actin, the deposition of extracellular matrix molecules such as collagen, and the distortion of normal tissue architecture, which, in the case of pulmonary fibrosis, leads to the loss of gas exchange and normal lung function (24). Our findings demonstrate that partially ADA<sup>/−/−</sup> mice spontaneously develop features of pulmonary fibrosis, including a pronounced increase in α-smooth muscle actin-positive myofibroblasts, excessive collagen deposition in the distal airways, and pronounced enlargement of the distal air spaces. These alterations occurred in association with pronounced adenosine accumulation, suggesting that adenosine may access pathways that maintain or promote the progression of pulmonary fibrosis. This is supported further by recent observations that lowering adenosine levels during the progression of pulmonary fibrosis can halt and even reverse aspects of pulmonary inflammation and fibrosis in completely ADA<sup>/−/−</sup> mice (6). Alveolar epithelial cell injury is an early and consistent finding in pulmonary fibrosis (38). Interestingly, extracellular adenosine is readily produced by airway epithelial cells (17). Hence, our observation of profibrotic actions of adenosine in the lung provides a compelling link between early damage to the airway epithelium and ensuing fibrosis.

The mechanisms by which adenosine elevations lead to pulmonary fibrosis are not clear but may pertain to the activation of profibrotic pathways in the lung. Perhaps the best studied mediator of fibrosis is TGF-β1 (34). Levels of TGF-β1 are elevated in the lungs of patients with pulmonary fibrosis (5), and investigations in vitro and in animal models demonstrate that TGF-β1 is sufficient to induce features of pulmonary fibrosis, including accumulation of myofibroblasts and collagen production (9, 21, 35). Our studies demonstrate that TGF-β1 levels are elevated in the lungs of partially ADA<sup>/−/−</sup> mice, suggesting that this molecule may contribute to the
increased abundance of myofibroblasts and collagen deposition. The direct stimulation of TGF-β1 by adenosine has not been shown; however, a recent study in completely ADA−/− mice demonstrated that the use of exogenous ADA enzyme therapy to lower adenosine levels in the lung could halt the production of TGF-β1 and, subsequently, resolve pulmonary fibrosis (6). Together, these findings suggest that adenosine-dependent elevations of TGF-β1 may represent an important mechanism for controlling pulmonary fibrosis.

An emerging hypothesis in the regulation of fibrosis is that imbalances in Th1 and Th2 cytokine responses can lead to an abnormal response to injury and, hence, fibrosis (34, 41). This hypothesis has emerged from studies demonstrating that Th1 cytokines, such as IFN-γ, are decreased in the lungs of patients with pulmonary fibrosis (30), whereas Th2 cytokines, such as IL-4, IL-5, and IL-13, are elevated (15, 40). Furthermore, in vitro studies and animal models have shown that these Th2 cytokines are able to activate features of fibrosis (14, 32, 45). Consistent with this hypothesis, we found that levels of IFN-γ were decreased in the lungs of partially ADA−/− mice, whereas levels of the Th2 cytokines IL-4, IL-5, and IL-13 were elevated. Among these Th2 cytokines, IL-13 is gaining particular attention with regard to its ability to promote pulmonary fibrosis. Overexpression of IL-13 in the lungs of transgenic mice leads to a pronounced accumulation of myofibroblasts and collagen around the bronchial airways (43), suggesting that IL-13 can access profibrotic pathways. Subsequent analysis of these mice demonstrated that activation of the TGF-β1 signaling pathway by IL-13 is the likely mechanism involved in this process (22). Interestingly, adenosine elevations have also been shown to be important in the pulmonary fibrosis in this IL-13 model. Adenosine levels are elevated in the lungs of mice overexpressing IL-13, and treatment of these mice with ADA can lower lung adenosine levels and, thereby, lead to the resolution of pulmonary fibrosis (3). Also of interest are the findings that adenosine regulates the levels of IL-13 production in vitro (31) and in vivo (3). Thus the ability of adenosine to regulate the production of Th2 cytokines in the lung may represent an important mechanism for the regulation of pulmonary fibrosis.

Adenosine exerts its effects by engaging cell surface ARs (13). Through the differential activation of ARs and downstream signaling pathways, this signaling nucleoside can exert tissue-protective (23) and tissue-destructive effects (1). With regard to tissue-destructive effects and chronic lung disease, attention has turned to the A2BAR. Engagement of this receptor in the human mast cell hmc-1 can directly promote the production of IL-4 and IL-13 (31), which may have important implications in the regulation of disorders where these cytokines are involved, such as asthma, chronic obstructive pulmonary disease, and pulmonary fibrosis. In addition, engagement of the A2BAR can lead to the production of IL-6 in multiple cell types, including pulmonary fibroblasts (44) and airway smooth muscle (43). IL-6 has also been implicated in the regulation of pulmonary fibrosis (19). A recent study has demonstrated that A2BAR engagement together with hypoxia can promote the differentiation of primary human lung fibroblasts into myofibroblasts through a pathway that involves IL-6 (44), suggesting that the A2BAR may be an important conduit for adenosine-mediated pulmonary fibrosis. The present study demonstrates that A2BAR levels are present in the lungs of partially ADA−/− mice exhibiting pulmonary fibrosis. Myofibroblasts (44) and macrophages (42) express the A2BAR. Thus elevations in A2BAR levels in the lungs of ADA−/− mice may be due to increases in these cell populations. Alternatively, increased levels of the A2BAR may result from cytokine-dependent upregulation of this receptor (26) in immune or nonimmune cells in the lung. Whatever the mechanism of increased A2BAR levels, increased signaling through this low-affinity, profibrotic receptor may be an important feature of fibrosis in environments where endogenous adenosine levels are chronically elevated.

In addition to pulmonary fibrosis, this study revealed fibrosis in the liver and kidney of partially ADA−/− mice, suggesting that systemic elevations of adenosine may access profibrotic pathways in multiple tissues. This finding implies that adenosine signaling may play a role in the pathogenesis of fibrosis in many disorders, including hepatic fibrosis and cirrhosis and chronic renal scarring in patients with glomerulonephritis.

In summary, we demonstrate that mice genetically engineered to possess low levels of ADA enzyme activity develop features of pulmonary and tissue fibrosis in association with elevated adenosine levels. The description of fibrosis in this model is consistent with other models of cytokine-induced fibrosis (3) and completely ADA−/− mice (6), which together support the hypothesis that adenosine is a signaling molecule that is produced in response to damage and can, in turn, elicit profibrotic activities in vivo. It is not known whether adenosine levels are elevated in the lungs of patients with pulmonary fibrosis; however, because adenosine is produced in response to tissue damage, elevated adenosine in these patients is probable. Indeed, adenosine levels are elevated in the lungs of asthmatic patients (10, 18), who also exhibit features of pulmonary fibrosis (11). Examining adenosine levels in patients with pulmonary fibrosis as well as establishing the involvement of AR mechanisms associated with adenosine-induced pulmonary fibrosis may provide new drug targets for the treatment of fibrotic disorders.

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