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*J Immunol* 2012; 189:3707-3713; Prepublished online 5 September 2012;
doi: 10.4049/jimmunol.1201207
http://www.jimmunol.org/content/189/7/3707

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A2B Adenosine Receptor Expression by Myeloid Cells Is Proinflammatory in Murine Allergic-Airway Inflammation

Bryan G. Belikoff,*1 Louis J. Vaickus,*1 Michail Sitkovsky,† and Daniel G. Remick*

Asthma is a chronic condition with high morbidity and healthcare costs, and cockroach allergens are an established cause of urban pediatric asthma. A better understanding of cell types involved in promoting lung inflammation could provide new targets for the treatment of chronic pulmonary disease. Because of its role in regulating myeloid cell-dependent inflammatory processes, we examined A2B R expression by myeloid cells in a cockroach allergen model of murine asthma-like pulmonary inflammation. Both systemic and myeloid tissue-specific A2B R deletion significantly decreased pulmonary inflammatory cell recruitment, airway mucin production, and proinflammatory cytokine secretion after final allergen challenge in sensitized mice. A2B R deficiency resulted in a dramatic reduction on Th2-type airways responses with decreased pulmonary eosinophilia without augmenting MPO, myeloperoxidase; PAS, periodic acid–Schiff; WT, wild type.

Abbreviations used in this article: A2A R, A2A adenosine receptor; A2B R, A2B adenosine receptor (A2B R), and A3 adenosine receptor subtype interactions. A2B Rs function as potent anti-inflammatory receptors in response to endogenously generated adenosine. The A2A R provides a negative regulatory feedback loop to limit inflammatory processes. The anti-inflammatory effects of A2A R engagement by adenosine are mediated by increases in intracellular cAMP levels (1). The chronic inflammatory mediator IL-13 is a central player in the pathogenesis of asthma and is shown to act synergistically with adenosine (2, 3). Adenosine is a known mediator of eosinophil airway influx secondary to AMP inhalation in patients with asthma (4, 5). In addition to the role of the A2A R, past studies show a central role for A2B R in the development of chronic pulmonary inflammation.

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Received for publication April 26, 2012. Accepted for publication August 4, 2012.

This work was supported by the National Institutes of Health (Grant R01 GM 097320 to M.S.; Grants R01 GM 82962, R01 ES 13358, and T32 AR07309 to D.G.R.).

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Over the last several years, adenosine has gained attention as an important mediator of inflammatory processes. Adenosine levels increase locally and systemically in response to cell stress, infection, trauma, and chronic inflammation in humans and in animal models of disease. Adenosine drives proinflammatory and anti-inflammatory processes depending on adenosine A1, A2A adenosine receptor (A2A R), A2B adenosine receptor (A2B R), and A3 adenosine receptor subtype interactions. A2A Rs function as potent anti-inflammatory receptors in response to endogenously generated adenosine. The A2A R provides a negative regulatory feedback loop to limit inflammatory processes. The anti-inflammatory effects of A2A R engagement by adenosine are mediated by increases in intracellular cAMP levels (1). The chronic inflammatory mediator IL-13 is a central player in the pathogenesis of asthma and is shown to act synergistically with adenosine (2, 3). Adenosine is a known mediator of eosinophil airway influx secondary to AMP inhalation in patients with asthma (4, 5). In addition to the role of the A2A R, past studies show a central role for A2B R in the development of chronic pulmonary inflammation.

Systemic deletion of the A2B R in mice attenuates chronic pulmonary inflammation, together with pulmonary levels of inflammatory mediators IL-4, IL-5, and IL-13 (6). Furthermore, adenosine deaminase-deficient mice develop severe pulmonary fibrosis that is prevented with exogenous administration of adenosine deaminase or an A2B R antagonist, indicating a role for adenosine signaling through A2B R in the development of this phenotype (7). The ability of the low-affinity A2B R to mediate proinflammatory pathways may be attributed to their action as a G protein-coupled receptor. Importantly, a recent study using mice that lack the A2B R in an allergy-induced model of chronic lung inflammation showed a deleterious role for A2B R signaling in airway remodeling and inflammation (6). Mice that lack A2B R also had decreased levels of circulating IgE and airway eosinophilia, providing further evidence of A2B R involvement in allergic inflammation (6).

A number of immune and nonimmune cell types have been implicated in the pathogenesis of chronic inflammation in asthma. Specifically, the wide receptor distribution of the A2B R on numerous cell types, not surprisingly, makes it difficult to delineate the cell types responsible for A2B R involvement in the pathogenesis of asthma. The generation of A2B R chimeric mice furthered our understanding of A2B R distribution patterns showing high A2B Rs expression on monocytes, endothelial cells, and vascular smooth muscle cells (8). Indeed, the discovery that A2B R activation on mast cells promotes degranulation and proinflammatory cytokine secretion increased interest in the development of A2B R antagonist for the treatment of asthma and other inflammatory airway diseases (2, 3, 9, 10). Furthermore, A2B R function to promote differentiation of lung fibroblasts to a myofibroblast phenotype implicating their involvement with nonimmune-mediated airway remodeling associated with chronic pulmonary inflammation such as asthma (11).

The wide cellular distribution and multiple inflammatory properties of the A2B R add to the complexity of this receptor involvement in airway disease. Early studies showed the A2B R ability to limit inflammation in models of acute vascular injury that describes A2B R’s anti-inflammatory property. Conversely, a recent study by
Zaynagetdinov et al. (6) determined a proinflammatory role for A$_{2B}$ Rs during chronic pulmonary inflammation using an allergen-induced model. This study was limited by the use of systemic A$_{2B}$ R knockout (KO) mice, thereby leaving the cell types responsible for this phenotype to be determined. More recent studies demonstrate high levels of A$_{2B}$ R expression on hypoxia-treated dendritic cells inducing the cells toward a Th2-polarizing phenotype, further strengthening our interest in the role of A$_{2B}$ Rs and asthma (12). Our study sought to determine whether A$_{2B}$ R expression by myeloid cells is involved in the pathogenesis of chronic airway inflammation. We hypothesized that A$_{2B}$ R expression on immune cell types of the myeloid lineage, which include neutrophils, monocytes, eosinophils, and basophils, play a central role in the pathogenesis of chronic pulmonary inflammation. These findings may have implications for the mode of adenosine receptor modulator drug delivery. Therefore, we made use of mice lacking A$_{2B}$ Rs on the myeloid cells lineage (lysozyme M promoter-driven Cre recombinase [LysM] KO) using A$_{2B}$ R-floxed/floxed LysM cre$^{+/-}$ transgenic mice (13), and established a mouse model of airway inflammation using German cockroach extract to induce airway inflammation via direct inhalation (14–17). Our results support the hypothesis that A$_{2B}$ R expression on myeloid cells is partially responsible for the pathogenesis of chronic airway inflammation in asthma.

Materials and Methods

Experimental mice

Female mice 8–12 wk old were used for all experiments. C57BL/6 mice were used as age-matched controls and were given 1 wk to acclimate to the pathogen-free housing facility at Northeastern University (Boston, MA). Ozgene developed the A$_{2B}$ R-deficient mice on a C57BL/6 background as described previously (13). Myeloid tissue-specific A$_{2B}$ R KO mice were used to determine the myeloid cell A$_{2B}$ R-dependent contributions to allergic-type inflammation. Myeloid tissue-specific A$_{2B}$ R KO mice were generated and validated using the Cre/loxP system as described previously (13). All animal experiments were conducted in accordance with Institutional Animal Care and Use Committee guidelines of Northeastern University.

Allergen challenge and asthma induction

On days 0, 14, and 21, mice received direct hypopharyngeal challenges of 4, 2, and 1 µg lipopolysaccharide- and whole-body German cockroach allergen (CRA; Greer, Lenoir, NC) in 50 µl sterile PBS using our previously described protocol (17, 18). In brief, mice were lightly anesthetized with isoflurane and suspended from the front incisors on an inclined board. Using forceps, the tongue was gently extended and held against the lower mandible. The allergen solution was delivered to the hypopharynx in two separate 25 µl aliquots. Inhalation of the solution was confirmed by inspiratory sounds and previous radiological studies (data not shown). In these studies, mice were lightly anesthetized and given a radio-opaque dye hypopharyngeally either with the tongue extended (epiglottis held shut) or with the tongue in place (epiglottis unencumbered), and real-time x-ray videos were taken showing the progression and final location of the dye, allowing distinction between esophageal/gastric and tracheobronchial progression. With in 5 min of dosing, all mice resumed normal activity. Mice received a total of three pulmonary CRA challenges at days 0, 14, and 21, and then were examined and sacrificed at 24 h after final allergen challenge for analysis.

Sacrifice and tissue collection

Mice were anesthetized with ketamine/xylazine and sacrificed by exsanguination following by cervical dislocation. The lungs were lavaged with 2 ml warm HBSS in 250 µl aliquots. This bronchoalveolar lavage (BAL) fluid was centrifuged to pellet the cells. The cell pellets were combined, counted, placed on slides, and a differential was performed. The right lung of each mouse was homogenized and used to assess eosinophil peroxidase (EPO) and neutrophil-specific peroxidase activity and cytokine analysis. In brief, lung homogenates (LHs) were centrifuged to separate tissue components from supernatant. Supernatants were reserved for cytokine/chemokine analysis. LH cell pellets were treated with cetyltrimethylammonium chloride or hexadecyltrimethylammonium bromide and sonicated to release eosinophil and neutrophil granules, respectively. Activity was measured as described later. The left lung was fixed in 70% ethanol for histological analysis.

Assessment of LH cytokines and chemokines

Cytokines and chemokines in LH samples were assessed by sandwich ELISA using biotin-linked detection Abs and streptavidin HRP development as previously described (19).

Assessment of EPO and myeloperoxidase

LH were treated with cetyltrimethylammonium chloride or hexadecyltrimethylammonium bromide and treated as described earlier. Activity was measured as previously described (18). Measurements were taken without the addition of degranulating agents to measure release representing ongoing allergen-induced eosinophil/neutrophil activation in the lung tissue.

Histological analysis

Fixed lungs were sent to the Boston University School of Medicine, Department of Pathology and Laboratory Medicine Core Services for histology sectioning and staining. Image analysis for the periodic acid–Schiff (PAS)-stained area was performed using ImageJ freeware (http://rsweb.nih.gov/ij/). In brief, whole-lung images were captured at ×2 magnification. These images were subjected to background subtraction and color deconvolution. The magenta-separated image was converted to a black-and-white image, and the total PAS (black)-stained area was calculated.

Statistical analysis

All data are presented as the mean ± SEM, with the numbers of animals provided in the figure legend. Groups of data were analyzed by ANOVA with a Dunnett’s or Bonferroni post hoc analysis to compare groups if appropriate. Student $t$ test was used to compare data between two groups when appropriate. The groups used for comparison are listed in the figure legends. All statistical analyses were performed using GraphPad Prism 4.0 software.

Results

To determine the degree of allergic pulmonary inflammation in the wild type (WT) mice, systemic A$_{2B}$ R and A$_{2B}$ RLysM KO mice were used to examine multiple mediators and metrics of asthma-like pulmonary inflammation. First, we assessed pulmonary mucous production because airways mucous hypersecretion is a characteristic feature of asthma-associated inflammation in humans. Histological sections of fixed whole left lungs were stained with PAS for mucus, which stains the mucus within the epithelial cells dark magenta. The A$_{2B}$ R KO and A$_{2B}$ RLysM KO mice showed markedly diminished mucous staining 24 h after the final allergen challenge in representative images (Fig. 1A). The total mucous-stained area in the entire section of the lung was then quantitated using

![FIGURE 1. Airways mucous production. (A) PAS stain in lung histological sections of WT, A$_{2B}$R KO, and LysM KO groups of mice. Each panel is a representative image; the mucin is stained magenta within the airway epithelial cells. All images are the same magnification. (B) Quantification of the total area of PAS-stained mucus using ImageJ photoanalysis software. A$_{2B}$R mice showed significant decreases in airways mucous production compared as with WT. Values are mean ± SEM of 2 independent experiments for WT ($n = 5$), A$_{2B}$R KO ($n = 6$), and LysM KO ($n = 6$). The $p$ values are indicated in the figure.](http://www.jimmunol.org/)}
image analysis software that showed that there was significantly less mucous-stained area in the A2B R KO or the A2B R LysM KO animals as compared with their WT mice (Fig. 1B).

Lung eosinophilia is a widely accepted characteristic of the asthmatic lung (20). These inflammatory cells can typically be recovered in the BAL and also observed in the lung parenchyma of asthmatic patients. In mice, eosinophils are recruited to the lung via eotaxins 1 and 2, as well as a number of other cytokines and chemokines. In our experiments, CRAs produced significantly more eotaxin 1 and 2 in the lung parenchyma in WT mice compared with either of the A2B R KO groups of mice (Fig. 2A, 2B). The number of peribronchial eosinophils was also decreased in the A2B R KO and A2B R LysM KO mice by routine light microscopy (Fig. 2C). Total lung eosinophils were quantified by measuring lung EPO to confirm the histologic findings. EPO levels were significantly reduced in both groups of A2B R KO mice compared with the WT mice (Fig. 2D). Notably, EPO levels were measured after BAL had been performed. We also evaluated the number of eosinophils in the BAL fluid recovered 24 h after the last allergen challenge. Cytospin preparations showed reduced influx of eosinophils (representative photomicrographs in Fig. 2E). Quantification of the slides demonstrated a significant reduction of eosinophil recruitment to the bronchoalveolar spaces in both the A2B R KO and A2B R LysM KO mice compared with WT mice (Fig. 2F).

In addition to pulmonary eosinophilia, infiltration by neutrophils is often observed in asthmatic patients, typically in the most therapeutically recalcitrant cases. In mice, neutrophils are recruited by a number of cytokines, chemokines, and other mediators including complement fragments and leukotriene metabolites. BAL neutrophils and LH neutrophil-specific myeloperoxidase (MPO) activity did not differ significantly among the experimental groups (Fig. 3A, 3B). We measured the lung levels of two important CXC chemokines, keratinocyte-derived chemokine (KC) and MIP-2, murine analogs of IL-8, and found no significant differences between WT and A2B R LysM KO mice. We did find a significant reduction in KC and MIP-2 levels in systemic A2B R KO mice compared with WT mice, albeit a modest reduction (Fig. 3C, 3D). No significant differences in RANTES levels were found between all experimental groups (Fig. 3E).

Macrophages are the only inflammatory cells present in normal lungs in the form of resident alveolar cells. During inflammatory insults, these resident macrophages perform a number of immune responses, including the production of cytokines and chemokines that recruit other immune cells to the site of inflammation. In our experiments, we measured the lung levels of two important CXC chemokines, keratinocyte-derived chemokine (KC) and MIP-2, murine analogs of IL-8, and found no significant differences between WT and A2B R LysM KO mice. We did find a significant reduction in KC and MIP-2 levels in systemic A2B R KO mice compared with WT mice, albeit a modest reduction (Fig. 3C, 3D). No significant differences in RANTES levels were found between all experimental groups (Fig. 3E).

**FIGURE 2.** Pulmonary eosinophil recruitment and infiltration. LH concentrations of eotaxins 1 (A) and 2 (B) in WT (n = 5), A2B R KO (n = 6), and LysM KO (n = 6) mice. (C) Representative images of parenchymal eosinophils in H&E sections of whole left lung. All images are the same magnification. (D) LH supernatant EPO activity in WT, A2B R KO, and LysM KO mice. (E) Representative cytospin photomicrographs of BAL cells including eosinophils from the indicated mice. All images are the same magnification. (F) Absolute eosinophil counts in the BAL fluid. The A2B R-deficient groups of mice demonstrated markedly reduced eosinophil infiltration into the BAL and lung parenchyma, and concurrent diminished production of eosinophil chemoattractants. (A, B, D, and F) Values are the mean ± SEM of two independent experiments. (D and F) n = 9 for WT, n = 10 for A2B R KO, and n = 10 for LysM. The p values are indicated in the figure.
functions including Ag processing and presentation and phagocytosis. As the inflammatory response continues, the alveolar macrophages are augmented by and replaced with fresh circulating monocytes recruited by various inflammatory mediators. In our experiments, there was a trend toward decreased alveolar macrophages in the BAL of A2B R KO mice and significantly reduced macrophage levels in the lung of the A2B R LysM KO mice compared with WT mice (Fig. 4A). Lymphocytes, similar to eosinophils and neutrophils, are typically not present in the healthy lung. Although granulocytes tend to arrive early in the inflammatory timeline, lymphocytes typically arrive many hours to days after the initial insult and orchestrate the Ag-specific response (21, 22). These cells are attracted by a number of mediators and themselves produce a complex array of Th1, Th2, and other cytokines and chemokines. In our model, the A2B R-deficient groups of mice exhibited significantly fewer BAL lymphocytes as compared with the WT mice after final allergen challenge (Fig. 4B).

To complete the evaluation of the impact of differential A2B R expression, we examined the production of a number of classical Th2 and proinflammatory/immunomodulatory cytokines. IL-4, IL-5, and IL-13 are produced principally by activated lymphocytes and are traditionally considered to be part of the Th2 classification. These cytokines have a number of effects including the promotion of an allergic-type, eosinophil predominant inflammatory response (23–25). In our experiments, the Th2 cytokines were significantly diminished in the A2B R-deficient mice as compared with the WT group (Fig. 5A–C) after final allergen challenge. In addition, we examined the production of proinflammatory mediators that are considered essential in the development of asthma. IL-17, the signature cytokine produced by Th17 cells, plays a critical role in the development of the asthmatic response. IL-17 was significantly reduced in both the A2B R KO and the A2B R LysM KO mice after allergen challenge (Fig. 5D). TNF-α has been shown to be a potent mediator of several parameters in CRA-induced asthma (26). Interestingly, lack of A2B R that resulted in decreased inflammation also resulted in decreased levels of TNF-α (Fig. 5E).

**FIGURE 3.** Pulmonary neutrophil infiltration. Absolute counts of neutrophils recovered from the BAL fluid (A). LH supernatant MPO activity (B). No significant differences were observed in the infiltration of neutrophils into the airspaces or lung parenchyma between the experimental groups. Values are the mean ± SEM of two independent experiments with n = 9 for WT, n = 10 for A2B R KO, and n = 10 for LysM KO. LH concentrations of KC (C), MIP-2 (D), and RANTES (E) in WT, A2B R KO, and LysM KO groups of mice. (C–E) Values are mean ± SEM of WT (n = 5), A2B R KO (n = 6), and LysM KO (n = 6). The p values are indicated in the figure.

**FIGURE 4.** Pulmonary macrophage and lymphocyte infiltration. Absolute counts of macrophages (A) and lymphocytes (B) recovered in the BAL fluid of WT, A2B R KO, and LysM KO groups of mice. Macrophages recovered in BAL trended toward decreased but were only significantly depressed in the LysM KO mice. In contrast, lymphocyte levels were markedly reduced in the BAL fluid of A2B R-deficient groups of mice. Values are the mean ± SEM of two independent experiments with n = 9 for WT, n = 10 for A2B R KO, and n = 10 for LysM KO. The p values are indicated in the figure.

**Discussion**

The outcome of adenosine activation of inflammatory pathways is complex and depends on the stimulus for inflammation, as well as the adenosine receptor subtype activated. Adenosine–A2B R interactions promote immune-mediator release such as histamine and cytokines to direct proinflammatory and anti-inflammatory outcomes. Despite the overall immunomodulatory role of A2B Rs in inflammation, A2B R-mediated mechanisms that control inflammatory
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outcomes may differ within the same tissue compartment. For instance, the downstream effects of A2B R activation tend to be pro-inflammatory during chronic pulmonary inflammation (in the OVA asthma model) and anti-inflammatory during acute pulmonary inflammation (after LPS exposure) (6, 7, 27, 28).

Cell types that drive allergic-type inflammation such as T cells and myeloid cells secrete proinflammatory cytokines (IL-4, IL-5, IL-13) and chemokines (CC and CXC) in the lungs of sensitized mice when re-exposed to the same allergen and may be regulated by A2B R stimulation. It is known that A2B R-mediated pathology in allergic pulmonary inflammation includes airways eosinophilia and mucous production after OVA peptide inhalation in sensitized mice (6). In vitro studies show that A2B R contributes to the proinflammatory adenosine signaling on mast cells as part of the allergic response (3). However, the relative contribution of A2B Rs and proinflammatory mediators secreted in response to adenosine–A2B R interactions may be responsible.

Past studies have shown that the role for neutrophils in the development of A2B R-mediated inflammatory disease is limited, whereas the A2A Rs predominate in this respect (13, 31). Airway neutrophilia results when infused OVA-specific Th1 cells are challenged with aerosolized OVA in mice. Conversely, airway eosinophilia results when infused OVA-specific Th2 cells are challenged with aerosolized OVA in mice (32). In our studies, final challenge with cockroach Ag resulted in a mixed airway response with BAL fluid cells consisting of eosinophils and neutrophils. The mixture of allergens (cockroach proteins Blag1 and Blag2) and innate immune activators (LPS, chitin, proteases) combine to elicit an allergen-specific Th2 response in addition to an innate Th1 inflammatory response. OVA models use a simplified allergen mixture that contains low quantities of the LPS, chitin, and proteases than found in CRA, and thus elicit a cleaner and more isolated Th2 response. However, OVA is not a real-world allergen, whereas a significant percentage of urban asthmatic children are demonstrably bronchially allergic to CRA, which is encountered as a complex mix of allergens and innate immune activators as in our model (33, 34). Accordingly, we found a minimal contribution of A2B R regulatory function on airways neutrophilia by measured lung MPO activity and absolute number of alveolar neutrophils recovered from allergic-challenged sensitized WT mice compared with systemic A2B R KO, and A2B R LysM KO mice. The limited response of A2B R deletion on airways neutrophilia together with decreased airways eosinophilia and IL-4, IL-5, and IL-13, and in the lungs of myeloid-specific A2B R KO mice suggests that downstream effects of A2B R activation on myeloid cells regulate Th2-type–dependent airway inflammation. The effect of A2B R ablation to decrease proinflammatory cytokines measured in the lungs of all groups of A2B R KO mice likely reflects the overall decrease in pulmonary macrophages and lymphocytes that orchestrate Th2 responses. The data also show specificity to the lack of A2B R because not all inflammatory parameters were reduced.

We determined chemokine levels in the lungs of allergic mice after allergen challenge. Eotaxins are CC chemokines that are potent activators and chemoattractants of both eosinophils and Th2 lymphocytes (35, 36). Whereas Th1-dependent airway neutrophilia is CXC chemokine dependent (32), we found significant

FIGURE 5. Th2 and proinflammatory/immunomodulatory cytokines. LH concentrations of the Th2 cytokines IL-4 (A), IL-5 (B), and IL-13 (C) all demonstrated a significant decrease in the Th2 cytokines. In addition, the proinflammatory cytokines IL-17 (D) and TNF-α (E) were decreased in the A2B R and LysM KO mice. The classic Th1 cytokine, IFN-γ, was also significantly decreased in the KO groups (F). Values are the mean ± SEM of two independent experiments with WT (n = 5), A2B R KO (n = 6), and LysM KO (n = 6). The p values are indicated in the figure.
airway eosinophilia associated with dramatic increases in both eotaxins 1 and 2 in sensitized WT mice compared with A2B R KO and A2B R LysM KO mice challenged with allergen. We did not detect significant reductions in CX3C chemokine KC and MIP-2 in mice lacking A2B Rs on myeloid cells. The presence of neutrophils in the BAL fluid recovered in A2B R-deficient mice suggest other unmeasured neutrophil chemotactic proteins are responsible for airway neutrophilia that are A2B R independent in our model. Adenosine receptor redundancy may provide an explanation for the decrease in measured Th2 cytokines in the lungs of systemic A2B RK Oa n d A2B R LysM KO mice compared with WT mice suggest that A2B R expression by myeloid cells play a role. The cellular source of eotaxin after A2B R stimulation is unknown but may include resident cells such as epithelial as well as mononuclear cells recruited to the site of inflammation. Alternatively, decreased levels of IL-4 may be responsible for the decrease in eosinophils levels, because IL-4 is known to stimulate the secretion of eotaxin from type II epithelial cells (37). Taken together, our results suggest that eotaxin regulation by A2B Rs is a major mechanism for promoting tissue inflammation. Our studies better define A2B R expression on myeloid cells as an independent contributor to the pathogenesis of allergen-induced pulmonary inflammation and indicate that blocking adenosine-A2B R interactions on myeloid cells may be an effective immunomodulatory therapy to treat allergic-type inflammatory disease. Furthermore, our findings suggest that using nanoparticle delivery of adenosine immunomodulatory therapy to act on myeloid tissue may be a promising approach for the treatment of patients with asthma. Whether disruption of A2B R activation on myeloid cells using nanoparticle technology can be achieved remains to be determined.

Acknowledgments
We thank Akio Ohira for invaluable laboratory instruction and Stephen Hatfield for help maintaining mutant mouse lines used in this manuscript.

Disclosures
The authors have no financial conflicts of interest.

References
Thorax 65: 151–155.
Am. J. Pathol. 177: 1861–1869.
Application in a Brown Norway rat model of allergic pulmonary inflammation. 
Resp. Rev. 2: 150–156.


