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Identification of A_3 Receptor- and Mast Cell-Dependent and -Independent Components of Adenosine-Mediated Airway Responsiveness in Mice[1]

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Adenosine-induced bronchoconstriction is a well-recognized feature of atopic asthma. Adenosine acts through four different G protein-coupled receptors to produce a myriad of physiological effects. To examine the contribution of the A_3 adenosine receptor to adenosine-induced bronchoconstriction and to assess the contribution of mast cells to this process, we quantified airway responsiveness to aerosolized adenosine in wild-type, A_3 receptor-deficient, and mast cell-deficient mice. Compared with the robust airway responses elicited by adenosine in wild-type mice, both A_3-deficient and mast cell-deficient mice exhibited a significantly attenuated response compared with their respective wild-type controls. Histological examination of the airways 4 h after adenosine exposure revealed extensive degranulation of airway mast cells as well as infiltration of neutrophils in wild-type mice, whereas these findings were much diminished in A_3-deficient mice and were not different from those in PBS-treated controls. These data indicate that the airway responses to aerosolized adenosine in mice occur largely through A_3 receptor activation and that mast cells contribute significantly to these responses, but that activation of additional adenosine receptors on a cell type(s) other than mast cells also contributes to adenosine-induced airway responsiveness in mice. Finally, our findings indicate that adenosine exposure can result in A_3-dependent airway inflammation, as reflected in neutrophil recruitment, as well as alterations in airway function. *The Journal of Immunology,* 2003, 170: 331–337.

The endogenous nucleoside adenosine can influence many physiological processes, in most cases by activating G protein-coupled receptors on target cells (1). Adenosine can be released by many cell types, including mast cells, and also can be generated extracellularly from ATP (2, 3). Several lines of evidence suggest that adenosine may contribute to the pathophysiology of a variety of diseases, including myocardial infarction, cancer, asthma, and chronic obstructive pulmonary disease (4–7).

The potential role of adenosine in asthma and the mechanisms by which it might influence airway function in this disorder have been studied intensively (6, 8). In 1983 it was recognized that aerosolized adenosine produced bronchoconstriction in asthmatics, but not in normal volunteers (9). Moreover, airway responsiveness to adenosine has been shown to reflect the degree of underlying airway inflammation in a more sensitive fashion than does responsiveness to other agents that can induce nonspecific airway hyperreactivity in subjects with asthma, such as methacholine (10). In addition to its capacity to produce bronchoconstriction, adenosine has been shown to amplify the inflammatory response in some animal models of asthma following sensitization and challenge (11, 12). In genetically engineered mice with elevated levels of adenosine due to adenosine deaminase (ADA)[3] deficiency, eosinophilic lung inflammation and mucus hypersecretion develop, and these animals die from respiratory failure at 3 wk of age (13). These studies coupled with the observation that adenosine levels are elevated in the lungs of subjects with asthma suggest that endogenously produced adenosine may contribute to the pathophysiology of this disorder (14).

Studies in both humans and animals have suggested that adenosine-induced bronchoconstriction can occur indirectly, in part through mediator release from mast cells (15). However, the relative contributions of the proposed mast cell-dependent and mast cell-independent components of adenosine-induced bronchoconstriction differ among species (16). For example, rabbits are thought to have a large mast cell-independent component, reflecting direct actions of adenosine on airway smooth muscle (ASM) (17), whereas findings in humans (18, 19) and rats (20, 21) indicate that these species may have a large mast cell-dependent component to adenosine responsiveness.

There is also uncertainty about which receptors account for adenosine's effects on airway function. Adenosine can act through any of four distinct G protein-coupled receptors, and various studies have implicated three of these four receptors (A_1, A_{2b}, and A_3) in adenosine-induced bronchoconstriction. While studies using pharmacological reagents and antisense DNA suggest that the A_1 receptor is responsible for the mast cell-independent component of adenosine-induced bronchoconstriction (17, 22–24), there has been considerable controversy about the receptor(s) involved in adenosine-induced mast cell degranulation. For example, in vitro

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[3] Abbreviations used in this paper: ADA, adenosine deaminase; ASM, airway smooth muscle.

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pharmacological studies employing a human mast cell line and a dog mastocytoma line have supported a role for the A_{2b} receptor in adenosine-induced mast cell activation (25–27), whereas a number of in vitro and in vivo studies with rodents suggest that the A_3 receptor is involved (28, 29). Several explanations may account for these discrepancies, including incomplete selectivity of the adenosine analogs used, different signaling pathways among mast cells from various tissues (or that have been generated in vitro using different methods), and species differences. However, it remains unclear to what extent the A_{2b} and A_3 adenosine receptors contribute to adenosine-induced mast cell degranulation in asthmatics.

A useful approach for assigning specific physiological roles to particular receptors has been to examine responses to adenosine in mouse lines lacking each adenosine receptor. We previously used mice lacking the A_3 receptor to identify an important role for that receptor in adenosine-induced degranulation of skin mast cells in vivo and in the ensuing adenosine-induced and mast cell-dependent enhancement of cutaneous vascular permeability (30). Here we use wild-type, A_3-deficient, and mast cell-deficient mice to assess the importance of the A_3 receptor and mast cells in airway responsiveness to adenosine.

Materials and Methods

Experimental animals

All studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals as well as the institutional animal care and use committee guidelines of University of North Carolina and Stanford University. Mast cell-deficient mice (WBB6F$_1$/J-*Kit*^{W}/*Kit*$^{W\text{-}v}$; 8–12 wk old), and their congenic normal littermates (WBB6F$_1$/J-*Kit*$^{+/+}$) were purchased from The Jackson Laboratory (Bar Harbor, ME). Compared with the congenic wild-type mice, adult WBB6F$_1$/J-*Kit*^{W}/*Kit*$^{W\text{-}v}$ contain <1.0% of the number of skin mast cells and essentially no detectable mature mast cells in the respiratory system (trachea, bronchi, and lungs) and many other anatomical sites (31). Mice deficient in the A_3 adenosine receptor ($A_3^{-/-}$) were generated as previously described (32). The A_3 null mutation was backcrossed six generations onto the C57BL/6 background, and $A_3^{-/-}$ and $A_3^{+/+}$ controls were obtained by intercrossing $A_3^{+/-}$ heterozygotes. All animals were genotyped by Southern blot analysis as described previously and were used between 5–9 mo of age (32). All experimental animals were matched for gender and age within each experiment.

Measurement of airway responsiveness in conscious mice

Mice were placed in a whole body plethysmograph (Buxco Electronics, Troy, NY), and baseline measurements of Penh were obtained. Penh is a dimensionless index calculated from inspiratory pressures, expiratory pressures, and expiratory time and has been shown to correlate with direct measures of pulmonary resistance in mechanically ventilated animals (33). Penh was then measured in response to aerosols of PBS vehicle or adenosine.

Histological evaluation of mast cell degranulation and airway inflammation

Four hours following exposure to 5 min of PBS or adenosine (6 mg/ml) aerosol in the whole body plethysmograph, mice were euthanized with an overdose of sodium pentobarbital (150 mg/kg). Lungs were inflated with Karnovsky's-2 fixative (2% paraformaldehyde, 2.5% glutaraldehyde, 0.1 M sodium cacodylate buffer, and 0.025% $CaCl_2$) (34, 35), the trachea was tied off with suture, and the trachea and lungs were removed en bloc and immediately placed in Karnovsky's-2 fixative (20/1, v/v) at room temperature for 4–6 h. Samples were then placed at 4°C for 1 h before being transferred into 0.1 M sodium cacodylate buffer and returned to 4°C until processing. Samples were shipped on wet ice to Stanford University, where they were processed into 1-μm, Epon-embedded, Giemsa-stained sections (34–36). Sections taken through the trachea were coded so that the observer was not aware of the identity of the individual specimens and were examined at ×1000 by light microscope. Mast cells were classified as extensively degranulated (>50% of the cytoplasmic granules exhibiting fusion, staining alternations, and/or extrusion from the cell), moderately degranulated (10–50% of the granules exhibiting fusion or discharge), or normal (<10% of the granules exhibiting alterations). The numbers of neutrophils infiltrating in the trachea (in the interstitium, in the epithelium, or near the surface of the epithelium in the lumen) were also quantified and expressed as neutrophils per square millimeter of the trachea (34–36).

Statistical analysis

Data are presented as the mean ± SEM. Statistical significance was assessed by ANOVA for adenosine dose-response comparison between WBB6F$_1$/J-*Kit*^{W}/*Kit*$^{W\text{-}v}$ and WBB6F$_1$/J-*Kit*$^{+/+}$ mice and for comparisons of the responses of various groups of mice to PBS or adenosine over time. Student's *t* test was used for all other analyses.

Results

Adenosine-induced airway responsiveness is diminished in mast cell-deficient mice

To test the contribution of mast cells to adenosine-induced airway responsiveness in mice, we exposed mast cell-deficient (WBB6F$_1$/J-*Kit*^{W}/*Kit*$^{W\text{-}v}$) mice and their congenic normal littermates (WBB6F$_1$/J-*Kit*$^{+/+}$ mice) to aerosolized adenosine and measured airway responsiveness by whole body plethysmography. As shown in Fig. 1*A*, responsiveness to various concentrations of adenosine was observed in both groups of animals, but the response was significantly attenuated (by ~50%) in mast cell-deficient animals

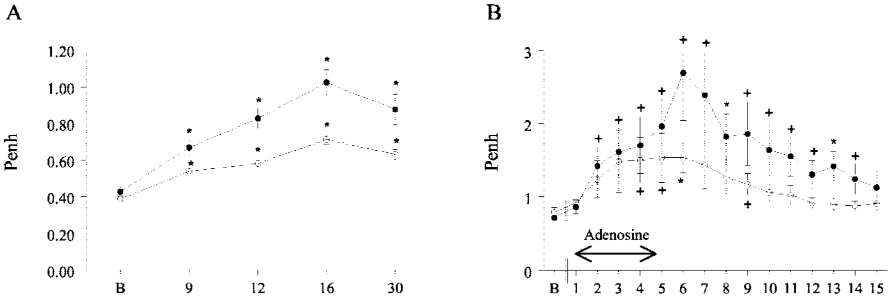

FIGURE 1. Reduced airway responsiveness to adenosine in mast cell-deficient mice. *A*, Mice were exposed to adenosine aerosol for 3 min at each indicated concentration, and Penh was recorded over a 10-min period from the start of each aerosolization. Data represent the mean Penh over each 10-min period ± SEM. ○, mast cell-deficient mice (WBB6F$_1$/J-*Kit*^{W}/*Kit*$^{W\text{-}v}$); ●, wild-type controls (WBB6F$_1$/J-*Kit*$^{+/+}$; $n = 18$/group); B, baseline. *, $p < 0.01$ (vs baseline value for mice of the same genotype). The dose response of *Kit*^{W}/*Kit*$^{W\text{-}v}$ mice was significantly different ($p < 0.001$) from that of wild-type mice by ANOVA. *B*, Airway function (Penh) was measured before, during, and after a 5-min aerosolization of adenosine at 6 mg/ml. ○, WBB6F$_1$/J-*Kit*^{W}/*Kit*$^{W\text{-}v}$ mice; ●, WBB6F$_1$/J-*Kit*$^{+/+}$ mice ($n = 8$/group). Data represent the mean Penh over a 5-min baseline period (B), and then mean Penh over each minute during a 5-min adenosine aerosol challenge and following the challenge. +, $p < 0.05$; *, $p < 0.01$ (vs baseline value for mice of the same genotype). The response of *Kit*^{W}/*Kit*$^{W\text{-}v}$ mice from the beginning of adenosine aerosolization through the 15th min was significantly different ($p < 0.001$) from that of the wild-type mice by ANOVA.

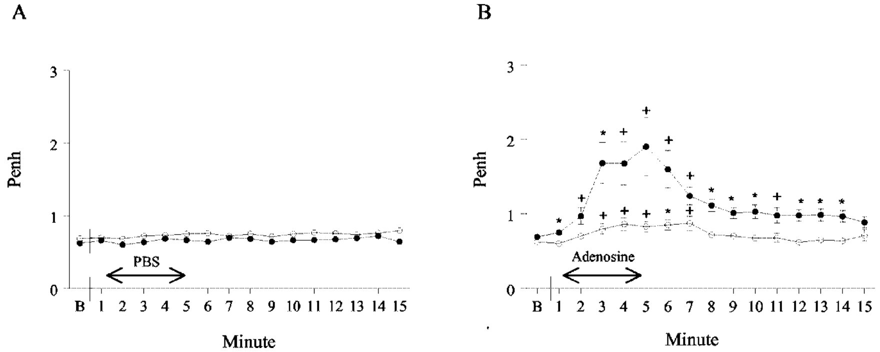

FIGURE 2. Reduced airway responsiveness to adenosine in $A_3^{-/-}$ mice. Airway function (Penh) was measured before, during, and after a 5-min aerosolization of PBS (*A*) or adenosine at 6 mg/ml (*B*). ○, $A_3^{-/-}$ mice; ●, $A_3^{+/+}$ mice ($n = 14$/group). Data represent mean Penh over a 5-min baseline period (B), and then mean Penh over each minute during a 5-min PBS or adenosine aerosol challenge and following the challenge. +, $p < 0.05$; *, $p < 0.01$ (vs baseline value for mice of the same genotype). The response of $A_3^{-/-}$ mice from the beginning of adenosine aerosolization through the 15th min was significantly different ($p < 0.001$) from that of the wild-type mice by ANOVA.

($p = 0.0007$). When the response to 5-min exposure to a single concentration of adenosine (6 mg/ml) was examined (Fig. 1*B*), similar results were obtained. Also, we noted that the adenosine-induced enhancement of Penh rapidly diminished after discontinuation of the aerosol challenge with this agent (Fig. 1*B*). These findings show that there are both mast cell-dependent and mast cell-independent components to adenosine-induced airway responsiveness in $WBB6F_1$/J mice.

$A_3^{-/-}$ mice exhibit attenuated adenosine-induced airway responsiveness

To test the contribution of the A_3 receptor to adenosine-induced airway responsiveness, we evaluated the responses of wild-type and $A_3^{-/-}$ mice to 5-min aerosols of PBS or adenosine (6 mg/ml). PBS produced no changes in airway responsiveness in $A_3^{+/+}$ ($p = 0.11–0.71$) or $A_3^{-/-}$ ($p = 0.22–0.94$) mice compared with baseline measurements (Fig. 2*A*). Adenosine aerosolization resulted in significant increases in Penh in $A_3^{+/+}$ mice, and this response was markedly attenuated in $A_3^{-/-}$ animals (Fig. 2*B*). However, a statistically significant rise in Penh above baseline was still observed in $A_3^{-/-}$ mice ($p < 0.01$) in response to adenosine, suggesting the presence of an A_3-independent component to the response (Fig. 2*B*).

Adenosine-induced mast cell degranulation and neutrophil recruitment are A_3 dependent

To assess the extent to which the observed airway responsiveness to adenosine was associated with activation of mast cells, we looked for histological evidence of mast cell activation in the same animals that had been studied physiologically. Four hours following adenosine or PBS exposure, lung tissue was obtained from all animals to quantify mast cell degranulation and neutrophil accumulation (as additional evidence of mast cell activation) (36). In wild-type mice, significantly more degranulated mast cells were observed in the adenosine- vs PBS-treated group (40.5 ± 4.3 vs 16.1 ± 3.7%; $p = 0.00005$; Figs. 3*A* and 4*A* vs Fig. 4*B*). By contrast, the percentage of degranulated mast cells in adenosine-exposed $A_3^{-/-}$ mice was no different from that in PBS-treated $A_3^{-/-}$ animals (17.8 ± 3.4 vs 16.8 ± 3.1%; Figs. 3*A* and 4*C* vs Fig. 4*D*). The numbers of mast cells present in the tracheae were not significantly different between groups: adenosine-treated $A_3^{+/+}$, 16.1 ± 2.1/mm^2; PBS-treated $A_3^{+/+}$, 18.2 ± 2.6/mm^2; adenosine-treated $A_3^{-/-}$, 19.8 ± 2.6/mm^2; and PBS-treated $A_3^{-/-}$, 20.2 ± 1.9/mm^2.

Since mast cell degranulation typically results in inflammatory cell influx due to the release by mast cells of diverse proinflammatory mediators (36–38), we quantified neutrophil accumulation in the airways 4 h after adenosine exposure. As shown in Figs. 3*B* and 5, $A_3^{+/+}$ mice treated with aerosolized adenosine exhibited more neutrophils (polymorphonuclear leukocytes) in the trachea than did PBS-treated, $A_3^{+/+}$, wild-type mice (74.5 ± 13.4 vs 33.9 ± 6.9/mm^2; $p = 0.01$) or adenosine-treated $A_3^{-/-}$ mice (74.5 ± 13.4 vs 25.2 ± 4.4/mm^2; $p = 0.002$). No significant difference in neutrophil numbers was observed between adenosine- and PBS treated $A_3^{-/-}$ mice (25.2 ± 4.4 vs 29.3 ± 3.2/mm^2). At 4 h after adenosine treatment, neutrophils were observed in $A_3^{+/+}$ mice in blood vessel lumens (in close proximity to the vascular endothelium), in the interstitial tissue of the trachea, within the tracheal epithelium, and in the tracheal lumen, in close proximity to the epithelial surface (Fig. 5, *A* and *B*). We further examined the tissue for any other changes associated with the degranulation of the mast cell and neutrophil recruitment. No difference in the composition or integrity of the epithelia was observed by simple histological analysis, nor were changes in the numbers or characteristics of other populations of leukocytes apparent.

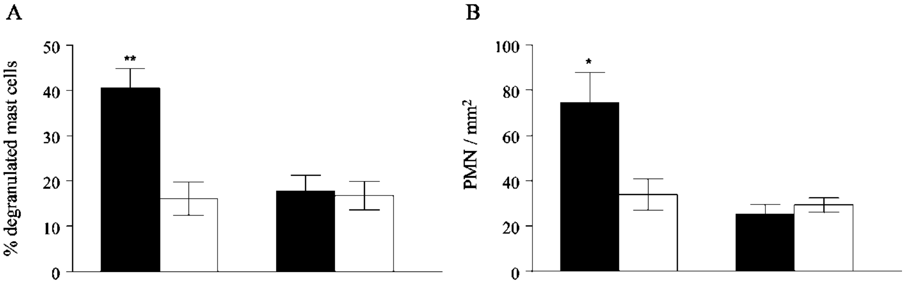

FIGURE 3. Percentage of degranulated mast cells (*A*) and number of neutrophils (*B*) in the tracheae of $A_3^{+/+}$ and $A_3^{-/-}$ mice 4 h after a 5-min exposure to aerosolized adenosine at 6 mg/ml (■) or PBS (□). Data represent the mean number of extensively degranulated mast cells (*A*) or the mean number of neutrophils (PMN, polymorphonuclear leukocytes) per square millimeter; *B*) ± SEM ($n = 14$/group). *, $p \leq 0.01$; **, $p < 0.001$ (vs either PBS-challenged mice of the same genotype or adenosine-challenged mice of the other genotype).

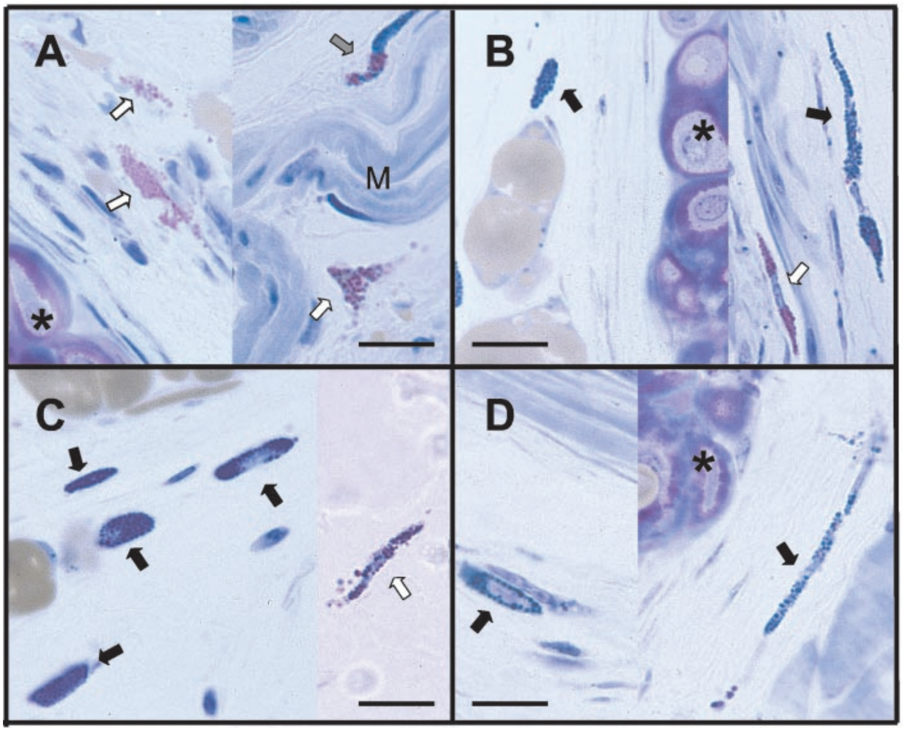

FIGURE 4. Adenosine induces increased degranulation of mast cells in the trachea of $A_3^{+/+}$ vs $A_3^{-/-}$ mice. Mast cells in the trachea of $A_3^{+/+}$ mice (*A* and *B*) or $A_3^{-/-}$ mice (*C* and *D*) 4 h after a 5-min exposure to aerosolized adenosine or PBS. Many mast cells that exhibit extensive or moderate degranulation (indicated by open arrows or gray arrow, respectively) are present in specimens from $A_3^{+/+}$ mice treated with adenosine (*A*), whereas $A_3^{+/+}$ mice treated with PBS (*B*) and $A_3^{-/-}$ mice treated with either adenosine (*C*) or PBS (*D*) exhibit many mast cells with no sign of degranulation (solid arrows) as well as occasional degranulated mast cells (open arrows). $*$, tracheal cartilage; M, tracheal muscle. *A–D* are photomicrographs of 1 μM, Epon-embedded, Giemsa-stained sections (magnification, ×1000). Scale bars in *A–D* = 20 mm.

These data show that adenosine-specific degranulation of airway mast cells and adenosine-specific neutrophil infiltration at sites of mast cell degranulation require expression of the A_3 receptor. These findings thus support the hypothesis that the mast cell-dependent component of adenosine-induced airway responsiveness and the associated neutrophil infiltration occur in mice through activation of A_3 receptors on mast cells.

Adenosine-induced neutrophil recruitment is mast cell dependent

To confirm that neutrophil recruitment was the result of mast cell activation by adenosine, we exposed mast cell-deficient (WBB6F_1/J-$\mathit{Kit}^{W}/\mathit{Kit}^{W\text{-}v}$) mice and their congenic normal littermates (WBB6F_1/J-$\mathit{Kit}^{+/+}$ mice) to aerosolized adenosine (6 mg/ml) for 5 min and examined neutrophil recruitment to the lung 4 h postexposure. As shown in Fig. 6, more neutrophils were observed in the tracheae of adenosine-treated, wild-type than in adenosine-treated, mast cell-deficient mice (69.67 ± 7.6 vs 38.41 ± 5.32; $p = 0.0044$)). No differences were observed among wild-type, PBS-treated; mast cell-deficient, PBS-treated; and mast cell-deficient, adenosine-treated groups (32.05 ± 4.23 vs 35.82 ± 6.36 vs 38.41 ± 5.32). These findings show that adenosine-induced neutrophil recruitment to the lung is mast cell dependent and are consistent with a

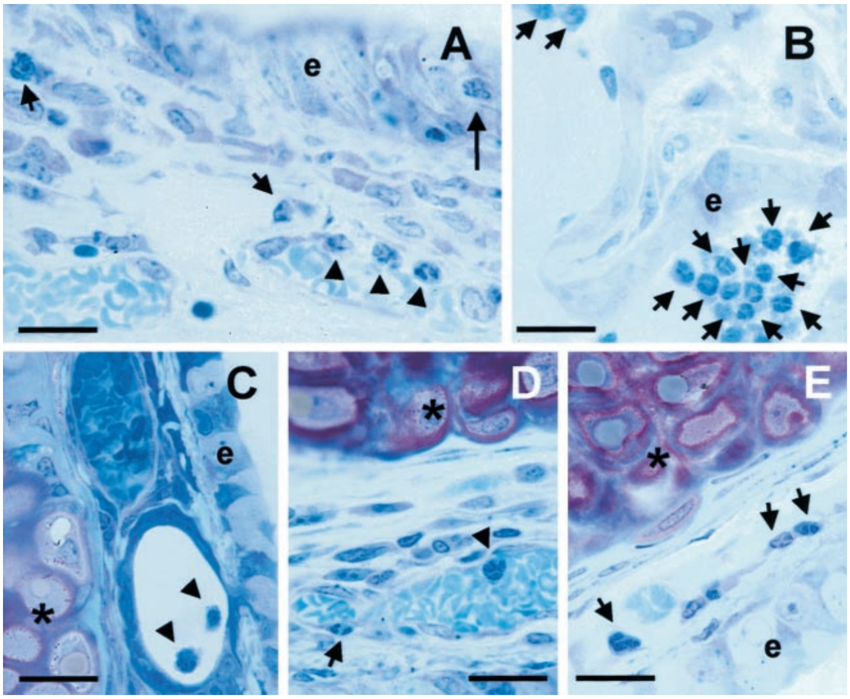

FIGURE 5. Adenosine induces enhanced neutrophil recruitment in the trachea of $A_3^{+/+}$ vs $A_3^{-/-}$ mice. Sections of trachea of $A_3^{+/+}$ mice or $A_3^{-/-}$ mice 4 h after exposure to aerosolized adenosine or PBS. *A* and *B*, $A_3^{+/+}$ mice treated with adenosine exhibit neutrophils, in *A*, near the endothelial cells in the lumen of a small blood vessel (arrowheads), in the interstitium (short arrows) and within the epithelium (long arrow) and, in *B*, near the lumenal surface of the epithelium (short arrows). A few intravascular (arrowheads) or interstitial (short arrows) neutrophils are also present in specimens from $A_3^{+/+}$ mice treated with PBS (*C*), $A_3^{-/-}$ mice treated with adenosine (*D*) and $A_3^{-/-}$ mice treated with PBS (*E*) are shown. e, tracheal epithelium; $*$, tracheal cartilage. *A–E* are photomicrographs of 1-μM, Epon-embedded, Giemsa-stained sections (magnification, ×1000). Scale bars in *A–E* = 20 mm.

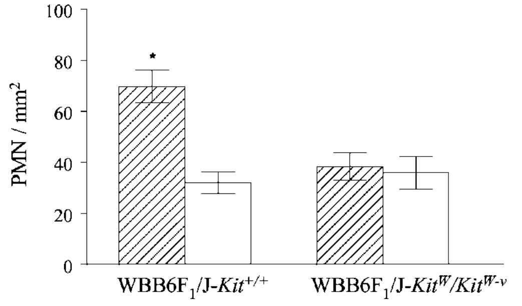

FIGURE 6. Adenosine-mediated neutrophil recruitment is mast cell dependent. The number of neutrophils in the tracheae of wild-type (WBB6F$_1$/J-*Kit*$^{+/+}$) and mast cell-deficient (WBB6F$_1$/J-*Kit*^{W}/*Kit*$^{W\text{-}v}$) mice 4 h after a 5-min exposure to aerosolized adenosine at 6 mg/ml (▨) or PBS (☐). Data represent the mean number of neutrophils (polymorphonuclear leukocytes (PMN)) per square millimeter ± SEM (n = 7/group). *, $p < 0.01$ (vs adenosine-treated *Kit*^{W}/*Kit*$^{W\text{-}v}$).

model in which mast cell activation by adenosine, via the A_3 receptor, results in neutrophil recruitment via chemoattractant release from stimulated mast cells.

Discussion

Adenosine has potent bronchoconstrictor effects on the airways of asthmatics and of many patients with chronic obstructive pulmonary disease. Here we show, using A_3-deficient mice, that physiological changes consistent with airway constriction are mediated to a large extent by the binding of adenosine to the A_3 receptor. Histological evidence of adenosine-dependent airway mast cell degranulation in wild-type, but not $A_3^{-/-}$, mice together with evidence of significantly attenuated airway responses to adenosine in mast cell-deficient mice suggest that adenosine-dependent mast cell activation is required for the full expression of adenosine-induced airway responsiveness in the mouse.

We observed that the administration of adenosine by aerosol could trigger degranulation of airway mast cells in vivo without additional stimuli of mast cell activation. This finding is consistent with observations by ourselves and others that adenosine alone can induce degranulation of mast cells in other tissues and organs in vivo, including the skin (28–30). These findings are also consistent with human studies showing adenosine-induced histamine release from bronchoalveolar lavage mast cells (39). However, these observations do contrast with those of some other studies (2, 30, 40, 41), including one of our own studies in which we showed that while adenosine, acting via the A_3 receptor, could enhance the degranulation of bone marrow mast cells in response to IgE and specific Ag, adenosine alone was ineffective at mediating bone marrow mast cell degranulation (30). However, in this issue Zhong et al. (42) demonstrate that adenosine can induce degranulation of mast cells that have been derived in vitro from mouse pulmonary mast cells, a result consistent with the findings in our in vivo studies of mouse airway mast cells in situ.

Taken together these observations indicate that the responsiveness of distinct mast cell populations in vitro to adenosine-induced degranulation can vary, perhaps because of differences in the species or anatomical site of origin of the mast cells or as a result of differences in the methods used to isolate the cells and/or to maintain and expand these mast cells in vitro. However, we show that for mouse airway mast cells, adenosine-dependent degranulation can be induced in vivo in the absence of other stimuli of mast cell activation, such as IgE and specific Ag. The capacity of adenosine to evoke mast cell degranulation in vivo without additional stimuli supports an important role for this mediator as an activator of mast cells in asthma and perhaps other disorders.

The observation that mast cell degranulation in response to adenosine is dramatically decreased in mice lacking the A_3 receptor is consistent with a model in which adenosine mediates degranulation of these cells directly by activation of the A_3 receptor on mast cells. This model is also supported by the observation by Zhong et al. (42) that pharmacological reagents with high specificity for the A_3 receptor can induce histamine release by cultured primary mouse lung mast cells. However, we cannot formally rule out the possibility that in vivo adenosine can activate airway mast cells by an indirect mechanism(s) as well.

Nevertheless, we clearly show that adenosine can induce increased airway responsiveness in mice and that this increase is largely dependent on both the presence of mast cells and the expression of the A_3 receptor. These findings together with the observed A_3-dependent degranulation of mast cells in vivo suggest a model in which the full expression of bronchoconstriction in response to adenosine requires the release of bronchoactive mediators by mast cells upon their activation by an A_3 receptor-dependent mechanism(s). Furthermore, this proposed mechanism is consistent with the findings that 1) IgE-dependent mast cell activation can result in mast cell-dependent bronchoconstriction in the mouse (35); and 2) the airway hyper-responsiveness to methacholine that follows anti-IgE challenge in mice is mast cell dependent (43). However, the specific mast cell-derived mediators that may contribute to the effects of adenosine challenge on airway responsiveness in the mouse remain to be defined.

Our findings in mice may have clinical relevance in that there is substantial indirect evidence that mast cells can contribute to adenosine-induced bronchoconstriction in humans. In vitro studies have shown that adenosine can potentiate the release of histamine and leukotrienes from immunologically activated human mast cells obtained from lung parenchyma (41) and that adenosine can independently induce histamine release from human bronchoalveolar lavage mast cells (39). Moreover, adenosine-induced contractions of isolated bronchi from asthmatics can be blocked by leukotriene and histamine antagonists (44). In vivo, adenosine-induced bronchoconstriction can be attenuated by drugs that block mast cell degranulation (nedocromil sodium) (45) and by drugs that block mast cell-derived products (antihistamines) (19) capable of producing bronchoconstriction in humans. Finally, concomitant bronchoconstriction and mast cell mediator release have been shown following the endobronchial installation of AMP in asthmatics (18).

While adenosine produces bronchoconstriction in the asthmatic airway, it has little effect on the airway caliber of normal individuals. A possible explanation for this observation is suggested by recent histological studies showing that the location of mast cells is markedly altered in the asthmatic airway. While in the healthy lung mast cells are found primarily in the submucosa, this study showed that mast cells infiltrate the ASM of asthmatics (46). It is easy to imagine that under these circumstances mast cell degranulation might have a more pronounced effect on airway smooth muscle tone. Alternatively the increased responsiveness of the asthmatic to adenosine might reflect increased expression of the A_3 receptors on mast cells in the inflamed airway. Support for this hypothesis comes from studies showing that while in situ expression of the A_3 receptor was not detected in normal lung, specific hybridization was present in mast cells in the airway wall in asthmatic lung. (47).

In our studies, unlike the human studies, we found a significant increase in airway responsiveness in naive mice following adenosine exposure. Our ability to measure changes in response to adenosine without induction of an inflammatory response could simply reflect the sensitivity of the system for measuring changes in the airways of mice vs humans. For example, the whole body plethysmograph used in our studies is sensitive to changes in airway caliber not only in the lower airways but also in the nasal passages and larynx, regions rich in mast cells. We would therefore expect that the response to adenosine might be significantly increased in the inflamed mouse airway. Alternatively, the ability to measure this response in the naive mouse may reflect anatomical differences in the distribution of the mast cells and/or differences in the expression levels of adenosine receptors between the two species.

We also show that exposure of the mouse airway to adenosine can lead to increased inflammation, specifically the recruitment of neutrophils, and that this process is dependent on A_3 expression. We also show that this neutrophil recruitment is mast cell dependent, supporting the hypothesis that activation of A_3 receptors on mast cells by adenosine stimulates the mast cell to produce a number of chemokines, cytokines, and lipid mediators that can contribute to neutrophil chemotaxis (38). While additional work will be required to fully define the mechanisms of adenosine-induced neutrophil recruitment in this setting, our data show that activation of the A_3 receptor plays a critical role in this response.

Chronic elevations of adenosine in the ADA-deficient mice are associated with several dramatic histopathological changes in the lung, including eosinophilia, macrophage activation, and goblet cell hyperplasia (13). In contrast, mast cell degranulation and neutrophil influx were the only histological changes observed in our study following acute exposure of the airways to aerosolized adenosine. There are a number possible explanations for these differences. The changes in the ADA-deficient mice might reflect alterations and adaptive changes on the part of the organism to loss of the receptor throughout development. Second, it is possible that inhaled adenosine does not reach the levels in all tissue compartments achieved in animals lacking ADA. Finally, it is possible that continual chronic exposure of the mice to inhaled adenosine (if achievable) would, given enough time, lead to changes similar to those observed in ADA-deficient mice. In this regard it is interesting to speculate that the changes we observed in the acutely exposed animal may represent the initiating events that eventually lead to the dramatic abnormalities observed in ADA-deficient mice, suggesting that the mast cell may have an important initiating role in the development of airway disease. Second, our findings suggest that the early recruitment of neutrophils, an effector cell not typically associated with allergic airway disease, may play an important role early in disease pathogenesis. It is interesting that while the eosinophil is classically associated with mild to moderate asthma, neutrophils are increased in the airways of severe, steroid-dependent asthmatics and are a prominent feature of patients dying from sudden-onset fatal asthma (48, 49).

While our studies support important roles for mast cells and the A_3 receptor in the full expression of adenosine-induced changes in airway responsiveness, they also indicate that other cell types and receptors contribute to this response. Statistically significant changes in airway responsiveness were observed in both mast cell-deficient and A_3-deficient mice. The cells that account for the mast cell-independent component of adenosine's ability to induce airway responses (via either direct or indirect mechanisms) remain to be determined, but candidates include ASM, epithelia, and nerves. Studies of human airway tissue suggest that one or several of these cell types could contribute to adenosine-induced bronchoconstriction. While human ASM cultures exposed to adenosine predominantly show elevations of cAMP via A_{2b}, evidence for A_1-mediated effects were demonstrated, and it is possible that in the asthmatic lung the differential contribution of A_1 vs A_{2b} to ASM function is altered (50). Cultured airway epithelial cells express A_{2a}, A_{2b}, and A_3 adenosine receptors (51), and mediators released from these cells upon activation by adenosine could influence airway tone. Finally, it has been demonstrated that adenosine-induced bronchoconstriction in humans can be attenuated by local anesthetics or anticholinergics, suggesting the involvement of neural pathways in adenosine-induced actions on human airway function (52–54).

Human alveolar macrophages, primary cultured tracheal epithelial cells, and eosinophils and mast cells from asthmatic lung have all been shown to express the A_3 receptor, but the contribution of this adenosine receptor subtype to immune cell function or to epithelial biology in humans is largely unknown (51, 55, 56). A functional role for the A_{2b} receptor on human lung mast cells has been suggested by pharmacological studies showing that an A_{2b} agonist can induce IL-8 secretion in a human mast cell line (25). However, high agonist concentrations were used to produce this effect (10 μM), which may have decreased receptor selectivity, and the human mast cell leukemia line used in that study exhibits many differences from normal, nonneoplastic human mast cells (57).

In summary, we show that adenosine can have a profound effect on airway tone and inflammation, and that A_3 receptors and mast cells contribute significantly to these processes. The ability of adenosine to activate mast cells in the absence of IgE and Ag has important implications. While basal levels of adenosine produced in the lung are likely to be insufficient to activate the A_3 receptor, adenosine levels increase dramatically during inflammation, and the level measured in the asthmatic airway is sufficient to activate the A_3 receptor (14). The infiltration of airway smooth muscle cells with mast cells in the asthmatic lung (46) coupled with this elevation in adenosine levels and subsequent activation of these cells via the A_3 receptor may play an important role in the pathogenesis of asthma as well as other diseases in which coordinate increases in adenosine and mast cells is observed.

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