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Adenosine-Dependent Airway Inflammation and Hyperresponsiveness in Partially Adenosine Deaminase-Deficient Mice

Janci L. Chunn,* Hays W. J. Young,* Suman K. Banerjee,* Giuseppe N. Colasurdo,† and Michael R. Blackburn‡*

Adenosine is a signaling nucleoside that is elevated in the lungs of asthmatics. We have engineered a mouse model that has elevated levels of adenosine as a result of the partial expression of the enzyme that metabolizes adenosine, adenosine deaminase (ADA). Mice with lowered levels of ADA enzymatic activity were generated by the ectopic expression of an ADA minigene in the gastrointestinal tract of otherwise ADA-deficient mice. These mice developed progressive lung inflammation and damage and died at 4–5 mo of age from respiratory distress. Associated with this phenotype was a progressive increase in lung adenosine levels. Examination of airway physiology at 6 wk of age revealed alterations in airway hyperresponsiveness. This was reversed following the lowering of adenosine levels using ADA enzyme therapy and also through the use of the adenosine receptor antagonist theophylline, implicating both the nucleoside and its receptors in airway physiological alterations. All four adenosine receptors were expressed in the lungs of both control and partially ADA-deficient mice. However, transcript levels for the A₁, A₂B, and A₃ adenosine receptors were significantly elevated in partially ADA-deficient lungs. There was a significant increase in alveolar macrophages, and monocyte chemoattractant protein-3 was found to be elevated in the bronchial epithelium of these mice, which may have important implications in the regulation of pulmonary inflammation and airway hyperresponsiveness. Collectively, these findings suggest that elevations in adenosine can directly impact lung inflammation and physiology. The Journal of Immunology, 2001, 167: 4676–4685.

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Abbreviations used in this paper: ADA, adenosine deaminase; BALF, bronchial alveolar lavage fluid; PEG-ADA, polyethylene glycol-modified ADA; MCP-3, monocyte chemoattractant protein-3; Penh, enhanced pause.
occlusion (21). The major inflammatory component found in the lungs is the accumulation of activated alveolar macrophages and the infiltration of eosinophils. This is followed by mucus hypersecretion and airway obstruction that is believed to lead to asphyxiation and death of the animals by 3 wk of age. Adenosine accumulates to very high levels in the lungs of these mice and the lung eosinophilia and mucus hypersecretion seen can be reversed by lowering adenosine levels using ADA enzyme therapy (21). The similarity of this phenotype to that seen in asthmatics, together with the association of inflammation and damage with elevated adenosine, makes this model useful for studying the role of elevated adenosine in lung inflammation and damage. However, the severe alveolar defects and the death of these animals at an early age have made assessment of the impact of elevated adenosine on airway physiology difficult.

In the current study we have characterized the impact of endogenously elevated lung adenosine on airway inflammation and physiology in a mouse model of partial ADA deficiency. Partially ADA-deficient mice were generated by the ectopic expression of an ADA minigene in the gastrointestinal tract of otherwise ADA-deficient mice. These mice do not exhibit the defects in alveogenesis seen in completely ADA-deficient mice. However, partially ADA-deficient mice developed lung inflammation and damage at a much later stage than that seen in completely ADA-deficient mice, and died from respiratory distress at 4–5 mo of age instead of 3 wk of age. Associated with this phenotype was a progressive increase in lung adenosine levels. Examination of airway physiology at 6 wk of age revealed an increase in airway responsiveness. These changes were shown to be dependent on elevated adenosine in that treatment with ADA enzyme therapy reversed these features in conjunction with lowering lung adenosine levels. Furthermore, treatment with the broad-spectrum adenosine receptor antagonist theophylline prevented airway hyperresponsiveness, implicating the involvement of adenosine receptors. All four of the adenosine receptors were shown to be present in control lungs, and transcript levels for the A1, A2B, and A3 adenosine receptors were elevated in the lungs of partially ADA-deficient mice. Elevations in the expression of the C-C chemokine monocyte chemotactrant protein-3 (MCP-3) were detected in the bronchial epithelium, which may be important in the regulation of pulmonary inflammation and airway hyperresponsiveness in this model. Collectively, these findings suggest that elevations in endogenous adenosine can directly impact lung inflammation and physiology in partially ADA-deficient mice. This model will prove useful in the study of specific mechanisms through which adenosine signaling regulates airway inflammation and physiology.

Materials and Methods

Transgenic mice

Male mice homozygous for the null Ada allele and carrying a placental-specific ADA minigene that allows for prenatal rescue were intercrossed with females heterozygous for the null Ada allele (22). Southern blot analysis of genomic DNA obtained from tails at weaning was used to determine the genotypes of the resulting progeny (21). Wild-type mice and mice heterozygous for the null Ada allele were used as controls. Mice were housed in contaminant-controlled environments to minimize pathogen exposure.

Histological analysis

Age-matched control and experimental animals were anesthetized and sacrificed. The lungs were perfused with 5–10 ml of PBS containing heparin and then infused with 0.5 ml of fixative (4% paraformaldehyde) and fixed overnight at 4°C. Fixed lungs were rinsed in PBS, dehydrated through graded ethanol washes, and embedded in paraffin. Sections (5 μm) were collected on slides and stained with H&E (Shandon Lipshaw, Pittsburgh, PA) according to the manufacturer’s instructions, or subjected to immunohistochemistry.

Airway physiology

Airway hyperresponsiveness was assessed by measuring the responsiveness to β-methylcholine (methylcholine) in conscious, unrestrained mice using a whole-body noninvasive plethysmograph (Buxco Electronics, Troy, NY) as described (23). This system estimates total pulmonary airflow in mice using a dimensionless parameter known as enhanced pause (Penh). Pressure differences were used to extrapolate Penh values, which are a function of the sum of the airflow in the upper and lower respiratory tracts during a respiratory cycle. This parameter has been shown to correlate with airway resistance measured by invasive techniques (23). Baseline Penh was determined by exposing mice to nebulized saline for 2 min and then recording and averaging Penh values for 3 min. The mice were then exposed to increasing concentrations of aerosolized methylcholine dissolved in saline. Methylcholine was aerosolized using an ultrasonic nebulizer, and the aerosol was drawn through the chamber at a constant rate for 2 min, after which Penh values were taken for 3 min and averaged. Methylcholine response curves for control animals were consistent with those shown previously for naive 129 mice (24).

Quantification of lung adenosine levels

Mice were anesthetized, the thoracic cavity was opened, and the lungs were rapidly removed and frozen in liquid nitrogen. Adenine nucleosides were extracted from frozen lungs using 0.4% perchloric acid as described (25), and adenosine was separated and quantified using reversed phase HPLC.

ADA enzyme therapy and determination of ADA enzymatic activity

Polyethylene glycol-modified ADA (PEG-ADA, Adagen) was kindly provided by Enzon (Piscataway, NJ). Mice were treated i.p. with 6.25 U of PEG-ADA (26, 27) (1 U defined as the amount necessary to convert 1 μM of adenosine to inosine per min at 25°C) immediately following initial airway physiology evaluation (day 1), and on days 3 and 6 following this. On day 7 airway physiology was measured again and the mice were then sacrificed to obtain blood, tissues, and bronchial alveolar lavage fluid (BALF). Levels of ADA in the tissues and blood were measured by zymogram analysis using procedures previously described (21, 27).

Theophylline treatments

Six-week-old control and partially ADA-deficient mice were given two i.p. injections, 3 h apart, of theophylline (10 mg/kg body weight) or PBS. Based on pharmacokinetic data (not shown), this dosage regimen maintains plasma theophylline levels at between 10 and 40 μM, which is sufficient to antagonize adenosine receptors but not to inhibit phosphodiesterases (28). Airway physiology was examined 1 h after the second injection of theophylline or PBS.

Bronchial alveolar lavage and cellular differentials

Mice were anesthetized and tracheally intubated with a blunt-ended 21-gauge needle. Lungs were lavaged with 1–2 ml of PBS and the recovered BALF was processed as previously described (21) for the determination of cellular differentials. Briefly, total cell counts were performed on initial lavaged aliquots and cellular differentials (200 cells/sample) were conducted on cell pellets resuspended in PBS, cytoplasmic stained with Diff-Quick (Dade Behring, Newark, DE).

Immunohistochemical localization of MCP-3

Paraffin-embedded tissues were sectioned (5 μm), exposed to two changes of histoclear, and rehydrated in a series of graded alcohols to water. Ag unmasking was performed before MCP-3 localization using target retrieval solution following the manufacturer’s guidelines (DAKO, Carpinteria, CA). Endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide for 5–10 min. MCP-3 immunohistochemistry and blocking procedures were followed according to the manufacturer’s guidelines using goat IgG VECTASTAIN Elite ABC kit (Vector Laboratories, Burlingame, CA). MCP-3 localization was performed by incubating slides for 30 min at room temperature with a 1:4 dilution of goat anti-mouse MCP-3 Ab (R&D Systems, Minneapolis, MN) as primary Ab. After incubation with appropriate biotinylated secondary Abs, the slides were incubated with avidin-biotinylated peroxidase complex (Vector Laboratories) for 30 min. The slides were developed using 3,3’-diaminobenzidine-tetrachloride (DAKO) for 7–10 min, dehydrated, and mounted.
Quantitative real-time RT-PCR

Quantitative real-time RT-PCR was performed using a 7700 Sequence Detector (Applied Biosystems, Foster City, CA) (29). Specific quantitative assays for the various adenosine receptors and MCP-3 were developed using Primer Express software (Applied Biosystems) following the recommended guidelines based on sequences from GenBank. The sequences of all oligonucleotides used are given in Table I. Total RNA was isolated from whole lung tissue using the TRIzol reagent from Life Technologies/BRL followed by DNase treatment to eliminate potential genomic DNA contamination. This was followed by cDNA synthesis and real-time PCR using established protocols (30). The resulting data were analyzed using sequence detector system software (Applied Biosystems) with TAMRA as the reference dye. The excitation of TAMRA was readily detected in blood and most tissues of wild-type mice, but was found only in the small and large intestine of ADA-deficient mice. These mice were therefore referred to as partially ADA-deficient mice.

Results

ADA enzymatic activity is found only in the distal gastrointestinal tract of partially ADA-deficient mice

ADA-deficient fetuses die prenatally due to the absence of ADA in their placentas (22). ADA-deficient fetuses were rescued from pre-natal lethality by the expression of an ADA minigene in their placentas (21, 22). With the loss of these placentas, these rescued pups were completely ADA-deficient and went on to die at 3 wk of age from severe pulmonary inflammation and damage that was linked to pronounced elevations in lung adenosine levels (21, 26). The severity of this phenotype and the early death of the mice made examination of the impact of adenosine on lung physiology difficult. We have recently identified an independent line of rescued ADA-deficient mice that were not completely ADA-deficient, but contained ectopic expression of the placental ADA minigene in the gastrointestinal tract (Fig. 1). The small and large intestines of these animals were the only tissues found to express the ADA minigene on this otherwise ADA-deficient background. Therefore, these animals were referred to as partially ADA-deficient mice. Unlike completely ADA-deficient mice, which die at 3 wk of age (21), partially ADA-deficient mice appeared relatively healthy unti the third month of life, when evidence of respiratory distress became evident. Partially ADA-deficient mice died between 4 and 5 mo of age from apparent respiratory distress. Therefore, expression of ADA in the gastrointestinal tract of otherwise ADA-deficient mice could extend the lifespan of these mice, making them amenable to the analysis of airway physiology.

Partially ADA-deficient mice develop severe lung inflammation and damage

Histological analysis of the lungs of partially ADA-deficient mice was conducted to characterize lung inflammation and damage in this model. At 6 wk of age the lungs of partially ADA-deficient mice appeared normal, except for the accumulation of macrophages in the alveolar spaces (compare Fig. 2, A and B). At 10 wk of age, partially ADA-deficient lungs exhibited severe lung inflammation, characterized by the presence of activated alveolar macrophages, perivascular and peribronchial accumulation of leukocytes, and pronounced alveolitis (Fig. 2C). By 12 wk of age, the lungs of partially ADA-deficient mice were severely inflamed and exhibited extensive bronchial plugging (Fig. 2D). Therefore, partially ADA-deficient mice develop progressive lung inflammation and damage, but at a much slower rate than do completely ADA-deficient mice.

![FIGURE 1. Distribution of ADA enzymatic activity in partially ADA-deficient mice. Various tissues were collected from 6-wk-old wild-type or ADA-deficient mice and equal amounts of protein (2 μg) were run on thin agarose gels followed by colorimetric detection of ADA enzyme activity. ADA enzymatic activity was readily detected in blood and most tissues of wild-type mice, but was found only in the small and large intestine of ADA-deficient mice. These mice are therefore referred to as partially ADA-deficient mice.](http://www.jimmunol.org/)

Table I. Primer pairs and internal probe sequences for murine adenosine receptors and MCP-3

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<th>Gene</th>
<th>Accession No.</th>
<th>Sequences</th>
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<td>β-Actin</td>
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<td>+1035-GCTCTGGCTCTTACCAACAT</td>
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<td></td>
<td>−1108-CCACGATCCACACAGATAC</td>
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<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt; receptor</td>
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<td>−814-ACACTTGTACACCGGGGCTCC</td>
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<td>+837-CCCTGTCTCTGGGCCCTACG</td>
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<td></td>
<td>+417FAM-CCCTGGGTCTTCGGCTTTGGCC</td>
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<tr>
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<td></td>
<td></td>
<td>−1170-GCAGGCTGAGAATAGGGTT</td>
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Adenosine levels are markedly elevated in the lungs of partially ADA-deficient mice

A major function of ADA is to control the levels of adenosine in tissues and cells. Hence, adenosine levels are commonly elevated in tissues of ADA-deficient humans and mice (21, 31). To determine the status of adenosine in the lungs of partially ADA-deficient mice, adenosine was quantified in whole lungs collected from control and partially ADA-deficient mice at 6 and 15 wk of age (Fig. 3A). At both stages, there was a marked increase in the levels of adenosine in the lungs of partially ADA-deficient mice. Furthermore, lung adenosine levels increased as the lung phenotype progressed. 2’-Deoxyadenosine, another substrate of ADA, was not detected in the lungs of control or partially ADA-deficient mice at any stage examined (data not shown). These findings demonstrated that adenosine levels were elevated in partially ADA-deficient mice, even at stages when there was not overt lung inflammation and damage.

Partially ADA-deficient mice exhibit increased basal Penh values and enhanced airway responsiveness that is reversed following ADA enzyme therapy

Exogenous adenosine has been shown to induce bronchoconstriction in asthmatics (17) and various animal models (32). To determine whether the endogenous accumulation of adenosine in the lungs was associated with alterations in airway physiology, airway hyperresponsiveness was measured in 6-wk-old partially ADA-deficient mice. The 6-wk time point was chosen because at this stage there was marked adenosine accumulation in the lungs (Fig. 3A) but relatively little lung inflammation and damage (Fig. 1B). Partially ADA-deficient mice exhibited an ~50% increase in baseline Penh over control animals (Fig. 4A). Furthermore, partially ADA-deficient mice demonstrated airway hyperresponsiveness as determined by a heightened responsiveness to methylcholine challenges (Fig. 4B). To confirm that these alterations in airway physiology were due to complications related to ADA deficiency, these parameters were examined in 6-wk-old partially ADA-deficient and control mice treated with ADA enzyme therapy (PEG-ADA treatment). PEG-ADA treatments had no effect on baseline Penh (Fig. 4A) or methylcholine responsiveness (Fig. 4B) in control mice. However, this enzyme therapy was able to reverse both the increase in baseline Penh (Fig. 4A) and the airway hyperresponsiveness (Fig. 4B) seen in untreated partially ADA-deficient mice. These findings demonstrated that the metabolic consequences associated with ADA-deficiency in this model lead to increased baseline Penh and airway hyperresponsiveness.

ADA enzyme therapy efficiently lowers adenosine levels in the lungs of partially ADA-deficient mice

In an attempt to correlate alterations in lung physiology with the metabolic disturbances seen in the lungs of partially ADA-deficient mice, adenosine levels were quantified in the lungs of 6-wk-old control and partially ADA-deficient mice treated with PEG-ADA. Results demonstrated that the ADA enzyme therapy was able to restore lung adenosine to control levels (Fig. 3B). These findings suggested that the increased baseline Penh and airway hyperresponsiveness seen were directly due to the accumulation of endogenous adenosine in the lungs of these animals.
Airway hyperresponsiveness in partially ADA-deficient mice is reversed following treatment with a nonselective adenosine receptor antagonist

The extracellular effects of adenosine are mediated through cell-surface adenosine receptors (1). To begin to determine whether the effects of elevated adenosine in the lungs of partially ADA-deficient mice were due to engagement of adenosine receptors, mice were treated with the broad-spectrum adenosine receptor antagonist theophylline. Six-week-old control or ADA-deficient mice were injected i.p. with either PBS or theophylline at a dosing regimen designed to maintain serum theophylline levels below 40 μM, a concentration favorable for adenosine receptor antagonism over phosphodiesterase inhibition (28). Theophylline treatment had no effect on baseline Penh (Fig. 5A) or methacholine responsiveness (Fig. 5B) in control mice, verifying a lack of effect of this dosage on phosphodiesterase inhibition that could potentially lead to bronchodilation. Theophylline treatment did not lower the increased baseline Penh seen in partially ADA-deficient mice (Fig. 5A); however, the airway hyperresponsiveness seen in these mice was reversed following theophylline treatment (Fig. 5B). These findings suggested that signaling through adenosine receptors was responsible for the adenosine-dependent airway hyperresponsiveness seen in partially ADA-deficient mice.

Adenosine receptor transcript levels are elevated in the lungs of partially ADA-deficient mice

Mammals are known to possess four adenosine receptors, the A₁, A₂A, A₂B, and A₃ adenosine receptors (1). Although these receptors have a wide tissue distribution, little attention has been given to the relative expression levels of these receptors in the lung. To quantify the expression of adenosine receptors in the lung, specific primers and probes were developed to conduct quantitative real-time RT-PCR (Table I). Adenosine receptor transcript levels were quantified in total cellular RNA extracts isolated from whole lungs of 6-wk-old control or partially ADA-deficient mice as well as control and ADA-deficient mice treated for 1 wk with PEG-ADA. Baseline Penh was increased in ADA-deficient mice, and treatment with PEG-ADA reversed baseline Penh values to control levels. Baseline Penh was monitored in control and ADA-deficient mice and mice treated with PEG-ADA for 1 wk with PEG-ADA. Baseline Penh was increased in ADA-deficient mice, and treatment with PEG-ADA reversed this airway hyperresponsiveness. Notably, Penh values in response to β-methacholine were significantly elevated in partially ADA-deficient mice, suggesting an increase in airway hyperresponsiveness. Treatment of partially ADA-deficient mice for 1 wk with PEG-ADA reversed this airway hyperresponsiveness. Values are given as mean percentage increase in Penh ± SEM. Value of n = 5-10 mice per group; *, significant difference at p ≤ 0.05 using Student’s t test.
examined at 15 wk of age to determine whether increases in adenosine receptor transcript levels were progressive in the lungs of partially ADA-deficient mice. Transcript levels of the $A_{2A}$ receptor did not change from 6 to 15 wk in partially ADA-deficient lungs (data not shown). However, 6-wk transcript levels for the $A_{1}$ (0.8), $A_{2B}$ (0.48), and $A_{3}$ (0.15%) receptors in partially ADA-deficient lungs increased to 1.72, 0.7, and 2.9% of $\beta$-actin, respectively, in 15-wk-old partially ADA-deficient lungs. These findings demonstrated that all of the adenosine receptors were expressed in the mouse lung and that receptor transcripts were elevated in the lungs of partially ADA-deficient mice, suggesting the potential for increased adenosine receptor signaling in this adenosine-rich environment.

Alveolar macrophages but not eosinophils are elevated in 6-wk-old ADA-deficient mice

Airway hyperresponsiveness has been associated with airway inflammation in humans and in various animal models (33). Histological examination demonstrated that there was an increase in alveolar macrophages in the lungs of partially ADA-deficient lungs at 6 wk of age (Fig. 2B). To more accurately characterize the inflammatory changes seen in these mice the cellularity of BALF was examined (Fig. 7). There was a >6-fold increase in the number of alveolar macrophages in BALF collected from partially ADA-deficient mice. This increase was reduced by 50% following PEG-ADA treatment. A significant increase in lymphocytes was also seen, but this increase was not altered by PEG-ADA treatment. Interestingly, there was no increase in neutrophils or eosinophils in the BALF of partially ADA-deficient mice at this stage. These findings suggested that the elevation in alveolar macrophages may be related to increases in lung adenosine levels.

MCP-3 expression is elevated in the bronchial epithelium of partially ADA-deficient mice

MCP-3 is a C-C chemokine that has been implicated in the regulation of lung inflammation (34). We have recently shown that expression of MCP-3 is closely linked to elevated adenosine levels in completely ADA-deficient mice, suggesting adenosine signaling may regulate the expression of this chemokine (35). In an attempt to determine whether increased MCP-3 expression was also associated with increased lung adenosine levels in partially ADA-deficient mice, we examined the expression of MCP-3 in the lungs of these mice using real-time RT-PCR (Fig. 8). Transcripts for MCP-3 were detected in total cellular RNA isolated from control lungs. There was a 5-fold increase in MCP-3 transcript levels in the lungs of partially ADA-deficient mice, and this increase was reversed following PEG-ADA treatment. MCP-3 immunoreactivity was localized to the bronchial epithelium (Fig. 9). Furthermore, the immunoreactivity was more intense in the bronchial epithelium of partially ADA-deficient mice (Fig. 9B) as compared with the immunoreactivity seen in the bronchial epithelium of control mice (Fig. 9A), or control mice treated with PEG-ADA (Fig. 9C), or partially ADA-deficient mice treated with PEG-ADA (Fig. 9D). These findings suggested that the elevated levels of adenosine in the lungs of partially ADA-deficient mice regulate the expression of MCP-3 in the bronchial epithelium.

Discussion

Elevations of the signaling nucleoside adenosine are seen in the lungs of asthmatics (9). However, the effects of adenosine on lung inflammation and physiology that have been examined in humans (17) and animal models (32) have involved challenges with exogenous adenosine or adenosine receptor agonists. Therefore, the effects of endogenously elevated adenosine on lung inflammation and physiology are not known. ADA is a purine catabolic enzyme that is responsible for controlling the levels of adenosine in tissues and cells, and hence ADA-deficient mice exhibit elevations in adenosine levels in many tissues including the lung (21). The ability to control adenosine levels in the lungs of ADA-deficient mice using a combination of genetic and biochemical manipulations has allowed us to assess the impact of endogenous adenosine on lung inflammation and physiology. At 6 wk of age there was a 20-fold increase in the levels of adenosine in the lungs of partially ADA-deficient mice. This increase was associated with elevations in alveolar macrophages and alterations in lung physiology, namely an increase in baseline Penh and airway hyperresponsiveness. These changes were found to be dependent on elevated adenosine levels in that lowering lung adenosine using ADA enzyme therapy was able to decrease macrophage accumulation and reverse increases in baseline Penh and airway hyperresponsiveness. Acute treatment with the nonselective adenosine receptor antagonist theophylline prevented airway hyperresponsiveness, indicating the involvement of adenosine receptors in this process. Thus, endogenous adenosine elevations are associated with specific alterations...
FIGURE 6. Adenosine receptor transcript levels in partially ADA-deficient lungs. Total cellular RNA was isolated from whole lungs of 6-wk-old control and ADA-deficient mice, and mice treated with PEG-ADA, as described in Materials and Methods. Real-time RT-PCR was used to quantify the transcript levels of each of the four adenosine receptors using specific primers and probes (see Table I). Values are normalized to β-actin transcript levels and are presented as the mean percentage of β-actin transcripts ± SEM. A, A₁; B, A₂A; C, A₂B; and D, A₃ adenosine receptor transcripts. Value of n = 5 samples per group; *, significant difference at p ≤ 0.05, using Student’s t test.

FIGURE 7. Total BALF cellularity in 6-wk-old partially ADA-deficient mice and mice treated with partial ADA enzyme therapy. BALF was collected from 6-wk-old control and ADA-deficient mice and mice treated with PEG-ADA. Cells were cytospun onto slides, stained, and counted. Values are given as mean total cells ± SEM. Value of n = 5–10 samples per group; *, significant difference at p ≤ 0.05 using Student’s t test.

FIGURE 8. Lung MCP-3 transcript levels in partially ADA-deficient lungs. Total cellular RNA was extracted from 6-wk-old control or partially ADA-deficient mice and mice treated with PEG-ADA. Real-time RT-PCR was used to quantify MCP-3 transcript levels using specific primers and probes (see Table I). Values are normalized to β-actin transcript levels and are presented as the mean percentage of β-actin transcripts ± SEM. The value of n = 5 samples per group; *, significant difference at p ≤ 0.05 using Student’s t test.

in lung inflammation and physiology that are likely mediated through adenosine receptors.

Adenosine mediates its effects by engaging G protein-coupled receptors on the surface of target cells (1). We found that all four of the adenosine receptors were expressed in the normal mouse lung, and that transcript levels for the A₁, A₂B, and A₃ adenosine receptors were elevated in partially ADA-deficient lungs. The greatest relative increase was seen for the A₃ receptor. Interestingly, transcript levels for this receptor have also been shown to be elevated in the lungs of humans suffering from lung inflammation (10), where expression was localized to eosinophils. At the stage when receptor transcript levels were examined, there was not an increase in eosinophils in the lung. However, there was an increase in alveolar macrophages, on which A₃ receptor activity has also been demonstrated (15, 36). Consistent with this, A₃ receptor transcripts and the number of alveolar macrophages in the lungs of partially ADA-deficient mice decreased following ADA enzyme therapy. These data suggest that the increase in A₃ transcript levels...
seen in 6-wk-old partially ADA-deficient mice may be accounted for by increased inflammation. This is supported by the observation that A₃ receptor transcripts are even higher in the lungs of 15-wk-old partially ADA-deficient mice that exhibit heightened inflammation. Additional studies are needed to localize the expression of the A₃ receptor in these mice to appreciate the functional significance of altered A₃ transcript levels in this model.

Elevation in A₁ and A₂B adenosine receptor transcripts in inflamed lungs was a novel observation and raises the question as to whether or not these increases are directly associated with the changes in lung physiology seen in partially ADA-deficient mice. In support of this possibility, acute treatments with theophylline were able to reverse the adenosine-dependent effects on airway hyperresponsiveness. Theophylline is a broad-spectrum adenosine receptor antagonist that has activity at the murine A₁, A₂A, and A₂B receptor, with little activity at the murine A₃ receptor (8). Therefore, engagement of the A₁, A₂A, or A₂B adenosine receptors may be responsible for the airway hyperresponsiveness seen. Whether the effect of receptor engagement in this model is a direct effect on airway smooth muscle or a secondary effect due to activation of other mediator cells (e.g., mast cells, nerves, airway epithelium, macrophages) cannot be determined from these studies. However, there is evidence that the A₂B adenosine receptor is expressed in human airway smooth muscle cells (37), and the A₁ adenosine receptor has been implicated in direct effects of adenosine on airway smooth muscle in rabbit models of asthma (38, 39). Clearly, determining the cell-specific expression of the adenosine receptors at both the message and protein level in the normal and inflamed lung will be paramount to understanding these issues. Furthermore, because the mechanisms of exogenous adenosine on bronchoconstriction differ depending on the species examined (32), it will be necessary to correlate our findings with adenosine receptor expression profiles in inflamed human lungs before attempting to design adenosine-based therapeutics.

MCP-3 belongs to a family of C-C chemokines that has attracted recent attention by its diverse role in lung inflammation (34, 40). MCP-3 was originally identified from cytokine-stimulated osteosarcoma cells by its ability to induce monocyte migration in vitro (41). Through its interactions with CCR-1 and CCR-3, MCP-3 functions as a potent monocyte and eosinophil chemoattractant (42, 43). We found that MCP-3 expression was elevated in the bronchial epithelium of partially ADA-deficient mice and that this elevation was reversed following ADA enzyme therapy. In addition, MCP-1, a closely related member of the C-C chemokine family, was also elevated in the bronchial epithelium of partially ADA-deficient mice (J. L. Chunn and M. R. Blackburn, unpublished data). These findings suggest that the regulation of chemokines may play an important role in the regulation of leukocyte accumulation in the lungs of these animals. In addition, these findings suggest that adenosine signaling may regulate the expression of MCP-3. Both the transcriptional and posttranscriptional regulation of MCP-3 expression may be mediated by cAMP signaling. The promoter region of the MCP-3 gene contains a cAMP response element (44) that may regulate transcription, and the 3′ untranslated region contains an adenosine-uridine-rich element (45), which may mediate mRNA degradation (46). Because adenosine can regulate cAMP levels by engaging its receptors (2), one can speculate that increased MCP-3 expression in the airways of partially ADA-deficient mice may be due to the elevated adenosine levels seen. Localizing adenosine receptor expression to the airways of ADA-deficient lungs in association with MCP-3 will help to strengthen this hypothesis.

FIGURE 9. Localization of MCP-3 protein in partially ADA-deficient lungs. Lungs from 6-wk-old control or partially ADA-deficient mice, or mice treated with PEG-ADA, were reacted with a polyclonal Ab to murine MCP-3 followed by peroxidase detection. A, Control lung. B, Lung from a partially ADA-deficient mouse. C, Lung from a control mouse treated with PEG-ADA. D, Lung from a partially ADA-deficient mouse treated with PEG-ADA. Arrows denote bronchial airway epithelium. Scale bar in D = 50 μm for all panels.
There is an outstanding literature base to suggest that adenosine plays a role in the regulation of inflammatory lung diseases such as asthma. In our model of partial ADA deficiency we have characterized specific changes in lung inflammation and physiology that are dependent on elevated lung adenosine levels. This model will prove useful for examining the specific mechanisms involved in these adenosine-dependent changes. As the specific expression patterns for the various adenosine receptors are elucidated, their function can be tested using specific pharmacologic or genetic approaches. For example, selective adenosine receptor antagonists can be used in this model in a similar manner as was the broad-spectrum adenosine receptor antagonist theophylline. In so doing, relevant targets for adenosine antagonism can be identified. In addition, mice deficient in the various adenosine receptors can be crossed onto the partially ADA-deficient background to assess the function of the various receptors in an adenosine-rich environment. The reversal of lung inflammation and airway hyperresponsiveness in association with lowering lung adenosine levels suggests that there may be an eventual therapeutic benefit to lowering the elevated levels of adenosine seen in the lungs of asthmatics by the use of ADA enzyme therapy. Continued work addressing this issue in ADA-deficient mouse models as well as other models of experimental asthma will be necessary before such ideas can be pursued.

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