Adenosine-Mediated Mast Cell Degranulation in Adenosine Deaminase-Deficient Mice

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ABSTRACT

Adenosine is a signaling nucleoside that has been suggested to play a role in asthma in part through its ability to influence mediator release from mast cells. Adenosine levels are elevated in the lungs of asthmatics, further implicating this molecule in the regulation of lung inflammation and suggesting that animal models exhibiting endogenous increases in adenosine will be useful for the analysis of adenosine function. Adenosine deaminase (ADA) is a purine catabolic enzyme responsible for regulating the levels of adenosine in tissues and cells. ADA-deficient mice develop lung inflammation and damage reminiscent of that seen in asthma in association with elevated adenosine levels. In the current study, we investigated the status of mast cells in ADA-deficient lungs. ADA-deficient mice exhibited extensive lung mast cell degranulation concurrent with elevated adenosine levels. ADA enzyme therapy prevented the accumulation of lung adenosine as well as mast cell degranulation, suggesting that this process was dependent on elevated lung adenosine levels. Consistent with this, treatment of ADA-deficient mice with broad spectrum adenosine receptor antagonists attenuated degranulation by 30 to 40%, supporting the involvement of adenosine receptor signaling. Moreover, these studies demonstrate the ability of endogenously generated adenosine to influence lung mast cell degranulation in a receptor-mediated manner and establish ADA-deficient mice as a model system to investigate the specific adenosine receptor responses involved in the degranulation of lung mast cells.

Asthma is a complex inflammatory disease of the airways. It is characterized by acute responses such as bronchoconstriction, as well as progressive lung eosinophilia and mucus hypersecretion that culminate in chronic remodeling of the airways (Elias et al., 1999). Various cell types including mast cells, T cells, eosinophils, macrophages, and epithelial cells participate in the production of signaling molecules that are thought to drive the inflammation and subsequent tissue damage associated with asthma. Efforts to understand the signaling mechanisms involved in these processes will help determine how this disease is manifested and guide new therapeutic directions for its treatment.

Adenosine is a ubiquitous signaling nucleoside that can elicit physiological effects by engaging G-protein-coupled receptors on target cells (Olah and Stiles, 1995). Four subtypes of adenosine receptors, A1, A2A, A2B, and A3, have been identified. Each receptor has a unique tissue distribution, ligand affinity, and signal transduction mechanism (Ralevic and Burnstock, 1998). Substantial clinical evidence suggests adenosine signaling plays an important role in asthma. This evidence includes the following: 1) detection of elevated adenosine levels in lavage fluid collected from asthmatics (Driver et al., 1993); 2) the observation that inhaled adenosine or its precursor AMP elicits bronchoconstriction in asthmatics but not nonasthmatics (Cushley et al., 1983b; Mann et al., 1986); 3) the altered adenosine receptor expression in patients with asthma (Walker et al., 1997); and 4) the therapeutic benefit of theophylline, an adenosine receptor antagonist, in this disease (Barnes and Pauwels, 1994). In addition to this clinical evidence, in vitro studies have shown that adenosine can influence cell types involved in asthma (reviewed by Jacobson and Bai, 1997; Fozard and Hannon, 1999). This includes adenosine’s ability to modulate mediator release from mast cells (Hughes et al., 1984; Peachell et al., 1991), influence eosinophil function (Knight et al., 1997), and stimulate mucus production by airway epithelial cells (Johnson and McNee, 1985). These findings suggest that adenosine signaling is important in influencing many cellular events in asthma.

Mast cells are bone marrow-derived inflammatory cells that can release mediators that have both immediate and chronic effects on airway constriction and inflammation (Shimizu and Schwartz, 1997). Upon stimulation, mast cells rapidly release preformed mediators such as histamine and tryptase, which are stored inside secretory granules. Lipid mediators and a variety of cytokines are produced and secreted over a more prolonged period. Increasing evidence...
suggests that adenosine can modulate mast cell degranulation (reviewed in Forsythe and Ennis, 1999). Adenosine, and adenosine analogs in vitro, have been shown to enhance mediator release from mast cells in response to challenge with a variety of stimuli (Marquardt et al., 1978; Church et al., 1983; Hughes et al., 1984; Marquardt et al., 1984; Peachell et al., 1991; Ramkumar et al., 1993). While adenosine alone seems to have no effect on mediator release from mast cells in the absence of antigen stimulation in vitro (Marquardt et al., 1978), a number of studies suggest that adenosine can initiate mast cell degranulation in the absence of additional stimuli in vivo (Doyle et al., 1994; Hannan et al., 1995; Tigani et al., 2000; Tilley et al., 2000).

The mechanisms through which adenosine mediates mast cell degranulation are not completely understood. Most in vitro studies suggest that the A2A and A3 adenosine receptors are predominantly involved in mediating adenosine’s effects on mast cells (Ramkumar et al., 1993; Feoktistov and Biaggoni, 1995; Auchampach et al., 1997; Gao et al., 2001). However, since adenosine is at best only a weak initiator of mast cell degranulation in vitro (Marquardt et al., 1978), a clear understanding of adenosine signaling in tissue mast cells will probably only come from in vivo studies. Toward this end, we have recently used a two-stage genetic engineering strategy to generate adenosine deaminase (ADA)-deficient mice (Blackburn et al., 1998). ADA controls the levels of adenosine in tissues and cells. Therefore, adenosine accumulates to high levels in the lungs of ADA-deficient mice (Blackburn et al., 2000b). These mice develop many of the histopathological and biochemical features seen in asthmatics, including lung eosinophilia and mucus hypersecretion (Blackburn et al., 2000b). These features are related to increases in lung adenosine levels in that lowering adenosine levels using ADA enzyme therapy leads to a rapid reduction in lung eosinophilia and mucus secretion. These findings demonstrate that the ADA-deficient mouse is a valuable model to study the physiological impact of elevated adenosine levels in vivo.

In the current study, we examined the status of mast cells in ADA-deficient lungs containing high adenosine levels. The number of toluidine blue-stained mast cells was found to decrease with age, suggesting a relationship between elevated adenosine and mast cells. The existence of c-kit-positive/toluidine blue-negative cells, and the prevention of the loss of toluidine blue staining by treatment with the mast cell stabilizer disodium cromoglycate, suggested that this decrease was due to mast cell degranulation. In addition, the attenuation of mast cell degranulation by the adenosine receptor antagonists theophylline, MRS-1220, and enprofylline suggested that adenosine receptors are involved in lung mast cell degranulation in this model.

**Materials and Methods**

**Transgenic Mice.** ADA-deficient mice were generated and genotyped as described previously (Wakamiya et al., 1995; Blackburn et al., 1998). Animals were on a mixed background of 129/Sv and FVB/N strains. Control mice were either wild-type (+/+), heterozygous for the null Ada allele (m1/+) as there was no phenotype seen in heterozygous animals (Blackburn et al., 1998). All mice were housed in cages equipped with microisolator lids and maintained under specific containment protocols.

**Toluidine Blue Staining and Mast Cell Counting.** Mice were sacrificed and the lungs infused with 0.25 to 0.5 ml of fixative (4% paraformaldehyde in PBS) prior to fixation overnight at 4°C. Fixed lung samples were rinsed in PBS, dehydrated, and embedded in paraffin according to standard techniques. Sections (5 μm) were collected on microscope slides and stained with toluidine blue. Toluidine blue staining was accomplished by immersing hydrated sections in a solution of 0.1% toluidine blue in 0.9% sodium chloride for 60 s followed by extensive rinsing in deionized water. Toluidine blue-positive mast cell numbers in lung tissues were determined by counting the number of stained cells in longitudinal sections through one mainstream bronchus. Multiple sections from each lung were analyzed to ensure that the entire length of the bronchus was examined. For quantification of dermal mast cells, toluidine blue-stained cells were counted in six randomly chosen 400× fields of ear cross sections.

**Quantification of Adenosine.** Lungs were removed from anesthetized mice and quickly frozen in liquid nitrogen. Adenine nucleosides were extracted from frozen lungs using 0.4 N perchloric acid and adenosine separated and quantified using reversed phase high-performance liquid chromatography (Knudsen et al., 1992). Adenosine levels were normalized to protein content and values are given as nanomoles per milligram of protein.

**ADA Enzyme Therapy.** Polyethylene glycol-modified ADA (PEG-ADA), also known as ADAGEN, was obtained from Enzon, Inc. (Piscataway, NJ). Mice were injected intramuscularly with dosages of PEG-ADA designed to deliver 100 to 500 units of PEG-ADA per kilogram of body weight (Blackburn et al., 2000a). Injections were started on postnatal day 1 and were given every 4 days up to postnatal day 17.

**Antagonists Treatments.** Theophylline, enprofylline, and MRS-1220 (Sigma, St. Louis, MO) were given to mice intraperitoneally once daily at a dose of 10 mg (theophylline, enprofylline) or 100 μg (MRS-1220) per kilogram of body weight. MRS-1220 was dissolved in dimethyl sulfoxide and diluted in phosphate-buffered saline (pH 7.4) before injection. Theophylline and enprofylline were dissolved in PBS. The dosages of theophylline and enprofylline used were chosen based on dosages used clinically to antagonize adenosine receptors (Feoktistov et al., 1998; Fozard and Hannan, 1999). In addition, 10 mg/kg/day theophylline was chosen because this dosage maintained plasma theophylline levels below 20 μM (data not shown), which is a concentration consistent with adenosine receptor antagonism but not phosphodiesterase inhibition. The dosage of MRS-1220 used was based on the effectiveness of this dosage on blocking adenosine-mediated lung inflammation in a guinea pig model (Spruntulis et al., 2000). Injections were started 4 days after the last PEG-ADA treatment. Mice were sacrificed and lung tissues were analyzed 13 to 15 days after stopping PEG-ADA treatment.

**Immunohistochemistry.** Tissues were fixed in 4% paraformaldehyde and embedded in paraffin, and 5-μm sections were incubated with 2 μg/ml polyclonal rabbit c-kit antibody (sc-168, Santa Cruz Biotechnology, Santa Cruz, CA) according to the manufacturer’s instructions. Secondary antibody (biotinylated goat anti-rabbit IgG from Vector Laboratories, Burlingame, CA) was detected using a Vectastain Elite ABC kit (Vector) and diaminobenzidine. Slides were counterstained lightly with toluidine blue to visualize the tissue and mast cell granules.

**Disodium Cromoglycate Treatment.** Disodium cromoglycate (Sigma) was continuously delivered to mice at a dose of 40 mg/kg/day using ALZET osmotic pumps. Disodium cromoglycate was dissolved in saline. Pumps were filled with disodium cromoglycate or saline and implanted subcutaneously into mice 2 days after the last PEG-ADA treatment. Mice were sacrificed and lung tissues were analyzed 13 to 15 days after stopping PEG-ADA treatment.

**Results**

**Toluidine Blue-Positive Mast Cells Are Not Found in the Lungs of 18-Day-Old ADA-Deficient Mice.** Mast cells release mediators that influence lung inflammation (Shimizu ...
and Schwartz, 1997). Moreover, the ADA substrate adenosine, which is elevated in ADA-deficient mice (Blackburn et al., 2000b), has been shown to stimulate and enhance mediator release from mast cells (Marquardt et al., 1978; Ramkumar et al., 1993; Fozard et al., 1996; Tigani et al., 2000). Mast cells were monitored using toluidine blue to examine the status of these cells in the lungs of ADA-deficient mice. Mast cells were readily detected in the dermis of control and ADA-deficient ears at post partum day 18 (Fig. 1, A and B). Mast cells were also detected in the bronchi of control mice at this stage (Fig. 1C). However, toluidine blue-positive mast cells were never detected in the airways of ADA-deficient lungs at day 18 (Fig. 1D). These results demonstrated that mast cells are severely and selectively affected in the lungs of ADA-deficient mice.

**Toluidine Blue-Positive Mast Cells Decrease with Age in ADA-Deficient Mice.** To determine whether the effects on mast cells were progressive, toluidine blue-positive mast cells were counted in the ears and lungs of control and ADA-deficient mice at different postnatal ages. Similar numbers of toluidine blue-positive mast cells were found in the lungs of control and ADA-deficient mice at birth; however, the number of lung mast cells was significantly reduced by post partum day 5, and they were undetectable by day 10 (Fig. 2A). The numbers of dermal mast cells also decreased with age in ADA-deficient mice, but at a later stage and to a lesser degree than seen in ADA-deficient lungs (Fig. 2B). These results demonstrated that the number of lung and dermal toluidine blue-positive mast cells are normal at birth but decrease rapidly in ADA-deficient mice.

**Lung Adenosine Levels Increase with Age in ADA-Deficient Lungs.** Adenosine was quantified in the lungs of control and ADA-deficient mice at different ages to determine whether decreases in toluidine blue-positive lung mast cells correlated with an increase in lung adenosine levels. At day 0, the levels of adenosine were similar in control and ADA-deficient lungs (Fig. 3). However, by day 5, adenosine levels in ADA-deficient lungs were significantly higher than those measured in control lungs, and levels continued to increase through day 18. These results demonstrated that the decrease in toluidine blue-positive lung mast cells was associated with increases in lung adenosine levels.

**Lung Adenosine Levels and Toluidine Blue-Positive Mast Cell Numbers Can Be Manipulated Using ADA Enzyme Therapy.** ADA enzyme therapy using PEG-ADA can prevent the metabolic consequences of ADA deficiency that include adenosine accumulation (Blackburn et al., 2000a,b). PEG-ADA was injected intramuscularly into both ADA-deficient and control mice every 4 days beginning at postnatal day 1 to determine whether systemically restoring ADA enzymatic activity could influence mast cells in ADA-deficient lungs. Eighteen-day-old ADA-deficient mice maintained on PEG-ADA had normal numbers of toluidine blue-positive mast cells in both lungs (Fig. 4A) and ears (data not shown). These findings indicated that ADA enzyme therapy could prevent the loss of toluidine blue-positive mast cells in ADA-deficient mice.

The half-life of PEG-ADA in mice is 4 days with the injection protocol used (Blackburn et al., 2000a). PEG-ADA treatments were stopped at day 18 to determine whether toluidine blue staining of mast cells would decrease after the removal of enzyme therapy. The number of toluidine blue-positive mast cells in ADA-deficient lungs began to decrease, relative to controls, 6 to 8 days after the cessation of enzyme therapy (Fig. 4B). Two weeks after stopping PEG-ADA treatment, mast cells were no longer detected in ADA-deficient lungs. These results demonstrated that ADA enzyme therapy could
be used to regulate mast cells in the lungs of ADA-deficient mice.

Adenosine levels were measured in lungs from PEG-ADA-treated mice in an attempt to correlate the loss of toluidine blue mast cell staining with adenosine levels. As expected, adenosine levels were increased greater than 5-fold in ADA-deficient lungs at day 18 (Fig. 5). There was a significant decrease in the fold elevations of adenosine in the lungs of day 18 ADA-deficient mice treated with PEG-ADA. However, 14 days after stopping PEG-ADA treatment, adenosine levels in ADA-deficient lungs had increased to levels 4-fold greater than those measured in control lungs (Fig. 5). These data demonstrated that PEG-ADA therapy could reduce adenosine accumulation in the lungs and suggest that high levels of adenosine lead to a decrease of toluidine blue-positive mast cells.

Evidence for Mast Cell Degranulation in ADA-Deficient Lungs. As mast cells degranulate, they release the contents of their granules that convey the metachromatic properties that allow them to be stained with toluidine blue. Therefore, degranulated mast cells show decreased or no staining with toluidine blue. However, degranulated mast cells can be recognized by monitoring the expression of mast cell surface molecules such as c-kit (Shimizu and Schwartz, 1985).
Immunohistochemistry for c-kit was used to begin to assess whether or not the disappearance of toluidine blue-positive mast cells in ADA-deficient lungs involved degranulation of these cells. Immunoperoxidase staining using a c-kit antibody, followed by toluidine blue staining, revealed that c-kit staining localizes to toluidine blue-positive mast cells in the airways of control mice (Fig. 6A). Examination of similar regions of ADA-deficient airways revealed the presence of c-kit-positive cells that did not stain with toluidine blue (Fig. 6C). These findings provided evidence that degranulation was the basis of the loss of toluidine blue-positive mast cells in the lungs of ADA-deficient mice.

To further investigate whether the disappearance of toluidine blue-positive mast cells was due to degranulation, ADA-deficient mice were treated with the mast cell stabilizer disodium cromoglycate. Control and ADA-deficient mice were maintained on PEG-ADA therapy from birth, and treatment was discontinued on day 18. ALZET implants, containing concentrations of disodium cromoglycate designed to sustain a dose of 40 mg/kg/day, were implanted 2 days after the last PEG-ADA treatment. Lung mast cells were quantified in saline- or cromolyn-treated control and ADA-deficient mice at 13 to 15 days after PEG-ADA therapy. As expected, there were no toluidine blue-positive mast cells in the lungs of ADA-deficient mice containing saline-filled ALZET pumps (Fig. 7); however, toluidine blue-positive mast cells were consistently found in the lungs of ADA-deficient mice treated with disodium cromoglycate. These findings suggested that degranulation was involved in the loss of toluidine blue-positive mast cells in ADA-deficient lungs.

Mast Cell Degranulation Is Mediated through Adenosine Receptors. Adenosine receptors, predominantly the $A_{2B}$ and $A_3$ subtypes, have been shown to be involved in adenosine-mediated mast cell degranulation (Marquardt et al., 1984; Feoktistov and Biaggioni, 1995; Auchampach et al., 1997; Salvatore et al., 2000). Theophylline, enprofylline, and MRS-1220, antagonists for adenosine receptors (Jacobson, 1998; Fozard and Hannon, 1999), were given to ADA-deficient mice to determine whether adenosine receptors were involved in the observed mast cell degranulation. Control and ADA-deficient mice were treated with PEG-ADA beginning at day 1, as described above, and treatment was discontinued at 18. Mice were then treated daily with the adenosine receptor antagonists starting on day 4 after the cessation of enzyme therapy. Theophylline, which blocks $A_1$, $A_{2A}$, and $A_{2B}$ receptors, and enprofylline, which is selective for $A_{2B}$ receptors (Fozard and Hannon, 1999), were given intraperitoneally at 10 mg/kg/day. MRS-1220 was given at a dosage of $100 \mu g/kg/day$. Although MRS-1220 was shown to be selective for the human $A_3$ adenosine receptor (Jacobson, 1998), its affinity to rat $A_3$ receptors is much lower (Kim et al., 1996) and was therefore considered as a nonselective adenosine receptor antagonist in these experiments. Lung mast cells were quantified in untreated or antagonist-treated ADA-deficient mice 14 to 16 days following the cessation of enzyme therapy (Fig. 8). ADA-deficient mice treated with theophylline, MRS-1220, or enprofylline all had significantly more mast cells compared with untreated ADA-deficient mice at the same stage. These experiments demonstrated that treatment with adenosine receptor antagonists could partially prevent mast cell degranulation in ADA-deficient lungs, suggesting the involvement of adenosine receptor signaling.

**Discussion**

Mediators released from lung mast cells influence both acute and chronic features of asthma (Shimizu and Schwartz, 1997). Immunohistochemistry for c-kit was used to begin to assess whether or not the disappearance of toluidine blue-positive mast cells in ADA-deficient lungs involved degranulation of these cells. Immunoperoxidase staining using a c-kit antibody, followed by toluidine blue staining, revealed that c-kit staining localizes to toluidine blue-positive mast cells in the airways of control mice (Fig. 6A). Examination of similar regions of ADA-deficient airways revealed the presence of c-kit-positive cells that did not stain with toluidine blue (Fig. 6C). These findings provided evidence that degranulation was the basis of the loss of toluidine blue-positive mast cells in the lungs of ADA-deficient mice.

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**Discussion**

Mediators released from lung mast cells influence both acute and chronic features of asthma (Shimizu and Schwartz,
The signaling nucleoside adenosine has been implicated to play a role in asthma, in part through its ability to promote or enhance mediator release from mast cells (Jacobson and Bai, 1997). Understanding the mechanisms that govern adenosine metabolism and site of action in the inflamed lung will help guide adenosine-based therapeutics for the treatment of asthma. We have generated a mouse model in which adenosine levels are markedly elevated in many tissues, including the lung (Blackburn et al., 1998; Blackburn et al., 2000b). This occurs because the mice are deficient in the purine catabolic enzyme, ADA, which is responsible for controlling the levels of adenosine in tissues and cells. These mice develop many histological features seen in the lungs of asthmatics, suggesting that they will be a useful model for studying the role of adenosine signaling in the inflamed lung (Blackburn et al., 2000b). In the current study, we demonstrated that ADA-deficient mice show extensive lung mast cell degranulation in association with elevated adenosine levels. Mast cell degranulation was evident at day 5, and mast cells were completely degranulated by day 10. Interestingly, lung inflammation is not detected in the lungs of ADA-deficient mice until day 15 (Blackburn et al., 2000b), suggesting that the mast cell degranulation seen was not secondary to adenosine’s effects on other inflammatory cells, but rather associated with precipitous increases in lung adenosine levels. This is supported further by the observation that ADA enzyme therapy prevented the accumulation of lung adenosine as well as mast cell degranulation. Moreover, treatment of ADA-deficient mice with broad-based adenosine receptor antagonists prevented 30 to 40% of the mast cell degranulation observed, suggesting the involvement of adenosine receptor signaling. These studies demonstrate the ability of endogenously generated adenosine to influence lung mast cell degranulation in a receptor-mediated manner and establish ADA-deficient mice as a model system to investigate the specific adenosine receptor responses involved in the degranulation of lung mast cells.

Degranulation of mast cells in ADA-deficient mice occurs in an apparent adenosine-dependent and allergen-independent manner. This is important in light of the observations that the action of adenosine on mast cells differs between in vitro and in vivo studies. Analysis in vitro suggests that adenosine alone induces minimal mast cell degranulation (Marquardt et al., 1978; Ramkumar et al., 1993). In contrast, analysis in vivo, in the current study and elsewhere (Doyle et al., 1994; Fozard et al., 1996; Tigani et al., 2000; Tilley et al., 2000), suggests that adenosine by itself is a potent stimulator of mast cell degranulation. It is possible that these inconsistencies represent differences in the origin of mast cells used for the in vitro studies. For example, bone marrow derived-mast cells, which are commonly used as a source of mast cells for in vitro analysis, may differ from tissue mast cells with regard to the specific expression of adenosine receptors. In addition, mast cells may differ in their responses to adenosine when removed from the context of a tissue environment and the various factors produced therein. These features highlight the importance of using in vivo models to analyze the role of adenosine in mast cells.

Murine mast cells express A_2A, A_2B, and A_3 adenosine receptors (Marquardt et al., 1994; Salvatore et al., 2000). Testing the functionality of these receptors, however, has been hindered by the absence of selective adenosine receptor antagonists. Recent genetic approaches have begun to shed light on the role of the A_3 adenosine receptor in murine mast cells (Salvatore et al., 2000). Tilley et al. (2000) demonstrated that adenosine, as well as its metabolite inosine, was able to activate cutaneous mast cells and in turn increase vasopermeability. These effects were not seen in mice deficient in the A_3 adenosine receptor, nor were bone marrow-derived mast...
cells from A3-deficient mice able to respond to adenosine even in the presence of antigen (Salvatore et al., 2000). These studies suggest that the A3 adenosine receptor is the sole receptor responsible for adenosine mediated mast cell degranulation in murine bone marrow-derived mast cells and cutaneous mast cells. Therefore, the degranulation of mast cells seen in ADA-deficient mice is probably mediated by engagement of the A3 adenosine receptor. In an attempt to assess A3 in our model, we treated mice with MRS-1220, which is a selective human A3 receptor antagonist (Jacobson, 1998). We saw a partial attenuation of mast cell degranulation; however, since MRS-1220 is not selective for the A3 receptor in the rat (Kim et al., 1996; Jacobson, 1998), we can not be sure that our observations in the mouse are attributed to A3 blockade. Definitive experiments await the availability of selective murine A3 receptor antagonists. In addition, it is possible that the expression of adenosine receptors may differ between cutaneous and lung mast cells, and it will therefore be important to clarify the expression of various receptors on lung mast cells as well as specifically test their function in our model.

There is precedence to suggest that other adenosine receptors may be involved in the degranulation of lung mast cells. One of the major lines of evidence for adenosine playing a role in asthma is the ability of inhaled adenosine or its precursor AMP to elicit bronchoconstriction in the asthmatic airway, but not in the nonasthmatic airway (Cushley et al., 1983b). This effect is believed to be related to the ability of adenosine to modulate mediator release from airway mast cells (Jacobson and Bai, 1997; Forsythe and Ennis, 1999). Moreover, there is evidence suggesting that the A2B adenosine receptor plays a role in mast cell degranulation in murine bone marrow-derived mast cells and specifically test their function in our model.

Engaging the A3 adenosine receptor. In an attempt to determine whether or not adenosine levels are elevated in the lungs of conventional animal models of allergen challenge. However, the concentrations of adenosine measured in the lungs of ADA-deficient mice are estimated to be between 100 and 150 μM (M. R. Blackburn, unpublished data). It is likely that these concentrations are high enough to engage both A3 and A2B receptors. Therefore, ADA-deficient mice will provide a useful experimental means to directly assess which receptor is involved in the degranulation of mast cells in lungs with elevated adenosine. Not only can additional pharmacology be conducted using selective adenosine receptor antagonists, but these mice can be intercrossed onto the A3 receptor-deficient background, and the A2B receptor-deficient background when it becomes available, allowing for the direct genetic assessment of receptor function in an adenosine-rich environment. In addition, the ability to control the levels of adenosine in the lungs of ADA-deficient mice using ADA enzyme therapy will provide a means for determining the levels of adenosine needed to induce mast cell degranulation in the lung. The ability to control lung aden- osine levels with ADA enzyme therapy and subsequently prevent mast cell degranulation, or the exacerbation of other inflammatory processes such as eosinophil chemotaxis or activation, would support ADA enzyme therapy as a novel therapeutic intervention for asthma.

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References


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