Adenosine induces fibronectin expression in lung epithelial cells: implications for airway remodeling

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Submitted 16 March 2005; accepted in final form 16 September 2005

Adenosine is an extracellular nucleoside that is elevated in tissues during hypoxia and ischemia reperfusion and has been implicated in asthma and other lung disorders. There, adenosine is considered an important modulator of physiological functions and inflammation, but its effects on matrix expression and turnover during tissue remodeling are unknown. We examined the effects of adenosine on lung epithelial cells with particular attention to the expression of fibronectin, a matrix glycoprotein highly expressed in injured tissues that has been implicated in wound healing. In A549 lung epithelial cells, we found that adenosine induced expression of fibronectin mRNA and protein in a dose- and time-dependent manner and found that the stimulatory effect of adenosine was inhibited by specific adenosine receptor antagonists. Adenosine stimulation was associated with increased levels of intracellular cAMP and with phosphorylation and DNA binding of the cAMP response element binding protein (CREB), known for its ability to stimulate fibronectin gene transcription. To confirm the latter, A549 cells were transfected with a DNA construct containing the human fibronectin promoter connected to a luciferase reporter gene. Adenosine stimulated transcription of the gene, and this effect was blocked by inhibitors of protein kinase activation. Finally, we tested primary lung fibroblasts and primary alveolar epithelial type II cells and found increased fibronectin expression in response to adenosine. Overall, our observations suggest that adenosine might modulate tissue remodeling by stimulating fibronectin expression in lung epithelial cells through induction of purinergic receptor-mediated signals that target CREB phosphorylation and stimulate fibronectin gene transcription.

extracellular matrix; signal transduction; gene transcription; lung injury; cyclic AMP; cAMP response element binding protein

ADENOSINE IS A PURINE NUCLEOSIDE that is produced by many cell types during normal metabolic activity and during physiological stresses associated with tissue inflammation (28, 30, 34). Once released, adenosine acts as a paracrine factor with effects on a variety of tissues in the cardiovascular, nervous, digestive, and immune systems (2, 19, 23, 42, 46), among others. Its generation in tissues is dependent on the balance between the activities of enzymes that catalyze its synthesis and metabolism. Adenosine is produced extracellularly by the action of membrane-bound 5′-nucleotidases on extracellular adenosine 5′-monophosphate (AMP), which is itself produced by the action of phosphatases on adenosine diphosphate (ADP) and adenosine triphosphate (ATP) (7, 32, 44). Adenosine metabolism occurs intracellularly and is mediated by the action of either adenosine kinase or adenosine deaminase, which converts adenosine to AMP or inosine, respectively (7, 32, 44). A third determinant of adenosine extracellular concentration appears to be inwardly directed transport across plasma membranes through nucleoside transporters (10).

The actions of adenosine are mediated through adenosine receptors belonging to the family of seven-transmembrane G protein-coupled cell-surface receptors. Adenosine receptors are expressed in many cell types including epithelial cells, mast cells, astrocytes, smooth muscle cells, and fibroblasts and have been classified into A1, A2A, A2B, and A3 (17, 28, 30, 34). The activation of adenosine receptors triggers a series of intracellular signals that modulate the expression of important soluble mediators such as IL-10, monocyte chemoattractant protein-1, IL-8, and IL-6 (16, 25, 42, 48) and affects the function of both cation and anion channels in tissue epithelia (26, 40, 41, 44), among other processes.

In the lung, most of the extracellular adenosine is derived from the cleavage of AMP by the enzyme 5′-nucleotidase (32, 44). In addition to its role in modulation of transepithelial electrolyte transport, adenosine has been implicated in the pathogenesis of obstructive airway diseases like asthma, an airway disorder associated with airway wall remodeling and inflammation, and characterized physiologically by airway hyperreactivity and bronchoconstriction. The association between adenosine and asthma is derived from studies showing a three- to fourfold increase in adenosine in the alveolar lining fluid of patients with asthma compared with control (15). The cause of this increase remains unknown, but it may be related to the increased generation of adenosine resulting from release of ATP from injured airway cells. Of note, aerosolized adenosine triggers bronchospasm in patients with asthma and other obstructive lung diseases (12, 29, 33).

Recently, it was demonstrated that mice with a null mutation of adenosine deaminase, the enzyme that degrades adenosine, show increased levels of tissue adenosine that are associated with inflammation (5, 11). Of note, these changes were also associated with evidence of tissue remodeling as exemplified by alveolar enlargement, airway wall thickening, mucus metaplasia, and tissue fibrosis (6). These studies suggest that not only does adenosine affect inflammation, but it can also modulate tissue remodeling. In view of the potential importance of...
this phenomenon, we investigated the effects of adenosine on tissue remodeling as it relates to extracellular matrix expression. This work focuses on fibronectin, a matrix glycoprotein highly expressed in injured tissues that has been implicated in wound healing and repair (24, 39). Fibronectin is increased in the alveolar lining fluid of smokers and subjects with chronic obstructive lung disease (43, 47). In the asthmatic airway, fibronectin deposition is increased in the subepithelial layer (37). These studies suggest that fibronectin is a sensitive marker of tissue injury and a key component in airway wall remodeling. Herein, we demonstrate that adenosine stimulates the biosynthesis of fibronectin through adenosine receptor-mediated activation of protein kinases and the induction of fibronectin gene transcription in A549 lung epithelial carcinoma cells and primary alveolar type II cells.

MATERIALS AND METHODS

Reagents. Adenosine was purchased from Calbiochem (La Jolla, CA). H-89, 3,7-dimethyl-1-propargylxanthine (DMPX; A2A and A2B receptor antagonist), theophylline (an A2B antagonist), rabbit anti-fibronectin (F-3648), goat anti-rabbit horseradish peroxidase (A-9169), and goat anti-rabbit FITC antibody (F-9887) were purchased from Sigma Chemical (St. Louis, MO). Erythro-9-(2-hydroxy-3-nonyl) adenine hydrochloride (phosphodiesterase II inhibitor, adenosine deaminase inhibitor) was purchased from AG Scientific (San Diego, CA). GAPDH antibody was purchased from Abcam (Cambridge, MA).

Cell culture. The human alveolar epithelial cell carcinoma line A549 (American Type Culture Collection, Rockville, MD) was grown in DMEM (Mediatech, Herndon, VA) supplemented with 10% heat-inactivated FBS (Atlanta Biologicals, Norcross, GA), 100 U/ml penicillin, and 100 U/ml streptomycin and incubated in 5% CO2 at 37°C. Primary alveolar epithelial cells were isolated as previously described (14). These studies were approved by the Animal Care and Use Committee. Briefly, rats were anesthetized, and a tracheostomy was placed followed by en bloc lung isolation. After buffer perfusion to remove intravascular blood elements, the lung airways were filled via the tracheostomy cannula with a solution containing porcine pancreatic elastase. The minced lung was then successively filtered through 100- and 20-m nylon mesh, and the recovered

mRNA analysis. A549 cells (1 × 106 cells/ml) were plated onto six-well plates and incubated in complete serum-free media for 24 h before the addition of adenosine (10–200 μM) for 1–6 h. Total RNA was isolated as previously described (31). The reverse transcription reactions of the extracted RNA were performed by combining the following reagents in a PCR reaction tube: 0.625 μM dNTP, 16 nmol random hexamer oligonucleotides (Roche Diagnostics, Indianapolis, IN), 5 μl First-Strand Buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl2; Invitrogen, Carlsbad, CA), 20 mM DTT, 200 units of reverse transcriptase enzyme, 0.5 μl RNasin (ribonuclease inhibitor; Promega, Madison, WI), and 1 μg extracted RNA in a total volume of 25 μl. Primers for PCR reactions were based on GenBank-published sequences and are as follows: human fibronectin forward primer (CCGTGGGCAACTCTTGT), reverse primer (TGGCGGATTGTGTG-GACAG); human β-actin forward primer (TGGAAGAAATCTG-GCACC), reverse primer (TGAGGTAGTCAGTCAGGTT); A1 purinergic receptor forward primer (GGGGCATGGAGGCAAGAC), reverse primer (CCACGCAACGATGAGA); A2B purinergic receptor forward primer (TCATCGAGTTGGCCCT), reverse primer (CTCTTGCTGGCCCCTAG); A2B purinergic receptor forward primer (CTCTCCGCGCCTGCTC), reverse primer (TGCGGCGACATGACCAACC). Reactions were performed using the following PCR protocol: 95°C for 30 s, 55°C for 30 s, 72°C for 1 min for 35 cycles. PCR products were resolved on 1% agarose gels and stained with ethidium bromide, band sizes were verified, and loading was controlled using the internal housekeeping gene β-actin.

Western blot analysis. A549 cells, rat type II lung epithelial cells, or primary lung fibroblasts (1 × 106 cells/ml) were treated with adenosine for 24–48 h, washed with PBS, and lysed in 1 ml of homogenization buffer [100 mM NaVa, 0.5 mM PMSF, 1% SDS, 0.5% sodium deoxycholate, 1 protease inhibitor cocktail tablet (Roche Diagnostics), in PBS, pH 7.4]. The resulting homogenate was centrifuged at 14,000 rpm for 5 min at 4°C. Protein concentration was determined by the Bradford method (8). The protein (50 μg) was mixed with an equal volume of 2X sample buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 5–10% mercaptoethanol, 0.004% bromophenol blue), boiled for 5 min, loaded onto a 5% SDS-polyacrylamide gel with a 3.5% stacking gel, and electrophoresed for 2 h at 150 V. The separated proteins were transferred onto nitrocellulose using a Bio-Rad Trans Blot semidry transfer apparatus for 1 h at 25 mA, blocked with Blotto [1X TBS (10 mM Tris-HCl, pH 8.0, 150 mM NaCl), 5% nonfat dry milk, 0.05% Tween 20] for 1 h at room temperature, and washed twice for 5 min with wash buffer, and incubated with a secondary rabbit antibody antibody raised against goat IgG conjugated to horseradish peroxidase (1:10,000 dilution) for 1 h at room temperature. Identically loaded blots used for loading controls were incubated with GAPDH (Abcam 9485; 1:4,000 dilution) primary antibodies. Blots were washed four times for 5 min in wash buffer, transferred to a Bio-Rad GS-800 calibrated laser densitometer (Hercules, CA).

Immunofluorescence. A549 cells (5 × 104 cells/ml) were plated onto Labtek eight-well permavox slides. Cells were incubated in complete serum-free media for 24 h before treatment with adenosine. After a 48-h induction with adenosine, the media were removed and cells were fixed with warm 4% paraformaldehyde for 30 min on ice. Cells were rinsed three times with 1X TBS and incubated in a humidified 5% CO2 incubator at 37°C for 1–3 wk to allow fibroblasts to migrate out of tissue sections. Primary mouse lung fibroblasts were placed in chambers two and eight passages when used in experiments. Cell viability was determined by trypan blue exclusion from four separate experiments.

The cells were incubated in complete serum-free media (Media-tech) for 24 h before being treated with adenosine. In inhibition experiments, cells were pretreated for 1 h with the following adenosine receptor antagonists: DMPX (10 μM), an A2A and A2B inhibitor, theophylline (10 μM), an A2B inhibitor, and MRS-1754 (10 μM), a specific A2B receptor antagonist. Cells were harvested and assayed for luminescence as described below.
10 min followed by immunofluorescence staining following a published procedure (38).

**Measurement of cAMP.** A549 cells (5 x 10⁴ cells/ml) were incubated in complete serum-free media for 24 h before treatment with adenosine (100 μM) for 30 min and 1, 2, or 4 h. Cells were harvested, washed with PBS (120 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer, pH 7.4), treated with 0.1 M HCl for 10 min, and centrifuged at 13,200 rpm for 10 min. Cell supernatants were tested for cAMP by the use of a cAMP enzyme immunoassay kit (Assay Designs, Ann Arbor, MI) following the manufacturer’s instructions.

**Examination of fibronectin gene transcription.** To evaluate for fibronectin gene transcription, the pFN(1.2kb)LUC promoter construct (27) was introduced into A549 cells via electroporation. Briefly, cells were washed with PBS and added to PBS supplemented with 10 mM HEPES, pH 7.4, to a final concentration of 1 x 10⁴ cells/ml. A549 cells were added to electroporation cuvettes (0.4-cm electrode gap) along with 40 μg of promoter construct plasmid DNA and 8 μg of pCL-neo mammalian expression vector (Promega) and subjected to 270 V and 950 μF (Gene Pulser II Electroporation System, Bio-Rad). Electroporated cells were pooled and plated into 25-cm² tissue culture flasks, and neomycin phosphotransferase gene-expressing cells were selected by the addition of 400 μg of Geneticin antibiotic (G418 sulfate; Life Technologies, Gaithersburg, MD) for a minimum of 2 wk. To obtain individual clones, cells were serially diluted into 96-well tissue culture plates. Single colonies were subsequently tested for luciferase activity; for this, cells were incubated in complete serum-free media for 24 h before the addition of adenosine (0–200 μM) for 0–24 h, harvested by cell scraper, washed with PBS, resuspended in 100 μl of cell lysis buffer (Promega), and sonicated. A 20-μl aliquot was then added to 80 μl of Luciferase Assay Reagent (Promega). Light intensity was measured using a Thermo-Labsystems Luminoskan Ascent microtiter plate luminometer. Results were recorded as normalized luciferase units and adjusted for total protein content that was measured using the Bradford method (8). The inhibitor enhanced fibronectin expression, and no further increase was noted with additional adenosine. These observations are consistent with a role for endogenous adenosine in regulation of fibronectin expression and a role for adenosine deaminase in metabolizing adenosine and preventing its actions in A549 cells (Fig. 2B).

**Adenosine stimulates fibronectin expression in A549 via purinergic receptors.** As reported by others, we found that A549 cells express several purinergic receptors including A1, A2A, and A2B; we failed to identify A3 receptors in these cells (44) (Fig. 3B, DMPX (A2A and A2B receptor antagonist) and theophylline (A2B receptor antagonist) diminished the stimulatory effect of adenosine on fibronectin expression. An A2B receptor-specific antagonist termed MRS-1754 also inhibited the effect (Fig. 3C).

**Adenosine stimulation is associated with activation of intracellular signals.** Purinergic receptor activation has been shown to elicit a number of intracellular events including activation of adenylate cyclase, increased concentration of cAMP, and activation of protein kinase A (17). This pathway appears relevant in our system since the exposure of A549 cells to adenosine triggered an intracellular accumulation of cAMP (Fig. 4A). Also, inhibition of protein kinase A with H-89 blocked the adenosine-induced fibronectin response in a dose-dependent fashion (Fig. 4B).

These intracellular events have been linked with, among other things, the activation of and an increase in DNA binding by CREB, a transcription factor known to stimulate fibronectin gene transcription (27). To test the role of CREB in our system, A549 cells were stimulated with adenosine followed by the extraction of nuclear protein and the performance of EMSA for CREB. As demonstrated in Fig. 4C, adenosine stimulation triggered an increased in DNA binding of CREB compared with control. In contrast, adenosine did not affect DNA binding. **RESULTS** Adenosine stimulates the expression of fibronectin in A549 cells. To determine whether adenosine is capable of stimulating fibronectin expression in lung epithelial cells, A549 cells were cultured in the presence of increasing concentrations of adenosine for different periods of time. As demonstrated in Fig. 1, adenosine stimulated the expression of fibronectin mRNA in these cells in a dose- and time-dependent fashion (Fig. 1A), and maximal levels of mRNA were detected at 4 h (Fig. 1B). This effect was associated with increased expression of fibronectin protein as determined by Western blotting (Fig. 1C) and confirmed by immunofluorescence staining (Fig. 1D). Adenosine stimulates transcription of fibronectin gene, and an inhibitor of adenosine deaminase mimics this effect. We explored whether the stimulatory effects of adenosine on fibronectin expression were related to induction of fibronectin gene transcription. To this end, A549 cells were stably transfected with pFN(1.2kb)Luc, a DNA construct containing the human fibronectin gene promoter fused to a luciferase reporter gene (27). Transfected A549 cells were stimulated with adenosine, and the cells were processed for their production of luciferase as a surrogate marker of fibronectin gene transcription. As presented in Fig. 2A, adenosine stimulated luciferase activity, indicating induction of gene transcription.

The concentration of adenosine surrounding lung epithelial cells is likely dependent on a number of factors, including adenosine metabolism by the enzyme adenosine deaminase. To determine the role of endogenous adenosine, we exposed cells to an inhibitor of adenosine deaminase and evaluated the effects of this intervention on fibronectin gene transcription. The inhibitor enhanced fibronectin expression, and no further increase was noted with additional adenosine. These observations are consistent with a role for endogenous adenosine in regulation of fibronectin expression and a role for adenosine deaminase in metabolizing adenosine and preventing its actions in A549 cells (Fig. 2B).
by the transcription factor AP-1. Densitometric analysis of bands for CREB and for AP-1 is depicted in figure.

**Effects of adenosine on fibronectin expression in primary lung cells.** To determine the relevance of our findings to lung cells, we harvested alveolar type II epithelial cells from rats and exposed them to adenosine as described above. This intervention stimulated the production of fibronectin protein as demonstrated by Western blot analysis and immunofluorescence staining (Fig. 5, A and B). We also tested primary lung fibroblasts isolated from mice transgenic with the full-length human fibronectin promoter connected to the luciferase reporter vector (45) and exposed them to adenosine. As observed for epithelial cells, adenosine stimulated the production of fibronectin protein in a dose-dependent manner in primary lung fibroblasts (Fig. 6A). Fibronectin gene transcription was also shown to be stimulated by adenosine in both a dose- and time-dependent manner (Fig. 6, B and C).

**DISCUSSION**

Adenosine is a signaling molecule produced by many cell types under normal conditions and is released in high concentrations at sites of tissue injury (2, 15, 28, 42, 46). In general, adenosine is thought to modulate inflammatory responses; however, its role in the control of tissue remodeling is unclear. Here, we demonstrate that adenosine can modulate the expres-
sion of the matrix glycoprotein fibronectin in A549 cells and in primary rat lung epithelial cells as well as in primary mouse lung fibroblasts. These observations suggest that high concentrations of adenosine in injured lungs and other tissues can stimulate the excessive expression and deposition of fibronectin. This finding is important when the many functions ascribed to fibronectin are considered, including its ability to promote the adhesion and migration/chemotaxis of immune cells, stimulate the proliferation of fibroblasts, enhance angiogenesis, and stimulate tumor cell growth, among other functions (3, 21, 24, 39).

Fibronectin is highly expressed in injured lungs where it contributes to repair responses elicited by a number of injurious agents (24, 39). Fibronectin is found elevated and has been implicated in the pathogenesis of obstructive lung diseases like asthma (9, 43, 47). Its expression in lung is stimulated by transforming growth factor-β (TGF-β) and other growth factors present in serum (27, 36). Our work suggests that adenosine is yet another stimulant of fibronectin expression in lung cells.

Adenosine stimulated the expression of fibronectin mRNA and protein in A549 cells in a dose- and time-dependent fashion. Adenosine induction of fibronectin mRNA was evi-

Fig. 2. Adenosine stimulates fibronectin gene transcription, and deaminase can mimic this effect. A: adenosine stimulates fibronectin gene transcription. A549 transfected cells (1 × 10⁶ cells/ml) were cultured in the presence of adenosine (100 μM) for 24 h. Afterward, the cells were harvested, and fibronectin gene transcription was measured by luminescence. B: an inhibitor of adenosine deaminase stimulates fibronectin gene transcription. A549 transfected cells (1 × 10⁶ cells/ml) were cultured in the presence of adenosine (100 μM) with or without a deaminase inhibitor (0–1 μM) for 24 h. Afterward, the cells were harvested, and fibronectin gene transcription was measured by luminescence. *Significant difference from control untreated cells (n = 4; P < 0.05).

Fig. 3. Role of adenosine receptors in adenosine-induced fibronectin expression. A: adenosine receptor mRNAs in A549 cells. A549 cells (1 × 10⁶ cells/ml) were incubated in complete serum-free media for 24 h before the addition of adenosine (100 μM) for 4 h. Total RNA was isolated and reverse transcribed using Superscript II reverse transcriptase and random hexamers. PCR reactions were set up using primers for purinergic receptors. PCR products were resolved on 1% agarose gels and stained with ethidium bromide, and band sizes were verified. B and C: adenosine-induced fibronectin expression is inhibited with adenosine receptor antagonists. A549 transfected cells (1 × 10⁶ cells/ml) were cultured in the presence of adenosine with or without adenosine receptor inhibitors for 24 h. Afterward, the cells were harvested, and fibronectin gene transcription was measured by luminescence. The adenosine receptor inhibitors for A1, A2A, and A2B inhibited the adenosine-stimulated expression of the fibronectin gene. 3,7-Dimethyl-1-propargylxanthine (DMPX; 10 μM) is an A2A and A2B inhibitor. Theophylline (Theo; 10 μM) is an A2B inhibitor. MRS-1754 (10 μM) is a specific A2B receptor antagonist. *Significant difference from adenosine-treated cells (n = 4; P < 0.001).
dent at 1 h followed by a peak at 4 h. Induction of fibronectin protein was evident at 48 h, which is consistent with observations made for fibronectin expression in other systems by this and other groups (27). Although the 10- and 50-μM concentrations of adenosine used in Fig. 1 A did significantly increase the expression of the fibronectin mRNA, we found more variability in our results using these lower doses. The higher dose of adenosine (100 μM) resulted in more consistent results in all assays, including the fibronectin gene expression assay and fibronectin Western blot analysis. We did not experience any cytotoxicity, at least by cell growth and viability experiments, with the doses used. Another interesting observation relates to the fact that fibronectin mRNA accumulation diminished at higher concentrations of adenosine (200 μM). One explanation for this is that, at higher concentrations, adenosine may affect other pathways that provide signals that counter its stimulatory effect on fibronectin gene transcription. However, defining the mechanism responsible for this occurrence will require further investigation.

To determine the effects of adenosine on fibronectin gene transcription, we tested A549 cells stably transfected with a DNA construct containing the human fibronectin promoter connected to a luciferase reporter gene. These studies revealed that the stimulation of fibronectin expression by adenosine was at least partly related to an effect on gene transcription. Transient transfection of mammalian cells is a technique with inherent variability due to the passage number, growth phase, and confluence of the cells in addition to the purity and quality of the plasmid DNA. Although we found that the relative light units of each experiment varied, the same relative fold increase or decrease in fibronectin promoter activity compared with the untreated control cells was the same in all cases.
Adenosine has been shown to act through G protein-coupled receptors that transmit intracellular signals responsible for differential gene expression. Increased cAMP levels, activation of protein kinase A, and induction of specific transcription factors have been demonstrated (44). Similar signals appeared to be working in our system. We found that A549 cells treated with adenosine showed increased intracellular concentrations of cAMP and that treatment of the cells with H-89, an inhibitor of protein kinase A, blocked the stimulatory effect of adenosine on fibronectin expression in a dose-dependent fashion. We also found that adenosine induced DNA binding by CREB, a transcription factor known for its ability to stimulate fibronectin gene transcription (27). In contrast, adenosine had no effect on DNA binding by AP-1.

Others have shown that the adenosine-stimulated intracellular signals are mediated through purinergic receptors A1, A2A,
A2B, or A3. We have confirmed the expression of three out of four of these receptors (A1, A2A, and A2B) in A549 cells and, consistent with their role in fibronectin induction by adenosine, we found that adenosine receptor antagonists prevented the stimulation of fibronectin. However, it should be highlighted that, in general, the methylxanthines and related compounds are weak adenosine receptor antagonists and may be nonspecific. Furthermore, studies suggest that A1, A2A, and A3 receptors can be activated by physiological concentrations of adenosine, whereas pathophysiological concentrations of adenosine are required to activate the A2B receptor (20). Therefore, although the inhibition of the processes studied with MRS-1754, a more selective inhibitor for A2B, strongly suggests a role for this receptor in mediating the effects of adenosine on fibronectin expression, further work will be required to determine the true role of this receptor in vivo.

The extracellular concentration of adenosine is dependent on its production as well as on its metabolism by enzymes like adenosine deaminase, a purine catabolic enzyme that promotes its conversion to inosine (7). To test the effect of endogenous adenosine in the absence of metabolism, we treated the transfected A549 cells with an adenosine deaminase inhibitor. Cells treated with the inhibitor showed an increase in fibronectin gene transcription compared with control cells, suggesting that regulation of extracellular adenosine concentration through its metabolism might be needed to maintain homeostasis. This is consistent with other work demonstrating that mice deficient in adenosine deaminase show a sixfold increase in constitutive fibronectin expression (1). Interestingly, these animals show upregulation of a number of other extracellular matrix-related genes including those encoding for collagens, proteoglycans, laminin, thrombospondin, cathepsins, urokinase-type plasminogen activator, and the profibrotic growth factor transforming growth factor-β. Together, these findings suggest that adenosine is an important regulator of tissue remodeling and that conditions associated with increased adenosine release or decreased metabolism might promote fibronectin expression.

Finally, we confirmed that the findings obtained with A549 cells were relevant to primary lung epithelial cells. More intriguing were our findings that adenosine could stimulate fibronectin expression in fibroblasts, suggesting that adenosine could affect lung cells other than epithelial cells.

**Implications for understanding tissue remodeling in lung disease.** Adenosine is generated in response to lung hypoxia and lung injury (32). Several studies have implicated adenosine in chronic lung diseases characterized by airflow limitation such as asthma (12, 15, 29, 33). However, the exact role of adenosine in vivo remains undefined. Its demonstrated anti-inflammatory properties suggest that it might have protective effects against tissue injury. However, its ability to stimulate mast cell degranulation, to activate eosinophils and other immune cells, and to induce cytokine expression in bronchial smooth muscle cells suggests that it might act to promote inflammation. The latter idea was strengthened by observations made in mice deficient in adenosine deaminase, an enzyme that, as stated above, serves to modulate levels of adenosine and deoxyadenosine in tissues and cells (5, 6, 11). The adenosine deaminase-deficient mice show accumulation of adenosine in the lungs and other organs. In the lungs of these animals, the concentration of adenosine was estimated to be ~100 μM. This is comparable to the levels detected in the bronchoalveolar lavage fluid of subjects with asthma (15). These animals develop pulmonary inflammation as exemplified by increased mast cell degranulation and the accumulation of macrophages and eosinophils in lung. Most notably, and consistent with the theme of this report, they also develop evidence of tissue remodeling. These animals developed respiratory failure and died prematurely by 3 wk of age, but in animals with partial adenosine deaminase deficiency, the abnormalities developed gradually, ultimately resulting in accumulation of macrophages, the development of alveolitis and fibrosis in the distal airways, and death by 5 mo of age.

Together, these studies suggest that the release of adenosine and/or a decrease in its metabolism might result in excessive stimulation of purinergic receptor-dependent signals that induce the differential expression of genes involved in control of tissue inflammation and remodeling. Therefore, targeting the mechanisms responsible for poor control of adenosine levels or for aberrant signaling through adenosine receptors might help preserve tissue function. In this regard, purified adenosine deaminase covalently linked to polyethylene glycol has been shown to lower systemic and tissue levels of adenosine in both adenosine deaminase-deficient humans and animals (4, 18, 22).

In conclusion, further work with these and related agents will be required to understand the true role of adenosine in lung and to elucidate ways by which to control its actions.

**GRANTS**

This work was supported by National Institute on Alcohol Abuse and Alcoholism Grant 1PS0AA183757 and a Merit Review grant (J. Roman).

**REFERENCES**


11. Chunn JL, Young HW, Banerjee SK, Colasurdo GN, and Blackburn MR. Adenosine-dependent airway inflammation and hyperresponsive-
ADENOSINE INDUCES FIBRONECTIN EXPRESSION IN LUNG EPITHELIUM


