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Adenosine-Activated Mast Cells Induce IgE Synthesis by B Lymphocytes: An $A_{2B}$-Mediated Process Involving Th2 Cytokines IL-4 and IL-13 with Implications for Asthma$^{1,2}$

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Adenosine provokes bronchoconstriction in asthmatics through acute activation of mast cells, but its potential role in chronic inflammation has not been adequately characterized. We hypothesized that adenosine up-regulates Th2 cytokines in mast cells, thus promoting IgE synthesis by B lymphocytes. We tested this hypothesis in human mast cells (HMC-1) expressing $A_{2A}, A_{2B},$ and $A_3$ adenosine receptors. The adenosine analog 5'-N-ethylcarboxamidoadenosine (NECA) (10 $\mu$M) increased mRNA expression of IL-1$\beta$, IL-3, IL-4, IL-8, and IL-13, but not IL-2 and IFN-$\gamma$. Up-regulation of IL-4 and IL-13 was verified using RT-PCR and ELISA; 10 $\mu$M NECA increased IL-13 concentrations in HMC-1 conditioned medium 28-fold, from 7.6 $\pm$ 0.3 to 215 $\pm$ 4 pg/ml, and increased IL-4 concentrations 6-fold, from 19.2 $\pm$ 0.1 to 117 $\pm$ 2 pg/ml. This effect was mediated by $A_{2B}$ receptors because neither the selective $A_{2A}$ agonist 2-p-(2-carboxyethyl)phenethylamino-NECA nor the selective $A_3$ agonist N$^\beta$-(3-iodobenzyl)-N$^\alpha$-methyl-5'-carbamoyladenosine reproduced it, and the selective $A_{2B}$ antagonist 3-isobutyl-8-pyrrolidinoxanthine prevented it. Constitutive expression of CD40 ligand on HMC-1 surface was not altered by NECA. Human B lymphocytes cocultured for 12 days with NECA-stimulated HMC-1 produced 870 $\pm$ 33 pg IgE per 10$^6$ B cells, whereas lymphocytes cocultured with nonstimulated HMC-1, or cultured alone in the absence or in the presence of NECA, produced no IgE. Thus, we demonstrated induction of IgE synthesis by the interaction between adenosine-stimulated mast cells and B lymphocytes, and suggest that this mechanism is involved in the amplification of the allergic inflammatory responses associated with asthma. The Journal of Immunology, 2004, 172: 7726–7733.

Adenosine is an intermediate product in the metabolism of ATP. Extracellular adenosine accumulates in inflamed areas due to its release from stressed or damaged cells. Adenosine exerts its action by binding to G protein-coupled adenosine receptors. Four subtypes of adenosine receptors have been cloned and classified as $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ receptors.

There is growing evidence that adenosine plays a role in asthma and chronic obstructive pulmonary disease (COPD), disorders associated with chronic lung inflammation. Elevated concentrations of adenosine are found in bronchoalveolar lavage fluid (1) and exhaled breath condensate (2) obtained from asthmatics.

Initial studies examining the potential role of adenosine in asthma focused primarily on its acute effects. Administration by inhalation of adenosine, or its precursor AMP, induces bronchospasm in patients with asthma and COPD, but not in normal controls (3, 4). Adenosine-induced bronchoconstriction is most likely mediated by activation of mast cells because this effect is blocked not only by adenosine receptor antagonists (5), but also by selective $H_2$ blockers (6, 7) and chromolyn sodium (8). Inhaled adenosine has been shown to increase levels of mast cell mediators such as histamine and tryptase in bronchoalveolar lavage fluid from asthmatics (9). Adenosine seems to participate early in the cascade of events, leading to triggering of bronchoconstriction, because adenosine-induced bronchoconstriction is also prevented by leukotriene inhibitors (10).

More recent studies suggest that responses to adenosine correlate with chronic inflammation. Adenosine hyperresponsiveness in asthma has been proposed to be superior as a clinical indicator of chronic airway inflammation than alternative indices. For example, AMP challenge improves with avoidance of allergens in children with allergic asthma, whereas metacholine does not (11), suggesting that AMP is better at reflecting airway inflammation. Also, anti-inflammatory treatment with steroids results in a greater improvement in the AMP test than the methacholine challenge (12, 13). The AMP test has also been used in patients with COPD, and response to this test seems to correlate with indices of inflammation (14).

It is possible, therefore, that adenosine not only induces the release of mast cell-derived mediators involved in acute bronchoconstrictor responses, but may also induce the release of proinflammatory cytokines. Recent studies in adenosine deaminase-deficient mice, characterized by elevated lung tissue levels of...
adeno-sine, strongly suggest a causal association between adeno-sine and an inflammatory phenotype (15, 16). These mice exhibit a lung phenotype with features of lung inflammation, including bronchial hyperresponsiveness, enhanced mucus secretion, increased IgE synthesis, and elevated levels of proinflammatory cyto-kines in bronchoalveolar lavage that could be reversed with ex-ogenous adenosine deaminase. Even more striking are the similarities in lung inflammatory phenotypes found between these mice with a null mutation of adenosine deaminase and mice over-expressing IL-13 (17).

IL-13 shares many biological properties with IL-4. Both are secreted by activated Th2 lymphocytes (18, 19) and mast cells (20–22). These cytokines are overproduced in asthma and play important roles in allergic reactions (23–26). One of the most im-portant functions of IL-4 and IL-13, related to mechanisms of al-lergic asthma and immediate-type hypersensitivity, is their ability to induce IgE synthesis in naïve B lymphocytes. IgG isotype switch- ing to IgE has been attributed to secretion of IL-4 and IL-13 by Th2 cells and to their provision of CD40 ligand (CD40L; CD154) through physical interaction with B cells (27, 28). More recently, mast cells also have been implicated in isotype switching of B cells (20, 29), a process that may occur in peripheral organs such as the lung. However, a potential role of adenosine and specific adeno-sine receptor subtypes in these processes has not been studied. To elucidate the role of adenosine in mast cell-mediated inflammation, our work, we tested the hypothesis that adenosine up-regulates Th2 cytokines in mast cells, and promotes IgE synthesis by B lymphocytes.

Materials and Methods
Cell culture and treatment conditions

Human mast cells-1 (HMC-1) were a generous gift from J. Buttefield (Mayo Clinic, Rochester, MN). Human cord blood CD19+ B cells were purchased from Poietics/Cambrex Bio Science (Walkersville, MD). Cells were maintained in Iscove's medium supplemented with 10% (v/v) FBS, 2 mM glutamine, 1× antibiotic-antimycotic mixture (Life Technologies, Gaithersburg, MD), and 1.2 mM α-thioglycerol under humidified atmo-sphere of air/CO2 (19:1) at 37°C in the presence of 10 μM NECA or its vehicle (DMSO). The culture medium was then collected by centrifugation at 200 × g for 10 min at 4°C. Secreted cytokines were assayed with RayBio Human Cytokine Array III (RayBiotech, Norcross, GA) in accordance with manufacturer's instruc-tions. The cytokine-specific Abs were incubated in HMC-1 conditioned medium for 2 h at room temperature to allow secreted cytokines to bind to their corresponding targets. Mem-branes were then washed and incubated with a mixture of cytokine-specific biotin-conjugated detection Abs for another 2 h. After washing, mem-branes were exposed to HRP-conjugated streptavidin for 1 h. The membranes were washed again, and the signals were visualized with an ECL method.

Determination of IL-4 and IL-13 levels in conditioned medium

HMC-1 (5 × 106 cells/ml) were incubated in serum-free Iscove's medium containing 2 mM glutamine, 1.2 mM α-thioglycerol, and 1 U/ml adenosine deaminase for 6 h under humidified atmosphere of air/CO2 (19:1) at 37°C with the reagents indicated in Results. At the end of this incubation period, the culture medium was collected by centrifugation at 200 × g for 10 min at 4°C. Cytokine concentrations were measured using IL-4 (eBioscience, San Diego, CA) and IL-13 (Cell Sciences, Norwood, MA) ELISA kits.

Determination of CD40L surface expression

CD40L (CD154) surface expression was determined by flow cytometry. HMC-1 cells were harvested by centrifugation at 200 × g for 10 min and resuspended at concentration of 106 cells/ml in PBS, pH 7.4, containing 1% BSA (PBS/BSA). Non-specific binding sites on cell surface were blocked by immersion of HMC-1 in the presence of 0.2% normal goat serum IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) in PBS/ BSA for 15 min at room temperature. Cells were then washed with PBS and resuspended at the same concentration in PBS/BSA containing 1 μg/ml anti-CD154 FITC-conjugated mouse anti-human mAb (BD Biosciences, San Jose, CA), or isotype-matched FITC-conjugated mouse IgG1 (BD Biosciences). After incubation for 30 min at room temperature, cells were washed twice with incubated in PBS/BSA containing 1 μg/ml propidium iodine for another 30 min. Stained cell suspensions were analyzed using a FACScalibur flow cytometer (BD Biosciences). Forward and side light scatter were used to select cell populations for analysis. Dead cells were excluded by electronic FACS gating.

Stimulation of IgE synthesis in human B lymphocytes

HMC-1 (5 × 106 cells/ml) were incubated in serum-free Iscove’s medium containing 2 mM glutamine, 1.2 mM α-thioglycerol, and 1 U/ml adenosine deaminase for 6 h under humidified atmosphere of air/CO2 (19:1) at 37°C in the presence of 10 μM NECA or its vehicle (DMSO). Conditioned medium was collected by centrifugation at 200 × g for 10 min, and fresh HMC-1 cells were resuspended in this conditioned medium, but at lower concentrations (105 cells/ml) to prevent B cell overgrowth. HMC-1 cell suspensions or equivalent volumes (100 μl) of identical control medium without cells were added to 106 human cord blood CD19+ B cells growing in 100 μl of Iscove's medium supplemented with 20% (v/v) FBS, 2 mM glutamine, 1× antibiotic-antimycotic mixture, 1.2 mM α-thioglycerol, and 1 U/ml adenosine deaminase. Stimulation of B cells with 10 ng of IL-4 and 2 μg/ml anti-CD40 mAb EA-5 (BioSource International, Camarillo, CA) was used as a positive control. Secreted IgE was measured with an ELISA kit.

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kit (Bethyl Laboratories, Montgomery, TX) in supernatants after incubation for 12 days under humidified atmosphere of air/CO$_2$ (19:1) at 37°C.

**Results**

Adenosine-induced expression of cytokines in human mast cells

We used a gene expression array assay as an initial step in screening of adenosine-induced expression of cytokines in HMC-1. Incubation for 3 h of HMC-1 in the presence of 10 μM NECA and 1 U/ml adenosine deaminase increased mRNA expression of the cytokines IL-1β, IL-3, IL-4, IL-8, and IL-13 by 2.4-1006, 4.1-1006, 3.7-1006, 6.7-1006, and 19.9-1006-fold, respectively, compared with cells incubated with vehicle and 1 U/ml adenosine deaminase (Fig. 1, A and B). The screening confirmed the previously reported adenosine-induced expression of IL-8. Up-regulation of IL-4 and IL-13 is an important new finding indicating that adenosine can stimulate generation of Th2 cytokines in human mast cells, potentially facilitating allergic reactions with implications for asthma. We therefore thought important to confirm adenosine-dependent increase in IL-4 and IL-13 mRNA expression after 3 h incubation with 10 μM NECA and 1 U/ml adenosine deaminase using a semiquantitative RT-PCR technique (Fig. 1C).

Finally, we used a cytokine protein array assay to detect cytokines secreted from mast cells stimulated with 10 μM NECA for 6 h. As seen in Fig. 1D, no significant cytokine secretion was detected in control medium collected from nonstimulated HMC-1. NECA-stimulated mast cells, however, secreted IL-3, IL-4, IL-8, and IL-13 into conditioned medium.

Table 1. Binding affinity of the adenosine agonists and A$_{2B}$ antagonist used in this study at human adenosine receptor subtypes (K$_D$ or K$_I$ values in μM)

<table>
<thead>
<tr>
<th></th>
<th>A$_1$</th>
<th>A$_{2A}$</th>
<th>A$_{2B}$</th>
<th>A$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NECA</td>
<td>0.014$^a$</td>
<td>0.02$^a$</td>
<td>0.33$^b$</td>
<td>0.006$^a$</td>
</tr>
<tr>
<td>CGS21680</td>
<td>0.29$^a$</td>
<td>0.027$^a$</td>
<td>361$^b$</td>
<td>0.067$^a$</td>
</tr>
<tr>
<td>IB-MECA</td>
<td>0.0037$^a$</td>
<td>2.5$^a$</td>
<td>54$^a$</td>
<td>0.0012$^a$</td>
</tr>
<tr>
<td>IPDX</td>
<td>24$^a$</td>
<td>36$^a$</td>
<td>0.47$^a$</td>
<td>53$^a$</td>
</tr>
</tbody>
</table>

$^a$ Data from Ref. 34.

$^b$ Data from Ref. 35.

$^c$ Data from Ref. 30.
A2B receptors stimulate IL-4 and IL-13 secretion

Because of the potential relevance of Th2 cytokines IL-4 and IL-13 to asthma, we focused next on characterizing the mechanisms of regulation of these cytokines by adenosine. We initially studied kinetics of IL-4 and IL-13 secretion to determine an optimal time point to use in pharmacological characterization of these processes. Incubation of HMC-1 in the presence of 10 μM NECA and 1 U/ml adenosine deaminase produced time-dependent increase in secreted IL-4 and IL-13 (Fig. 2). IL-4 concentrations in conditioned medium from HMC-1 incubated for 6 h in the absence or in the presence of 10 μM NECA were 19.2 ± 0.1 and 117.4 ± 1.7 pg/ml, respectively. Concentration of IL-4 in conditioned medium declined to 57.5 ± 2.8 pg/ml by 18 h in the presence of NECA. IL-13 concentrations in conditioned medium from HMC-1 incubated for 6 h in the absence or in the presence of 10 μM NECA were 7.6 ± 0.3 and 214.6 ± 4.0 pg/ml, respectively. After 18 h, IL-13 concentrations in conditioned medium remained unchanged at 10.8 ± 0.8 and 206.0 ± 1.2 pg/ml.

We then chose 4 h as a time point at which adenosine-induced secretion of IL-4 and IL-13 was submaximal to characterize the adenosine receptor(s) involved. We have previously shown that HMC-1 cells express A2a, A2b, and A3 receptors (31–33). Therefore, we analyzed the effects of the nonselective adenosine receptors agonist NECA, the selective A2a agonist CGS21680, and the selective A3 agonist IB-MECA (Table I) on secretion of IL-4 and IL-13 from HMC-1. As seen in the Fig. 3A, only the nonselective agonist NECA stimulated IL-13 secretion with an EC50 of 421 ± 18 nM, whereas CGS21680 and IB-MECA were not effective at submicromolar concentrations that selectively activate A2A and A3 receptors, respectively. Furthermore, increasing concentrations of the selective A2B antagonist IPDX, from 3 to 30 μM, produced parallel rightward shifts of the concentration-response curve of NECA (Fig. 3B). Schild analysis yielded a Ks value of 1.4 μM, close to that previously found for A2B-dependent IL-8 secretion in HMC-1 cells (30), and a slope close to unity (1.13 ± 0.02), suggesting that IPDX is a simple competitive antagonist of this process. These data confirm that A2B receptors are involved in the mediation of IL-13 secretion in human mast cells. Similarly, only the nonselective agonist NECA (EC50, 833 nM ± 53 nM), but not the selective A2a agonist CGS21680 or the selective A3 agonist IB-MECA stimulated IL-4 secretion (Fig. 3C). The selective A2B antagonist inhibited IL-4 secretion with a Ks value of 1.2 μM and a slope of 0.6 ± 0.1 (Fig. 3D).

In a separate set of experiments, we tested the effect of the natural ligand adenosine on IL-13 secretion. HMC-1 cells were incubated for 4 h with increasing concentrations of adenosine in the presence of adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride (5 μM). Adenosine induced concentration-dependent IL-13 secretion with an EC50 of 6.7 μM (Fig. 4).

Expression of surface CD40L in HMC-1

The initiation of IgE synthesis depends entirely on induction of STAT 6 triggered by IL-4 or IL-13 action on B lymphocytes (36). In addition to stimulation with either IL-4 or IL-13, the process of

FIGURE 3. Pharmacological analysis of IL-13 and IL-4 release from HMC-1. A, Concentration-response curves for IL-13 secretion induced by the nonselective agonist NECA (●), by the selective A2a agonist CGS21680 (○), or by the selective A3 agonist IB-MECA (■). Values are expressed as mean ± SEM (n = 3). B, Effect of the selective A2B antagonist IPDX on NECA-induced IL-13 secretion. Concentration-response curves were repeated in the absence and in the presence of increasing concentrations of IPDX, which produced a progressive shift to the right. Values are expressed as mean ± SEM (n = 3). Inset, Schild plot of antagonism of A2B receptors by IPDX. R represents the ratio of the agonist EC50 in the presence of antagonist to its EC50 in the absence of antagonist. C, Concentration-response curves for IL-4 secretion induced by the nonselective agonist NECA (●), by the selective A2a agonist CGS21680 (○), or by the selective A3 agonist IB-MECA (■). Values are expressed as mean ± SEM (n = 3). D, Effect of the selective A2B antagonist IPDX on NECA-induced IL-4 secretion. Concentration-response curves were repeated in the absence and in the presence of increasing concentrations of IPDX, which produced a progressive shift to the right. Values are expressed as mean ± SEM (n = 3). Inset, Schild plot of antagonism of A2B receptors by IPDX. R represents the ratio of the agonist EC50 in the presence of antagonist to its EC50 in the absence of antagonist.
Ig isotype switching also requires interaction of CD40 on the surface of B cells with CD40L located on another cell. HMC-1 cells have been shown to express CD40L on their surface (29). We investigated whether stimulation of adenosine receptors would affect expression of CD40L in HMC-1 cells. We confirmed expression of CD40L on HMC-1 surface using flow cytometry. As a positive control, we used cells incubated for 6 h in the presence of 10 nM PMA and 1 μM ionomycin. As seen in Fig. 5, these cells demonstrated an increased expression of CD40L. In contrast, incubation of HMC-1 in the presence of 10 μM NECA for 6 h did not change CD40L surface expression.

Effects of adenosine-activated HMC-1 on IgE synthesis by B cells in vitro

We used the induction of IgE synthesis by B cells as an in vitro model to investigate the biological relevance of A2B-mediated secretion of IL-13 and IL-4 from HMC-1. As seen in Fig. 6, B cells cocultured together with NECA-stimulated HMC-1 for 12 days produced 870 ± 33 pg of IgE per 10⁶ B cells, whereas B cells cocultured with nonstimulated HMC-1, or cultured alone in the absence or in the presence of NECA, produced virtually no IgE. As a positive control, we incubated B cells in the presence of 2 μg/ml anti-CD40 and 10 ng/ml IL-4, a concentration at least 150-fold higher than that generated by A2B receptor activation, for 12 days. This stimulation led to the synthesis of 2800 ± 600 pg of IgE per 10⁶ B cells (data not shown).

Discussion

There is growing evidence that adenosine plays an important role in respiratory disorders associated with lung inflammation such as asthma and COPD (for recent reviews, see Refs. 37 and 38). In this study, we present the first evidence that activation of adenosine receptors triggers IL-4 and IL-13 production in mast cells, and that this, in turn, induces IgE synthesis by B lymphocytes, thus providing a regulatory loop for amplification of allergic reactions.

Our initial screening of mRNA levels in NECA-stimulated HMC-1 cells yielded data indicating up-regulation of several cytokine genes. In addition to previously described up-regulation of IL-8 mRNA (33), we found increased messages for both IL-4 and IL-13. The up-regulation of IL-4 and IL-13 mRNA was confirmed using RT-PCR as a complementary technique. Furthermore, the screening of cytokine protein levels indicated the presence of both IL-4 and IL-13 secreted into medium collected from NECA-stimulated HMC-1 cells. Interestingly, both mRNA and protein screening techniques indicated up-regulation of IL-3 by adenosine. Up-regulation of IL-1β mRNA was also detected. IL-4 reportedly up-regulates IL-1β mRNA in HMC-1 (39), and it is possible that the increase in IL-1β mRNA observed in this study was secondary to secretion of IL-4 in our model. The absence of IL-1β signal on protein screen may indicate that either IL-1β is not secreted from NECA-stimulated HMC-1 or that IL-1β levels remained below detection limits. These screening data remain to be validated by complementary techniques. If confirmed, adenosine-induced up-regulation of IL-3 and IL-1β would more likely act synergistically with IL-4 and IL-13, because they also have been implicated in promoting Th2 response (40, 41). Resting HMC-1 cells do not secrete IL-2 and IFN-γ (42). Considering that Th1 and Th2 cytokines oppose each other’s actions in many aspects of the immune response, it is noteworthy that we did not observe up-regulation of Th1 cytokines IL-2 and IFN-γ in NECA-stimulated HMC-1 cells.
Adenosine regulates cellular functions by binding to four subtypes of G-protein-coupled adenosine receptors. HMC-1 cells express \(A_{2A}, A_{2B},\) and \(A_3\), but not \(A_1\) receptors \((33, 43)\). \(A_{2B}\) receptors were shown to induce synthesis of IL-8 and vascular endothelial growth factor, and \(A_3\) receptors up-regulate angiopoietin-2 production in HMC-1 cells \((33)\). The results of the present study indicate that IL-4 and IL-13 production is regulated by \(A_{2B}\) receptors. The nonselective adenosine analog NECA stimulated both IL-4 and IL-13 production with an EC\(_{50}\) in the submicromolar range, whereas the selective \(A_{2A}\) agonist CGS21680 and the selective \(A_3\) agonist IB-MECA were not effective at concentrations that maximally activate these receptors. Furthermore, the increase in IL-4 and IL-13 generation was blocked by the selective \(A_{2B}\) antagonist IPDx with \(K_\text{B}\) values identical to those previously reported for \(A_{2B}\)-dependent IL-8 secretion in the same cells \((30)\).

Stimulation of IL-4 and IL-13 via \(A_{2B}\) adenosine receptors in human mast cells is an important finding. \(A_{2B}\) receptors have a lower affinity compared with other receptor subtypes. Their role is more prominent in circumstances in which adenosine concentrations are significantly increased. Blackburn and colleagues \((15, 16)\) recently reported that elevated adenosine levels in lungs of adenosine deaminase-deficient mice induced an inflammatory phenotype resembling human asthma, including extensive mast cell degranulation, increase in airway hyperresponsiveness, mucus hypersecretion, and elevated IgE levels. Interaction between adenosine and IL-13 signaling systems was demonstrated in adenosine deaminase-deficient mice and transgenic mice overexpressing IL-13 that have a similar inflammatory phenotype. These studies highlight the role of adenosine in the development of pulmonary inflammation and provide strong evidence that adenosine is a potent stimulator of IL-13 release. In agreement with this, our data show that activation of \(A_{2B}\) adenosine receptors induces secretion of IL-13 as well as IL-4 in human mast cells.

Adenosine levels in interstitial fluids under physiological conditions are between 30 and 300 nM \((44, 45)\). These concentrations are sufficient to cause activation of all but \(A_{2B}\) adenosine receptors \((46)\). In inflammation, the combination of local hypoxia, cellular stress, and damage can cause 100- to 1000-fold increase of adenosine concentrations reaching the 10–100 \(\mu\)M range \((47)\). Adenosine levels in lungs of adenosine deaminase-deficient mice also reach high \((100–125 \, \mu\text{M})\) concentrations \((48)\). In the present work, we demonstrated that these levels of adenosine are sufficient to activate \(A_{2B}\) receptors and induce IL-13 secretion from HMC-1.

The adenosine receptor subtype mainly responsible for mast cell degranulation appears to be different depending on species studied \((49, 50)\). Murine mast cells, similar to HMC-1, express \(A_{2A}, A_{2B},\) and \(A_3\) subtypes of adenosine receptors \((51)\). Rodent \(A_1\) receptors have less homology with their human counterpart than any other adenosine receptor subtype and differ substantially in sensitivity to adenosine antagonists \((52)\). Murine \(A_3\) receptors degranulate mast cells \((51)\) and contribute to adenosine-induced bronchoconstriction in mice \((53)\). It is not known, however, whether \(A_3\) or \(A_{2B}\) adenosine receptors regulate cytokine production in mice. Considering that degranulation and cytokine production in mast cells can be triggered independently \((54–56)\), it is possible that these distinct events could be regulated via different adenosine receptor subtypes. To our knowledge, this hypothesis has not been tested.

IL-4 and IL-13 are pleiotropic cytokines exerting sometimes diverse, but often common actions (for review, see Ref. 57). Their concentrations are elevated in asthma \((58)\). One of the shared effects of these cytokines is induction of IgE synthesis in B lymphocytes, which in turn plays a crucial role in allergic reactions. Particularly, IgE binding to FcεRI receptors is a potent stimulus for mast cell degranulation and synthesis of multiple factors that contribute and exacerbate asthma symptoms. Th2 lymphocytes have been considered as the major source for IL-4 and IL-13, and help provide for IgE synthesis by B lymphocytes. However, mast cells and basophils have also been proposed to induce IgE synthesis in B cells. Activation of mast cells by IgE also stimulates production of IL-13 and IL-4 in mast cells \((20–22, 59)\), thus further amplifying an inflammatory cycle. In addition to IL-4 or IL-13, the induction of IgE synthesis from uncommitted naive B lymphocytes requires the engagement of the surface membrane CD40 expressed in B cells through physical contact with either activated Th cells or mast cells expressing CD40L (for review, see Ref. 60). Mast cells, including HMC-1, have been shown to constitutively express CD40L \((29)\). Our data show that HMC-1 surface expression of CD40L is preserved in the presence of adenosine agonists. Therefore, adenosine-stimulated HMC-1 fulfill all necessary requirements to initiate synthesis of IgE by B cells.

Indeed, coculturing of NECA-stimulated HMC-1 cells with B lymphocytes for 12 days results in IgE production. This effect cannot be explained by stimulation of adenosine receptors on B cells, because NECA did not induce IgE production when lymphocytes were cultured alone. This experiment demonstrates that adenosine-stimulated mast cells can induce IgE synthesis by B cells. Our data provide a mechanism that would explain the involvement of adenosine in the induction, maintenance, and amplification of allergic reactions.

Our results suggest that adenosine-induced IL-4, IL-13, and IgE production is relevant to the Th2-type inflammation observed in asthma and COPD. The initial polarization of naïve T cells into Th2 cells occurs under the influence of IL-4, which can be supplied by activated mast cells \((61)\). Elevated levels of IL-4 and IgE can act synergistically to increase mast cell FceRI expression and mediator release \((62)\), thus providing a positive feedback and multiplying the initial adenosine signal. IL-13 can contribute to lung inflammation by inducing mucus hypersecretion, eosinophilia, and airway hyperreactivity \((26, 63–65)\). Considering that IL-13 is also an important regulator of tissue remodeling \((66–68)\), our findings raise the possibility that adenosine contributes to other aspects of asthma and COPD. Finally, IL-13 itself was shown to elevate extracellular adenosine levels \((17)\) that can further perpetuate the inflammatory responses.

It should be noted that mast cells are heterogeneous and vary significantly not only between species, but also between their tissue locations. Depending on their environment, mast cells may develop different phenotypes. We performed our studies in HMC-1, a cell line that resembles human lung mast cells \((69)\), and expresses predominantly \(A_{2B}\) receptors \((31)\). In contrast, human mast cells developed from cord blood express predominantly \(A_{2A}\) adenosine receptors that inhibit their activation \((70)\). It also should be noted that inhaled adenosine induces bronchoconstriction by activating lung mast cells from asthmatics, but not from normal subjects \((37)\). Differential expression of adenosine receptor subtypes could be proposed as an explanation for this disparity. Thus, the major question to be answered remains: is \(A_{2B}\) receptor the key subtype determining the difference between human lung mast cells in asthmatics and normal subjects? The presence of \(A_{2B}\) receptors was shown in mast cells in bronchoalveolar lavage fluid obtained from asthmatics \((71)\). Comparative studies of mast cells in bronchoalveolar lavage from asthmatics and normal subjects may be one way to elucidate differences in regulation of mast cells by adenosine and ascertain the role of \(A_{2B}\) receptors in the mechanism of pulmonary inflammation. Another approach may involve simulating the inflammatory environment present in asthma and examining what effect this would have on expression of adenosine.
receptors in human mast cells. In recent years, techniques of human mast cells cultivating from blood progenitors have been introduced (for review, see Ref. 72), but it may be difficult to reproduce the milieu of cytokines and other factors present in asthmatic lungs. Studies of factors that change expression of adenosine subtypes in mast cells may help to approach this problem.

In summary, we present for the first time evidence that adenosine stimulates generation of IL-4 and IL-13 in mast cells. This effect is mediated via A2B adenosine receptors. Demonstration of induction of IgE synthesis by interaction of adenosine-stimulated mast cells and B lymphocytes implies that this mechanism may be involved in amplifying the allergic inflammatory response associated with asthma.

Acknowledgments

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References

