Adenosine A2B receptors are highly expressed on murine type II alveolar epithelial cells

Rebecca E. Cagnina,1,2 Susan I. Ramos,1,3 Melissa A. Marshall,1,3 Guoquan Wang,4 C. Renea Frazier,4 and Joel Linden1,3

1Robert M. Berne Cardiovascular Research Center, Departments of 2Molecular Physiology and Biological Physics and 3Internal Medicine, University of Virginia, Charlottesville, Virginia; and 4PGxHealth, LLC, a Division of Clinical Data, Incorporated, Charlottesville, Virginia

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Cagnina RE, Ramos SI, Marshall MA, Wang G, Frazier CR, Linden J. Adenosine A2B receptors are highly expressed on murine type II alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol 297: L467–L474, 2009. First published July 2, 2009; doi:10.1152/ajplung.90553.2008—The adenosine A2B receptor (A2BR) has a wide tissue distribution that includes fibroblasts and endothelial and epithelial cells. The recent generation of an A2BR−/− mouse constructed with a β-galactosidase (β-gal) reporter gene under control of the endogenous promoter has provided a valuable tool to quantify A2BR promoter activity (29). To determine the sites of expression of the A2BR receptor in the mouse lung, histological and flow cytometric analysis of β-gal reporter gene expression in various lung cell populations was performed. The major site of A2BR promoter activity was found to be the type II alveolar epithelial cells (AECs), identified by coexpression of prosurfactant protein C, with relatively less expression in alveolar macrophages, bronchial epithelial cells, and cells of the vasculature. Highly purified type II AECs were prepared by fluorescence-activated sorting of enhanced green fluorescent protein (eGFP)-positive cells from transgenic mice expressing eGFP under control of the surfactant protein C promoter (21). The type II cells expressed 89-fold higher A2BR mRNA than pulmonary leukocytes, and the A2BR was shown to be functional, as treatment of purified type II AECs with the nonspecific adenosine receptor agonist 5'-N-ethylcarboxamidoadenosine (NECA) induced an increase in intracellular cAMP greater than the 100 nM (8). In this study, we describe high density A2BR expression on type II AECs in the mouse lung that has previously been unrecognized, and we demonstrate that these receptors are functional on highly purified sorted cells expressing enhanced green fluorescent protein (eGFP) under control of the promoter for the type II AEC marker, surfactant protein C (SP-C-eGFP) (21). The identification of high density, functional A2BR expression on type II AECs suggests that adenosine may play an important role in regulating type II AEC function during periods of high pulmonary adenosine production, such as injury or infection.

MATERIALS AND METHODS

Mice. All experiments were approved by the Animal Care and Use Committee of the University of Virginia. Mice were 8–12 wk of age. C57BL/6 animals were purchased from Jackson Labs. A2BR−/− congenic to C57BL/6 were a gift of Dr. K. Ravid (Boston, MA). Heterozygotes (A2BR+/−) were prepared by breeding A2BR−/− mice with wild-type C57BL/6. SP-C-eGFP mice were the gift of Dr. M. O’Reilly (Rochester, NY) and are congenic to C57BL/6.

Inflation fixation. Mice were euthanized using an overdose of pentobarbital sodium. The pulmonary vasculature was perfused free of blood, and the trachea was cannulated. The lungs were inflation-fixed using 4% paraformaldehyde for 15 min at 25 cmH2O. The lungs were then excised en bloc and immersed in 5 ml 4% paraformaldehyde for an additional 15 min. They were then rinsed in PBS for 25 min.

β-gal staining. Fixed tissues were immersed in 5 ml of X-gal staining buffer (5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 2 mM MgCl2, 1 mg/ml X-gal, 0.2% Nonidet P-40, and 0.1% deoxycholate in PBS). Tissues were rocked at room temperature overnight while protected from light, rinsed in PBS, immersed in 70% ethanol, and processed for paraffin embedding. Embedded tissues were sectioned and counterstained using hematoxylin or subjected to immunohistochemistry.
Immunohistochemistry. Five-micrometer paraffin sections were rehydrated, quenched with aqueous hydrogen peroxide (0.45%) for 15 min, and washed with deionized H₂O followed by PBS with 0.5% fish skin gelatin (PBS-FSG), 10% normal rabbit or donkey serum, and Vector avidin block for 60 min. Primary rat anti-Mac-2 antibody (Cedarlane) diluted 1:10,000 or primary anti-surfactant protein C (Chemicon) diluted 1:1,500 was applied overnight at 4°C. Sections were washed in PBS-FSG 4 × 5 min. Biotinylated rabbit anti-rat (Vector) or donkey anti-rabbit (Jackson ImmunoResearch) secondary antibody was applied for 60 min at room temperature. After washing 4 × 5 min in PBS, the sections were incubated in Vector ABC Elite solution for 30 min. DAB substrate (Dako) was used (5 min) to visualize staining. Sections were counterstained with hematoxylin I.

Cell dissociation. The method for cell dissociation and alveolar type II cell enrichment was adapted from Corti et al. (4). Briefly, mice were anesthetized using pentobarbital sodium, and the lungs were perfused free of blood with PBS. The trachea was cannulated, and 3 ml of dispase (BD Biosciences) was instilled followed immediately by 1 ml of 1% low-melt agarose. Ice-cold saline-soaked gauze was placed over the exposed lung for 2 min. Lungs were removed en bloc and immersed in 2 ml of dispase and incubated at 37°C for 45 min. Lung tissue was separated from bronchial structures and rocked gently in DMEM + 10 mM HEPES + 10%FBS (Gibco) for 5 min. Tissue was then subject to serial filtration through 100- and 40-μm cell strainers (BD Biosciences) followed by 20-μm nylon gauze (Nytex). Single cell suspensions were centrifuged at 300 g for 8 min, resuspended in DMEM + 10 mM HEPES + 10%FBS, and incubated on α-CD45- and α-CD16/32- (clone 30-F11, 93, eBioscience) coated plates for 2 h. Nonadhered cells were gently removed and used for subsequent studies. For RT-PCR studies in which a population of leukocytes was desired, the subtraction step on antibody-coated plates was omitted.

Fluorescein di-β-D-galactopyranoside staining. Fluorescein di-β-D-galactopyranoside (FDG; Sigma-Aldrich) is a substrate for β-gal that can be used to identify β-gal-positive cells by flow cytometry. Cells were centrifuged at 300 g for 8 min, and the red blood cells were lysed (RBC lysis buffer, Sigma-Aldrich). Remaining cells were resuspended in staining buffer (PBS + 1% BSA + 300 μM chloroquine) and incubated at 37°C. Cells were loaded with FDG by hypotonic loading. An equal volume of prewarmed 2 mM FDG in H₂O was added to the cell suspension and allowed to incubate for 1 min before quenching with 10 vol of ice-cold staining buffer. Cells were centrifuged at 300 g for 8 min at 4°C and resuspended in ice-cold staining buffer. Stained cells were held on ice until ready for FACS analysis.

Fluorescence-activated cell sorting. Live cells were sorted based on fluorescence or eGFP fluorescence, and viability was assessed using 2 μg/ml propidium iodide (Invitrogen). Sorted cells used for RT-PCR experiments were also labeled with APC α-CD45 (clone 30-F11, eBioscience). Fluorescent-activated cell sorting (FACS) was performed using a Becton Dickinson FACSVantage SE Turbo Sorter with DIVA Option. Some populations of sorted cells were applied to a slide using a Cytospin (Thermo-Shandon) at a concentration of 25,000–50,000 cells/slide for immunohistochemistry, whereas others were used for subsequent assays.

Quantitative RT-PCR. Sorted populations of cells were resuspended in 1 ml of TRI-Reagent (Ambion), and RNA was isolated according to the manufacturer’s protocol. cDNA was obtained using the Quantitect Reverse Transcription Kit (Qiagen). RT-PCR was performed using the iQ SYBR Green Supermix (Bio-Rad) on a Bio-Rad iCycler IQ thermal cycler. Primers for the A2BR were forward 5′-gctgctgctgctgctgca-3′ and reverse 5′-cctcctgctcctgcact-3′; primers for the A1R were forward 5′-ctctcaggctgaggcag-3′ and reverse 5′-ccacaggagggctacag-3′; primers for the A2AR were forward 5′-tgctgctgctgctgctgca-3′ and reverse 5′-gccggtcctgcctgcctg-3′; and primers for the A3R were forward 5′-gctgctgctgctgctgca-3′ and reverse 5′-tgctgctgctgctgctgca-3′. Primers for the GAPDH housekeeping gene were forward 5′-ttcaccagctgaggaagc-3′ and reverse 5′-gctgctgctgctgctgca-3′. Data analysis and fold-change estimation were performed using the ΔΔCₚ method.

Radioisotopic binding assays. The methods used for radioligand binding studies have been described previously (25). In brief, all four subtypes of recombinant human and murine adenosine receptors were stably expressed in HEK-293 cells or CHO-K1 cells. Crude membrane preparations from transfected cells were diluted in HE buffer (50 mM HEPES, 1 mM EDTA, pH 7.4) at concentrations between 2 and 50 μg/tube in a volume of 150 μl and adenosine deaminase was added at 2 U/ml. Dilutions of antagonist compounds were prepared at 10x final concentrations in HE containing 10% DMSO. Radioligands were as follows: [35S]N6-aminobenzyladenosine ([35S]-ABA) for A₁ and A₃ receptors and [3H]-4-(2-[7-Amino-2-(2-furyl)]-[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino)ethyl)phenol ([3H]-ZM241385) for A₂A receptors. [3H]-flurooro-N-(5-2,3,6,7-tetrahydro-2,6-dioxo-1,3-di(2,3-ditritiumpropyl)-1H-purin-8-yl)pyridin-2-yl)-N-(2-methoxyethyl)pyridine-3-carboxamide ([3H]ATL-852) was characterized as an improved radioligand for A₃ receptors. Diluted antagonists (25 μl) were added to each membrane sample (150 μl). The radioligand was added in a 75-μl volume, the tubes were incubated for 1.5 h at room temperature, filtered through glass fiber filters, and counted in either a Wallac 1470 Wizard Automatic Gamma Counter (125) or a Beckman LS6500 Multipurpose Scintillation Counter (13). Samples were assayed in triplicate. Nonspecific binding was measured in the presence of NECA (100 μM).

cAMP assay. Sorted cells were plated in serum-free SAGM (Lonza) + 1 U/ml adenosine deaminase (Roche) at a density of 50,000 cells/well in a 96-well plate and were allowed to recover for 2 h before stimulation. In some instances, ATL-802 (100 nM) was added for 20 min before agonist stimulation. NECA (10 μM) or the β-adrenergic agonist isoproterenol (10 μM) was used to stimulate cells, and all wells received 10 μM rolipram to inhibit phosphodiesterase activity simultaneously with agonist. Stimulation was allowed to proceed for 15 min, and cAMP accumulation was measured by cAMP-Screen Immunoassay System (Applied Biosystems).

RESULTS

The A2BR promoter is highly active in type II AEC in murine lung. The location of A2BR receptor promoter activity in mouse lung was evaluated by immunohistochemistry and reporter gene expression. The A2BR gene knockin mouse model contains the prokaryotic reporter gene β-gal, under control of the endogenous A2BR promoter (29). Staining of naïve A2BR mouse lungs for β-gal revealed the highest expression in cuboidal, junctional cells of the alveolus with no background staining evident in C57BL/6 WT mice (Fig. 1A). Prosurfactant protein C (pro-SP-C), a unprocessed form of surfactant protein C, is a specific marker for type II AEC, as these are the only cell types that express this protein. Immunohistochemistry of lung sections dually stained for α1-pro-SP-C and reverse 5′-galactopyranoside staining. Sections were counterstained with hematoxylin I. The A2B promoters were forward 5′-tccaccacccaggaagc-3′ and reverse 5′-ccgctgctgctgca-3′. Data analysis and fold-change estimation were performed using the ΔΔCₚ method.
A2B receptors on type II alveolar epithelial cells (AECs) are highly expressed in murine lung. A:Reporter gene expression for the adenosine A2B receptor is highly localized to type II alveolar epithelial cells (AEC) in murine lung. A: WT and A2BKO mice were stained for expression of the β-galactosidase (β-gal) reporter gene under the control of the endogenous adenosine A2B receptor (A2B) promoter in A2BKO mice. B: Immunostaining of wild-type (left) and A2BKO (right) lung sections for pro-SP-C expression following staining for β-gal. Positive staining for pro-SP-C colocalizes with β-galactosidase expression.

Type II AECs express high levels of A2B receptors. It is not possible to obtain pure type II AECs from wild-type mouse lung. To prepare a nearly pure population of type II AECs that express a normal complement of A2B receptors, we used a transgenic mouse model with eGFP under the control of the human surfactant protein C promoter (21). Using this model, a nearly pure population of type II AECs can be obtained by cell sorting. Two populations of cells were collected based on eGFP and CD45 expression. As expected, no cells were double positive for eGFP and the leukocyte marker, CD45. Sorted populations were subject to RT-PCR to quantify A2B mRNA expression, and relative levels of expression (ΔΔCt) in eGFP-positive cells were determined. Cells were separated and collected based on their fluorescence signal, and subjected to FACS. Cells were separated and collected based on their fluorescence signal and subsequently stained for prosurfactant protein C expression. Usually, cells that were positive for fluorescence also stained positive for prosurfactant protein C (Fig. 3). In triplicate experiments, 5.4 ± 1.3% of fluorescence-negative and 98 ± 2% of fluorescence-positive cells stained positive for prosurfactant C (P < 0.0001). However, rare fluorescence-negative cells also stained positive for prosurfactant protein C (Fig. 3). Similar results were seen with sorted cells from A2BKO mice (data not shown).
positive type II AECs were compared with the population of CD45-positive leukocytes. Type II AEC cells that were eGFP-positive had high relative expression of A2BR mRNA, expressing 89 ± 6.2-fold higher levels than CD45-positive leukocytes (P < 0.05). In addition, relative levels of the A1, A2A, and A3 adenosine receptor transcripts in type II AECs were assessed using RT-PCR and compared with A2BR transcript expression. In eGFP-positive type II AECs, the level of A2B receptor transcript was 3.5 ± 0.24-fold higher than A1R transcript, 34 ± 6.5-fold higher than A2AR transcript, and 79 ± 2.7-fold higher than A1R transcript, indicating that at the level of mRNA, the A2BR is the most abundant adenosine receptor transcript expressed in type II AECs.

ATL-802 is a selective antagonist of the mouse A2BR. Several potent and selective A2BR antagonists have been described in recent years. In general, xanthine compounds such as MRS1754 bind with lower potency and selectively to rodent than human A2B receptors (14). For this reason, we have searched for new radioligands and antagonists of mouse A2B receptors. A new radioligand, [3H]ATL-852, was found to bind to membranes derived from HEK-293 cells stably transfected with the recombinant mouse A2BR with a $B_{max}$ = 1,970 fmol/mg protein and a $K_d$ = 28.5 nM (Fig. 4, A and B). In a screen of novel antagonists, ATL-802 was identified as a new potent A2B-selective antagonist in competition binding assays to recombinant mouse A1, A2A, A2B, and A3 receptors. As shown in Fig. 4C and Table 1, ATL-802 is more selective for mouse than human A2BRs. The $K_i$ value for ATL-802 at the mouse A2BR was 8.6 ± 2.2 nM, and the antagonist exhibited excellent selectivity, 978-fold over the A2AR and greater than 1,000-fold over the A1 and A3 receptors.

The A2BR on type II AECs is functional and stimulates cAMP formation. To assess whether the A2B receptor on type II AECs is functional, dispersed cells from the lungs of SP-C-eGFP transgenic mice were sorted based on eGFP expression. Sorted cells were allowed to recover and subsequently stimulated with 10 μM NECA. Some cells were treated with 100 nM ATL-802, and cAMP formation was assessed following NECA-induced stimulation as evidence of A2BR activation. The β-adrenergic agonist isoproterenol (10 μM) was used as a positive control for $G_s$-stimulated cAMP accumulation. As shown in Fig. 5, eGFP-positive cells from SP-C-eGFP mice had increased cAMP formation in response to stimulation with NECA, and this response was inhibited almost completely by treatment with the selective A2B antagonist, ATL-802. These eGFP-positive cells also increased cAMP accumulation in response to stimulation with isoproterenol, but the magnitude was 3.1 times less than that seen with NECA stimulation. ATL-802 did not significantly block isoproterenol-induced cAMP accumulation. In the eGFP-negative population, stimulation with NECA also increased cAMP, and this response was also...
inhibited by ATL-802; however, the magnitude of response to NECA was significantly lower than in eGFP-positive cells.

**DISCUSSION**

In this study, we show evidence that the highest expression of A2B receptors in the lung is on type II airway epithelial cells. These receptors are functional, and when maximally activated, generate over 3 times more cAMP than maximally activated β-adrenergic receptors. We took advantage of the reporter gene construct in the A2B<sup>R<sup>−/−</sup> mouse model that permits use of β-gal expression as a marker for endogenous A2B<sup>R</sup> promoter activity. The same approach was used to identify A2B<sup>R</sup> promoter activity in the vasculature of several tissues (29). It can be argued that the A2B<sup>R</sup><sup>−/−</sup> mouse may have adapted to the loss of endogenous receptor. In that case, reporter gene expression might not be representative of A2B<sup>R</sup> expression in a wild-type mouse. For this reason, we also examined A2B heterozygotes (A2B<sup>R</sup><sup>+/−</sup>). The advantage of heterozygotes is that they possess one copy of the β-gal reporter gene as well as one copy of the functional A2B<sup>R</sup> gene. Hence, reporter gene expression in the setting of a mouse model with functional receptor expression can be determined. In all cases, reporter gene expression showed identical localization in A2B<sup>R</sup><sup>−/−</sup> and A2B<sup>R</sup><sup>+/−</sup> animals despite a decrease in intensity of β-gal staining in A2B<sup>R</sup><sup>−/−</sup> mice compared with A2B<sup>R</sup><sup>+/−</sup> mice. We also used the transgenic SP-C-eGFP mouse in which eGFP is under control of the human surfactant protein C promoter. This model is useful because it provides a method to isolate pure type II AECs and study A2B<sup>R</sup> expression and function in these cells that have unaltered adenosine receptors (22).

Initially, we identified the major source of A2B<sup>R</sup> promoter activity in the lung based on β-gal reporter gene expression on cells that appeared to have morphological characteristics of type II AECs. This conclusion was confirmed based on colocalization of β-gal reporter gene expression and immunostaining for the type II AEC marker, pro-SP-C, on fixed lung sections from naïve animals. This finding was further confirmed by isolating pure populations of dissociated, β-gal-positive cells from A2B<sup>R</sup><sup>−/−</sup> and A2B<sup>R</sup><sup>+/−</sup> animals followed by immunostaining for pro-SP-C. While staining in fixed tissue showed colocalization of β-gal and pro-SP-C staining in nearly every type II AEC examined, results from dissociated cell populations showed that while every β-gal-positive cell is also pro-SP-C positive, there is a small population of β-gal-negative cells that stain positively for pro-SP-C. This might be due to a limitation of the staining technique, as fluorescein can leak out of cells rapidly. Stained cells were held on ice to minimize this effect, but it is possible that the β-gal-negative/pro-SP-C-negative population is an artifact and that these cells do actually express the β-gal reporter. Alternatively, it is possible that not every type II AEC expresses the A2B<sup>R</sup> receptor at all times. Although this study supports the conclusion that most lung cells with high A2B<sup>R</sup> promoter activity are type II AECs (with the possible exception of a small fraction of large airway epithelial cells, Fig. 2), the converse might not necessarily be true, i.e., it is possible that not all type II AECs are positive for A2B<sup>R</sup> promoter activity at all times.

The A2B<sup>R</sup> has previously been documented to be expressed on multiple cell types in the mouse lung, and perhaps the greatest significance of this study is identifying the type II AEC as expressing higher levels of the A2B<sup>R</sup> than these other cell populations. Before this study, the principal site of expression for the A2B<sup>R</sup> has been thought to be bronchial epithelial cells of medium and large airways (2, 3, 19, 33). Functional A2B expression has also been demonstrated in alveolar macrophages, fibroblasts, and bronchial smooth muscle (2, 31, 32). In the initial characterization of the A2B<sup>R</sup><sup>−/−</sup> mouse, β-gal reporter characterization was not shown in the lung, but the authors concluded that the principal site of A2B<sup>R</sup> expression was the vasculature and macrophages, and it was stated that reporter activity was observed in the lung vasculature (29). Interestingly, in the current study, we saw no detectable re-
porter staining in alveolar macrophages or pulmonary vasculature.

It is notable that in the SP-C-eGFP mouse, only 8–10% of type II AECs express eGFP (30). For this reason, eGFP-positive cells can be used to prepare highly enriched type II AECs, but eGFP-negative cells cannot be used as a source of cells lacking type II AECs. Based on RT-PCR characterization of A2BR mRNA expression in a purified type II AEC population and a purified mixed leukocyte population from the SP-C-eGFP mouse, the A2BR was the most abundantly expressed adenosine receptor subtype in type II AECs, and eGFP-positive type II AECs expressed \( \frac{89}{89} \) higher levels of A2BR mRNA than purified leukocytes (primarily consisting of alveolar macrophages).

The A2B receptor on the type II AEC is functional since activation of this receptor induced a rise in cAMP. The A2BR classically signals through \( G_s \), and this is true in type II AECs as well. We were able to show that the NECA-induced rise in cAMP in type II AECs from the SP-C-eGFP transgenic mouse was due to activation of the A2B receptor and not the other adenosine receptor subtypes. We characterized a novel, selective antagonist of the mouse A2B receptor, ATL-802. The sorted, eGFP-positive type II AECs also showed an isoproterenol-induced rise in cAMP that was insensitive to inhibition with ATL-802. It has been well documented that \( \beta \)-adrenergic activation induces cAMP accumulation and subsequent surfactant release in type II AECs (11, 18, 20), yet activation of the A2B receptor in isolated type II AECs stimulated a 3.1-fold greater response in cAMP accumulation compared with \( \beta \)-adrenergic activation by a maximal concentration of isoproterenol, indicating a potentially greater role for the A2B receptor in regulation of type II AEC function. The eGFP-negative population also showed a NECA-

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<th>K_i (nM)</th>
<th>A_1</th>
<th>A_2A</th>
<th>A_2B</th>
<th>A_3</th>
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<tr>
<td>Mouse</td>
<td>9.583 ± 1,713</td>
<td>8.393 ± 2,228</td>
<td>8.58 ± 2.15</td>
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</tr>
<tr>
<td>Human</td>
<td>369 ± 40</td>
<td>654 ± 51</td>
<td>2.36 ± 0.245</td>
<td>&gt; 1 μM</td>
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K_i values are expressed as means ± SE (n = 3). Values were determined from competition radioligand binding experiments as described in MATERIALS AND METHODS.

Fig. 5. Activation of the A2B receptor elevates cAMP in a purified population of type II AECs. Single cell suspensions of lung from SP-C-eGFP mice were sorted based on eGFP expression and viability. Cells were allowed to recover overnight and were then stimulated with 10 μM NECA ± 100 nM ATL-802 or 10 μM isoproterenol ± 100 nM ATL-802. cAMP accumulation following stimulation was measured by immunoassay. Purified type II AECs (eGFP+) from SP-C-eGFP mice showed increased cAMP accumulation following NECA stimulation. This increase in cAMP was due to specific activation of the A2B receptor, as treatment with the antagonist ATL-802 inhibited this effect almost completely. Treatment with isoproterenol induced cAMP accumulation, but was unaffected by pretreatment with ATL-802. The assay was performed in triplicate and is representative of triplicate experiments. *P < 0.05 relative to all other groups; n.s., not significant, based on one-way ANOVA with Bonferroni’s multiple comparison test.
induced rise in cAMP that could be inhibited by ATL-802, but the magnitude of the NECA response was significantly lower than in the eGFP-positive cells. As previously mentioned, only \(~10\%\) of total type II AECs are positive for eGFP, and the smaller NECA response in eGFP-negative cells could be due in large part to type II AECs within that population. We can conclude that type II AECs have a functional A2B receptor and that A2B receptor activity is highest in this group of cells compared with other sorted populations.

Localization of A2B R expression to type II AECs is potentially functionally significant based on the known roles of both A2B R signaling and type II AEC biology. A2B R activity has long been theorized to control chloride and water secretion in pulmonary epithelial cells (12, 15). This was classically thought to be a function of larger airways, but the current study suggests an important role of type II AECs in controlling this effect. It was recently shown that the A2B R on a non-hematopoietic cell in the lung is responsible for alveolar fluid clearance following ventilator-induced lung injury (6). Based on the findings of the current study, we would predict that type II AECs are the primary regulators of fluid clearance, but further characterization of the type II cell in this model is necessary.

Type II cells are also known to regulate a variety of other functions, including surfactant production and secretion. It has been suggested that activation of the A2B R on the type II AEC induces surfactant release, but further pharmacological characterization is needed to completely understand this effect (10, 20). An interesting overlap of type II AEC and A2B R biology is in regulating immune responses. Type II AECs are known to actively participate in host defense by responding to pathogens through Toll-like receptors and actively secreting immune-modulating cytokines and chemokines (1, 13, 16, 26, 27). A2B R activation has been shown to have both pro- and anti-inflammatory effects, including controlling cytokine release and regulating vascular leak in response to injury (5, 28, 29, 32, 33). Further characterization of the A2B R on type II AEC is likely to reveal important downstream effects of this receptor, including modulation of the immune response to injury that may have broad implications in lung pathophysiology and disease.

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GRANTS

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