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A3 Adenosine Receptor Signaling Contributes to Airway Inflammation and Mucus Production in Adenosine Deaminase-Deficient Mice

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Adenosine signaling has been implicated in chronic lung diseases such as asthma and chronic obstructive pulmonary disease; however, the specific roles of the various adenosine receptors in processes central to these disorders are not well understood. In this study, we have investigated the role(s) of the A3 adenosine receptor in adenosine-dependent pulmonary inflammation observed in adenosine deaminase (ADA)-deficient mice. The A3 receptor (A3R) was found to be expressed in eosinophils and mucus-producing cells in the Airways of ADA-deficient mice. Treatment of ADA-deficient mice with MRS 1523, a selective A3R antagonist, prevented airway eosinophilia and mucus production. Similar findings were seen in the lungs of ADA/A3 double knockout mice. Although eosinophils were decreased in the Airways of ADA-deficient mice following antagonism or removal of the A3R, elevations in circulating and lung interstitial eosinophils persisted, suggesting signaling through the A3R is needed for the migration of eosinophils into the Airways. These findings identify an important role for the A3R in regulating lung eosinophilia and mucus production in an environment of elevated adenosine. The Journal of Immunology, 2004, 173: 1380–1389.

Asthma and chronic obstructive pulmonary disease (COPD) are lung diseases that afflict millions of individuals and result in billions of dollars in annual health care cost. In contrast to most injury and repair responses, the inflammation seen in these disorders is chronic and may last throughout the life of the afflicted individual. Although signaling pathways associated with the genesis of inflammation and the control of tissue remodeling have been described (1, 2), little is known about signaling pathways that serve to regulate the chronic nature of these diseases.

Adenosine is a potent and ubiquitous signaling nucleoside that is generated at sites of tissue damage and hypoxia. Accordingly, adenosine levels are elevated in the bronchoalveolar lavage fluid (BALF) (3) and exhaled breath condensate (4) of asthmatic patients where significant lung inflammation and hypoxia exist. These elevations suggest that adenosine signaling may regulate aspects of chronic lung disease. This hypothesis is supported by recent studies demonstrating that elevations in adenosine evoke signaling pathways that lead to chronic lung disease in mice (5–7). Additional evidence linking adenosine to asthma and COPD include the observations that exposing patients to exogenous adenosine can elicit bronchoconstriction, whereas adenosine has little effect on control subjects (8–11). However, relatively little is known about the function of adenosine signaling on specific cell types in the inflamed lung.

Adenosine engages distinct subtypes of P1 purinergic receptors (A1, A2A, A2B, and A3 adenosine receptors), which are seven-transmembrane proteins that couple to heterotrimeric G-proteins (12). Engagement of adenosine receptors is associated with both pro- and anti-inflammatory actions that are dictated by specific receptor subtypes, downstream signaling components and levels of available ligand (12–14). The A3 adenosine receptor A3R has emerged as an adenosine receptor subtype that may serve important regulatory roles in the inflamed lung. Walker et al. (15) demonstrated that transcript levels for the A3R are elevated in lung biopsies of patients with asthma or COPD, suggesting increased signaling through this receptor. Moreover, numerous in vivo and ex vivo studies suggest that A3R signaling can influence inflammatory cell types associated with asthma and COPD (16–22). In rodents, airway mast cells express the A3R (22) and engagement of this receptor can promote (22) or enhance (23, 24) mediator release from mast cells. In addition, human eosinophils express the A3R (25); however, the function of A3R signaling on this cell type remains controversial with both pro- (26) and anti- (17) inflammatory activities being reported. Expression of the A3R in the asthmatic lung (15) in association with adenosine elevations (3) suggests A3R engagement may regulate features of this disease. However, studies examining the importance of the A3R in inflamed lungs that exhibit elevations in adenosine are lacking.

Adenosine deaminase (ADA) is a purine catabolic enzyme that converts adenosine to inosine, and in so doing helps to regulate the levels of adenosine present in tissues and cells. Consistent with this, mice deficient in ADA exhibit elevations in adenosine in many organs including the lung (27). ADA-deficient mice develop
features of chronic lung disease in association with elevations in lung adenosine concentrations (5). Features seen include the accumulation of eosinophils and activated macrophages in the airways, mucus metaplasia in the bronchial airways, and airway destruction. Many of the pulmonary features seen in ADA-deficient mice are reversible following the lowering of lung adenosine levels using ADA enzyme therapy (5, 6, 28, 29), suggesting that adenosine signaling plays an important role in the exacerbation of pulmonary inflammation and airway remodeling. The current study examines the role(s) of the A3R in the airway inflammation seen in ADA-deficient mice. Findings suggest that the A3R plays a proinflammatory role in ADA-deficient airway disease by contributing to airway inflammation and mucus production.

Materials and Methods

Mice
ADA-deficient mice used for antagonist studies were on a 129sv-C57BL/6J-FVBn mixed background (30, 31), and genotypes were determined by PCR analysis of genomic DNA using the following primers: wild-type sense primer, 5′-CCTTGAAGGCCATGATTCTGTA-3′; wild-type antisense primer, 5′-AGGAATGGACCGGACCTTGAT-3′; and ADA minigene primers (trans-5, 9, 13, and 17 (0.625, 1.25, 2.5, 2.5, 2.5 U, respectively). ADA control mice. Findings suggest that the A3R plays a proinflammatory role in ADA-deficient airway disease by contributing to airway inflammation and mucus production.

RNA isolation, RT-PCR, and quantitative RT-PCR (Q-RT-PCR) analysis
Total RNA was isolated from whole lung tissue using TRIzol reagent (Invitrogen Life Technologies, Grand Island, NY). Total RNA was DNase treated (Invitrogen Life Technologies) and quality was assessed by electrophoresis through formaldehyde agarose gels. Lung RNA (1 μg) was used in a Superscript One Step RT-PCR (Invitrogen Life Technologies) with A3R or β-actin-specific primers following manufacturer’s instructions. Alternatively, transcript levels were quantified using real-time Q-RT-PCR. Adenosine receptor and β-actin transcripts were analyzed using TaqMan probes on the Smart Cycler (Cepheid, Sunnyvale, CA) (28). MacSac: 5′-GGGAAATGGT GCTTACATGACG-3′, antisense: 5′-TCTTGAAGGCCATGATTCTGTA-3′. The mean intensity of PAS/AB staining was determined by dividing the (area of PAS/AB staining) × (mean intensity of PAS/AB staining)) by the total area of the airway epithelium (5).

Histology and period acid Schiff (PAS)/AB staining
Mice were sacrificed and the lungs inflated with 0.5 ml of fixative (4% paraformaldehyde in PBS) before fixation overnight at 4°C. Fixed lung tissues were embedded in paraffin and 5-μm sections were stained with H&E using a Rapid Chrome staining kit (Thermo Shandon, Pittsburgh, PA). Similar sections were stained with PAS counterstained with Alcian blue (AB). The mucus index score was determined by dividing the (% of areas of PAS/AB staining) (mean intensity of PAS/AB staining)) by the total area of the airway epithelium (5).

Bronchoalveolar lavage and peripheral blood analysis
Mice were anesthetized, the trachea isolated by blunt dissection, and a small caliber catheter secured into the airways. Two volumes of 1 ml of PBS with 0.1% BSA were instilled and gently aspirated and pooled (BALF). Samples were then centrifuged at 2500 rpm for 5 min to recover cells. Cell pellets were resuspended in PBS and total cell counts determined using a hemocytometer. Aliquots were cytopsin and stained for cellular differentials (5). Whole blood was collected from the tail vein and smeared onto microscope slides for differentials. Total peripheral white blood cell counts were determined on hemolysed aliquots using a hemocytometer.

Immunochemical analysis
Deparaffinized sections were rehydrated in a series of graded alcohols to water. Endogenous peroxidase activity was blocked by incubation in 0.5% H2O2 in methanol (20 min). Slides were then treated with 15% N 1% normal rabbit serum in PBS (ARI) for 1 h at room temperature with a 1/3000 dilution of rabbit anti-mouse major basic protein 1 (mMBP-1) Ab (Ab) followed by development with a rabbit IgG Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA). Quantification of peribronchial eosinophils was assessed by analysis of 10 fields at ×20 magnification. The number of peribronchial eosinophils within a 0.6-mm2 area were counted using Image Pro Plus 4.0 analysis software (MediaCybernetics, Silver Spring, MD).
A3R SIGNALING IN THE LUNGS OF ADA-DEFICIENT MICE

Results

A3R transcripts in the lungs of ADA−/− mice

Total RNA was isolated from the lungs of 18-day-old mice and A3R transcripts were analyzed by RT-PCR. Although A3R transcripts were detectable in the lungs of both ADA control and ADA−/− mice, the prevalence of these transcripts increased in the lungs of ADA−/− mice (Fig. 1). These findings demonstrate that A3R transcript levels are elevated in the lungs of ADA−/− mice at a time when adenosine concentrations, eosinophil numbers, and mucus production are all increased in the lung (5).

Localization of A3R transcripts in the lungs of ADA−/− mice

Activation of the A3R can elicit various responses depending on the cell type and downstream signaling components involved (15–20, 22, 39). We examined the localization of A3R transcripts using in situ hybridization. A3R transcripts were not detectable in the lungs of ADA control mice (Fig. 2A), whereas, transcripts for the A3R were abundant in the mucus-containing epithelial cells of ADA−/− mice (Fig. 2, C and D). Expression of the A3R was also seen in areas of inflammation (data not shown). These findings demonstrate that the increases in the A3R transcripts in the lungs of ADA−/− mice are located at sites of inflammation and mucus metaplasia.

Expression of the A3R in mouse eosinophils

General localization of A3R transcripts in inflammatory cells from the lungs of ADA−/− mice suggests that eosinophils may express the A3R. However, the cellular resolution of A3R expression was difficult using the in situ hybridization technique. Therefore, we quantified A3R transcripts in isolated eosinophils to confirm expression in this cell type. An established model of OVA sensitization and challenge (33) was used to generate a large number of airway eosinophils. Wild-type mice sensitized to OVA and challenged with saline (o/s) exhibited predominantly macrophages in the BALF (Fig. 3A), whereas mice challenged with OVA (o/o) exhibited a robust increase in BALF eosinophils (Fig. 3A). A3R transcript levels were markedly increased in RNA extracts from BALF cell pellets of o/o mice (Fig. 3B), suggesting A3R transcripts are present in eosinophils recruited to mouse airways.

Eosinophil chemotaxis assay

Chemotactic capacity of ADA−/− BALF was assayed as described previously (37). Briefly, the migration of purified eosinophils from the blood of IL-5 transgenic mice (38) was investigated using 3-μm polycarbonate membrane transwell inserts in 24-well tissue culture polystyrene plates (Costar, Corning, NY). The inserts contained eosinophils (1 × 10^6) and 30 ng/ml recombinant mouse IL-5. Three eosinophil populations were examined: 1) purified eosinophils with no additional manipulation; 2) eosinophils pretreated with 100 nM IB-MECA; and 3) eosinophils treated initially with 5 μM MRS 1523 followed by 100 nM IB-MECA. The inserts were then placed in the wells containing 500 μl of medium alone or medium containing mouse eotaxin-1 (positive control), ADA control, or ADA−/− BALF. The plates were incubated at 37°C, 5% CO₂ for 90 min. The cells that had migrated to the lower chamber were collected and counted using a hemacytometer.
next examined A3R transcript levels in RNA isolated from eosinophils purified from the circulation of IL-5 transgenic mice (38). These eosinophils possessed no A1R transcript, and relatively low levels of A2AR and A2BR transcripts. In contrast, A3R transcripts were relatively abundant (Fig. 3C). Collectively, these data demonstrate that the A3R is expressed in mouse eosinophils recovered from both the circulation and the airways.

**Effects of A3R antagonism on lung inflammation in ADA−/− mice**

ADA−/− mice were treated with the A3R antagonist MRS 1523 to assess the contributions of A3R signaling to the adenosine-mediated pulmonary alterations in these mice. ADA−/− mice were maintained on ADA enzyme therapy until postnatal day 17 to allow the mice to mature to a size amenable to the implantation of osmotic pumps containing MRS 1523 (22). On postnatal day 19, mice were implanted with a s.c. osmotic pump containing either MRS 1523 (100 μg/kg/day) or vehicle (saline). On postnatal days 29–31, which was 12–14 days after the cessation of ADA enzyme therapy, adenosine levels were elevated in the lungs of ADA−/− mice (40). At this stage, various pulmonary endpoints were examined. ADA control mice treated with saline or MRS 1523 exhibited normal lung pathologies (Fig. 4, A and B), indicating that there

![Figure 3](https://example.com/figure3.png)

**FIGURE 3.** A3R transcripts are abundant in eosinophil-rich BALF pellets and circulating eosinophils. A, Cellular differentials were determined by counting 400 cells on Diff-quik-stained cytospun slides from BALF collected from OVA sensitized and saline challenged (O/S) and OVA sensitized and OVA challenged (O/O) mice. The cell types are presented as a percentage of cells ± SEM, n = 3. B, Total RNA was isolated from BALF cell pellets from O/S and O/O mice. RNA samples were subjected to Q-RT-PCR for the A3R. Values presented are mean ± SEM, n = 3. C, Total RNA was isolated from eosinophils collected from the circulation of IL-5 overexpressing transgenic mice. The RNA was examined by Q-RT-PCR to determine the levels of adenosine receptors in this cell type. Values are mean ± SEM, n = 3. (*, p ≤ 0.05, Student’s t test).

![Figure 4](https://example.com/figure4.png)

**FIGURE 4.** Blocking A3R signaling leads to a decrease in infiltrating inflammatory cells. ADA control and ADA−/− mice were implanted with osmotic pumps as described in Materials and Methods. A, At 29–31 days of age, the total number of cells recovered from the BALF was determined. Values are given as mean total cell counts × 10^5 ± SEM (*, p ≤ 0.05, Student’s t test). B, Cellular differentials were determined on aliquots of cells from 29 to 31-day-old ADA control and ADA−/− mice treated with saline or MRS 1523. Values are given as mean total cell counts × 10^5 ± SEM, n = 5. (*, p ≤ 0.05 ADA−/− saline vs ADA control saline; #, p ≤ 0.05 ADA−/− MRS 1523 vs ADA−/− saline using a Student’s t test).

![Figure 5](https://example.com/figure5.png)

**FIGURE 5.** MRS 1523 treatment decreases the number of eosinophils in the BALF of ADA−/− mice. ADA control and ADA−/− mice were implanted with osmotic pumps as described in Materials and Methods. A, Scale bar in A = 100 μm and applies to A–D.
were no adverse responses due to pump implantation or A3R antagonism. In contrast, ADA−/− mice treated with saline exhibited increased pulmonary inflammation (Fig. 4C), and this inflammation was diminished by MRS 1523 treatment (Fig. 4D). Total cells infiltrating the airways of ADA−/− mice increased (Fig. 5A), and this increase was prevented by MRS 1523 treatment. The most abundant cell types found in these airways were macrophages and eosinophils (Fig. 5B). Treatment with MRS 1523 was associated with a decrease in all cell types in the airways, with an almost complete ablation of eosinophils (Fig. 5B). These data demonstrate that treatment with the A3R antagonist MRS 1523 can attenuate pulmonary inflammation and eosinophil infiltration into the airways of ADA−/− mice.

**Localization of eosinophils in ADA−/− mice following antagonist treatment**

Absence of eosinophils in the airways of ADA−/− mice treated with MRS 1523 prompted us to examine circulating and lung interstitial eosinophil numbers to determine whether A3R antagonism was affecting eosinophil production or trafficking. Lung interstitial eosinophils were localized using an Ab against mMBP-1 that is selectively produced by eosinophils (41). Few mMBP-1-positive cells were observed in the peribronchial region of ADA control mice treated with saline or MRS 1523 (data not shown). In contrast, mMBP-1 positive eosinophils were readily detected in peribronchial regions of ADA−/− mice treated with saline (Fig. 6A). Interestingly, mMBP-1 positive eosinophils persisted in the peribronchial spaces of ADA−/− mice treated with MRS 1523 (Fig. 6B). Quantification of these findings demonstrated a significant decrease in interstitial lung eosinophilia in ADA−/− mice treated with MRS 1523; however, levels remained significantly elevated over control values (Fig. 6C). Examination of eosinophil levels in circulation revealed a significant increase in eosinophils in the circulation of ADA−/− mice treated with saline (Fig. 6D). This increase persisted in ADA−/− mice treated with MRS 1523, which was in marked contrast to the decreased eosinophilia seen in the BALF of these mice (Fig. 5B). Collectively, these data demonstrate that A3R antagonism does not affect the production of eosinophils in ADA−/− mice, and suggest that A3R signaling may impact eosinophil trafficking in the lungs.

**Effects of MRS 1523 on mucus production in the airways of ADA−/− mice**

Elevations in adenosine are associated with increased mucus production in the airways of ADA−/− mice (5). Mucus production was investigated in the airways of ADA−/− mice treated with control saline or saline and MRS 1523 vs ADA control saline and MRS 1523 using a Student’s t test. Values are presented as total eosinophils per mm3 ± SEM, n = 5. (*, p ≤ 0.05 ADA−/− saline and MRS 1523 vs ADA control saline; #, p ≤ 0.05 ADA−/− MRS 1523 vs ADA−/− saline using a Student’s t test).
MRS 1523 to determine the importance of A3R signaling in this process. ADA control mice treated with saline (A) or MRS 1523 (B). ADA−/− mice treated with saline (C) or MRS 1523 (D). Arrows denote mucus production. Scale bar in A = 100 μm and applies to A–D. E, Mucus production was quantified as described in Materials and Methods. Values are presented as mean mucus index score ± SEM, n = 5. (*, p ≤ 0.05 ADA−/− saline vs ADA control saline; #, p ≤ 0.05 ADA−/− MRS 1523 vs ADA−/− saline using a Student’s t test).

FIGURE 8. Persistent elevations in cytokines and chemokines following MRS 1523 treatment. A, Cytokine transcript levels were quantified in lungs of ADA control and ADA−/− mice treated with saline or MRS 1523 using SYBR Green-based Q-RT-PCR. B, Chemokine transcript levels were quantified using Q-RT-PCR. Values are presented as the percentage of β-actin ± SEM, n = 5. (*, p ≤ 0.05 ADA−/− vs ADA control saline using a Student’s t test).
the development of adenosine-induced mucus production in this model.

Cytokine and chemokine levels in the lungs of MRS 1523-treated mice

Recruitment of inflammatory cells to the lung is associated with increased expression of proinflammatory cytokines and chemokines (2). Cytokine and chemokine levels were examined to determine whether A3R antagonism could impact the expression of these mediators. Levels of IL-5, IL-13, and IL-6 were elevated in the lungs of ADA−−/− mice treated with saline, and levels continued to be elevated in the lungs of ADA−−/− mice treated with MRS 1523 (Fig. 8A). Similar observations were made regarding the levels of the chemokines MCP-3, TARC, and eotaxin 1 (Fig. 8B). These findings demonstrate that increases in proinflammatory cytokines and chemokines in the lungs of ADA−−/− mice occur in an A3R-independent manner.

Pulmonary phenotypes in ADA/A3 double knockout mice

ADA−−/− mice were crossed onto an A3−−/− background to generate ADA/A3 double knockout mice to genetically determine the contribution of the A3R to the lung disease seen in ADA−−/− mice. Similar to what was seen following A3R antagonism, removal of the A3R resulted in diminished lung inflammation in ADA−−/− mice.
mice (Fig. 9A). In particular, there was a substantial decrease in the number of airway macrophages and eosinophils. The number of eosinophils found in the interstitial spaces surrounding bronchioles of ADA/A3 double knockout mice were decreased (Fig. 9B); however, there was no decrease in the degree of eosinophilia in the circulation (Fig. 9C). In addition, cytokine and chemokine increases in the lungs of ADA/A3 double knockouts were similar to those seen in ADA−/− mice treated with MRS 1523 (data not shown). These findings provide genetic evidence that A3R signaling contributes to inflammation and eosinophil trafficking in the lungs of ADA−/− mice.

Mucus production was examined in ADA/A3 double knockout mice to confirm the involvement of the A3R in this attribute of the ADA−/− pulmonary phenotype. As was seen following MRS 1523 treatment, genetic removal of the A3R was associated with decreased mucus production in the lungs of ADA−/− mice (Fig. 9D). These findings reiterate the importance of A3R signaling in the production of mucus in the lungs of ADA−/− mice.

Ex vivo effects of A3R signaling on eosinophil chemotaxis

Previous studies have demonstrated that engagement of the A3R on human eosinophils can inhibit chemotaxis ex vivo (15, 18). To further examine the chemotactic properties in the lungs of ADA−/− mice, and to determine the impact of A3R signaling on mouse eosinophil chemotaxis ex vivo, purified mouse eosinophils were placed in chemotaxis chambers and the chemotactic index of BALF from ADA control and ADA−/− mice was determined. Purified eosinophils exhibited little chemotactic activity toward BALF from the lungs of ADA control mice; however, substantial chemotactic activity was seen in ADA−/− BALF (Fig. 10). Activation of the A3R by treatment with the A3R agonist IB-MECA decreased chemotaxis toward BALF from ADA−/− mice as well as toward the positive control eotaxin 1. This inhibition of chemotaxis was lost when eosinophils were incubated with the A3R antagonist MRS 1523 together with IB-MECA. These findings demonstrate that BALF from ADA−/− mice is chemotactic for eosinophils, and that A3 signaling can block this chemotaxis ex vivo.

Discussion

Adenosine is a signaling molecule that is generated in response to tissue damage and hypoxia. High levels of this nucleoside are found in the airways of asthmatics (3, 4), suggesting adenosine signaling may play a role in the exacerbation of this disease. However, the mechanisms by which adenosine influences aspects of lung disease are not fully appreciated. In the current study, we provide novel evidence that A3R signaling contributes to airway eosinophilia and mucus production in a model of ADA deficiency where lung adenosine levels are chronically elevated.

Transcript levels for the A3R are elevated in the lungs of asthma and COPD patients, where expression is localized to eosinophilic infiltrates (15). Similarly, we found that A3R transcripts are elevated in the lungs of ADA−/− mice where transcripts localized to sites of eosinophilic inflammation. Increased levels of A3R transcripts were also seen in the BALF of allergen-challenged mice where the predominant infiltrate was eosinophils, and analysis of eosinophils purified from peripheral blood revealed that the A3R is the most abundant adenosine receptor in eosinophils. It is not clear from these studies whether or not the levels of A3R, particularly at the cell surface, change in response to inflammatory cues; however, the expression of the A3R in both circulating and pulmonary eosinophils suggest that it may play a role in regulating eosinophil function. The exact role of the eosinophil in asthma is not fully understood; however, its presence in the airways of patients with severe asthma suggests that this cell may contribute to the exacerbation of airway disease by damaging epithelial cells and contributing to airway remodeling (1). Thus, the presence of the A3R on human eosinophils (16, 25), together with the high levels of adenosine found in the lungs of asthmatics (3, 4), suggest that A3 signaling may play an important role in regulating eosinophil function in the asthmatic lung.

ADA−/− mice exhibit adenosine-dependent airway and lung eosinophilia in association with elevations in lung adenosine levels (5). Contribution of A3 signaling to lung inflammation and damage in the lungs of ADA−/− mice was conducted using both pharmacologic and genetic approaches. The pharmacologic approach consisted of treatment of ADA−/− mice with a selective A3R antagonist (MRS 1523), which has been used successfully in vivo in mice (22, 34). The genetic approach entailed the generation of ADA/A3 double knockout mice. Similar results were seen with both methods and suggest that A3R signaling contributes to proinflammatory features of adenosine in this model. Closer examination of eosinophils revealed no change in the degree of peripheral blood eosinophilia in ADA−/− mice following disruption of A3 signaling suggesting this signaling pathway is not involved in the production or mobilization of eosinophils from the bone marrow. However, the reduction of BALF eosinophilia following antagonism and removal of the A3R suggests A3R signaling is involved in the migration of eosinophils from the circulation to the airways.

Diminished airway eosinophilia following the disruption of A3 signaling is in opposition to studies performed in human eosinophils ex vivo, where chemotaxis of purified eosinophils toward known chemotactic agents was blocked by A3R activation (15, 18). This discrepancy could be due to differences in mouse and human eosinophils or to differences attributed to the ex vivo nature of the chemotaxis experiments performed. Our examination of A3R signaling on mouse eosinophils ex vivo revealed that activation of the A3R decreases chemotaxis of murine eosinophils toward ADA−/− BALF as well as eotaxin I, suggesting that the impact of A3R signaling on migration ex vivo is similar in human and mouse eosinophils. Furthermore, our findings demonstrate that significant differences exist between the impact of A3R signaling on eosinophil migration ex vivo and in the whole animal. It is therefore likely that the diminished airway eosinophilia seen in the lungs of ADA−/− mice following disruption of A3R signaling is not a cell autonomous effect of eosinophils. Rather, disruption of A3R signaling in ADA−/− mice must impact the expression or activity of key regulatory molecules from other cells that express the A3R that in turn impact eosinophil migration. The A3R is expressed on murine mast
cells (22), airway macrophages (6), and airway epithelial cells (Fig. 2), all of which may produce regulatory molecules that can impact eosinophil migration. As an attempt to address this issue, the levels of key regulatory cytokines and chemokines were measured in the lungs of ADA−/− mice with and without disruption of A3R signaling. Levels of key eosinophil regulatory cytokines (IL-5, IL-13) and chemokines (eotaxin I, TARC, MCP-3) were elevated in ADA−/− lungs, whereas the levels were not diminished following antagonism or removal of the A3R, suggesting regulation of these molecules is not involved in preventing airway eosinophilia in response to disrupted A3R signaling. Additional studies are needed to determine the impact of key regulatory cytokines and chemokines in the lungs of ADA−/− mice. Whether or not the A3R is also expressed on mucus-producing cells of the asthmatic lung is not known, nor has the function to prevent the migration of eosinophils into the airways been studied in human asthmatics (3, 4). A3R antagonism may provide a mechanism for reducing airway eosinophilia.

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