

RESEARCH LETTER

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Reduced AIBP expression in bronchial epithelial cells of asthmatic patients: Potential therapeutic target

To the Editor,

Apolipoprotein A-I binding protein (AIBP; gene name *APOA1BP* or alternatively, *NAXE*) is a secreted protein,¹ which facilitates removal of excess cholesterol from activated cells, including primary alveolar macrophages, endothelial cells and microglia.^{2–4} We have demonstrated that the pulmonary surfactant can serve as a cholesterol acceptor when incubated with alveolar macrophages.⁴ In addition, ApoA-I is found in bronchoalveolar lavage fluid (BALF).⁵ These findings suggest that cholesterol efflux occurs not only in blood and tissues but also in the pulmonary airspace. AIBP binds to surfactant protein B and augments cholesterol efflux from alveolar macrophages to surfactant.⁴ This results in normalization of lipid raft content in the plasma membrane, reduced inflammatory signalling and reduced expression of inflammatory cytokines in alveolar macrophages. In response to inhaled LPS lung injury, AIBP is secreted into BALF.⁴ In addition, AIBP facilitates mitophagy, helps maintain mitochondrial function and reduces oxidative stress in macrophages.⁶ The hypothesis that AIBP expression serves to protect against inflammation implies that raising AIBP levels in the lung may have a therapeutic effect.

Because administration of AIBP, either as a recombinant protein or *via* adeno-associated virus delivery, produced anti-inflammatory and protective effects in neuroinflammation and neuropathic pain,⁷ vascular inflammation and atherosclerosis³ and acute lung injury⁴; in this work, we examined whether lung expression of endogenous AIBP is affected in asthma patients and if inhaled AIBP can reduce pulmonary inflammation and alleviate airway hyperresponsiveness in a mouse model of asthma.

Immunohistochemistry of post-mortem human lung tissue obtained from non-asthmatic subjects revealed a pattern of predominant AIBP protein expression in bronchial epithelial cells (Figure 1A). Interestingly, the AIBP expression was significantly reduced in the bronchial epithelial cells of post-mortem lungs from subjects with asthma (Figure 1B and C). In addition, primary bronchial epithelial cells isolated from post-mortem lungs of subjects with asthma had a significantly lower *APOA1BP* mRNA expression compared to that in non-asthmatic subjects (Figure 1D). Similar to human asthma, expression of endogenous AIBP in bronchial epithelium was significantly reduced in house dust mite (HDM)-challenged mice, compared with control mice that received intranasal PBS (Figure 1E–G). This pattern of reduced AIBP expression in bronchial epithelium following HDM challenge in mice was not observed in mouse acute lung injury model.⁴ In addition, the lung cell types expressing the

highest levels of AIBP differed in the two models, with the highest AIBP expression in acute lung injury observed in recruited inflammatory cells (i.e. neutrophils and macrophages).⁴ In contrast, the predominant recruited inflammatory cell following HDM challenge (i.e. eosinophils) did not express high levels of AIBP.

Because endogenous AIBP expression was reduced in asthma (Figure 1A–G) and because of the broad anti-inflammatory protections afforded by AIBP in the lung,⁴ and other tissues,^{3,7} we tested whether intranasal delivery of recombinant AIBP (RFT1081 produced by Raft Pharmaceuticals) will have a therapeutic effect in a mouse model of asthma.

Intranasal RFT1081 administration resulted in the maximal RFT1081 levels detected in BALF at 30 min, in lung tissue at 1 h and in blood at 2 h (Figure 1H and I). The half-life of RFT1081 in BAL and lung tissue was approximately 2 h, and no detectable RFT1081 in BALF, blood or lung was found at 24 h (Figure 1H and I). Based on RFT1081 pharmacokinetics, we chose to administer it 2 h before the administration of HDM.

Four weekly administrations of intranasal HDM in female mice induce lung eosinophilic inflammation and airway hyperresponsiveness (AHR) to methacholine challenge.⁸ Two doses of RFT1081, 2.5 and 25 µg, or vehicle (PBS) were administered to 8-week-old C57BL/6J female and male mice weekly, via intranasal instillation, 2 h before the intranasal HDM. Intranasal RFT1081 produced no apparent adverse effects. As expected, HDM-challenged mice pre-treated with PBS developed AHR. In contrast, RFT1081 pre-treatment reduced, in a dose-dependent manner, HDM-induced AHR, with the 25-µg dose resulting in nearly complete inhibition of AHR (Figure 2A and I). RFT1081 also induced dose-dependent reductions in HDM-induced BAL total cell count and eosinophils and lung eosinophilia (Figure 2B–D and J–L). RFT1081 significantly reduced, in a dose-dependent manner, expression of T2 cytokines IL-4, IL-13 and the alarmin IL-33 (Figure 2E–G and M–O); expression of IL-5 was below the detection limit; and thus, the response of IL-5 to RFT1081 could not be evaluated (not shown). The expression of the epithelial mucus gene *Muc5ac* was also significantly reduced by RFT1081 (Figure 2H and P).

Taken together, our studies demonstrate significantly reduced AIBP expression in human bronchial epithelial cells in asthmatics compared with non-asthmatics, as well as in bronchial epithelial cells following HDM challenge in a mouse model of asthma. These results correspond to findings of reduced ApoA-I levels in BALF of asthmatics compared with non-asthmatics.⁵ Because airway epithelial

inflammation is a major component of asthma,⁹ restoring levels of AIBP, which has anti-inflammatory properties, may present a novel therapeutic strategy for asthma. Our results with intranasal administration of RFT1081 (modified AIBP), showing a therapeutic effect in the acute HDM mouse model of asthma, support this proposition. As AIBP is expressed by airway epithelium, AIBP may regulate inhibitory pathways in airway epithelium important to eosinophilic inflammation and AHR. For example, we have demonstrated that AIBP inhibits IL-33, an epithelial cytokine which can promote eosinophilic inflammation in the airway.⁹ In addition, AIBP inhibits expression of the epithelial mucus gene *Muc5ac*, which may be of benefit to asthmatics with increased mucus if this observation can be validated in human studies. Further study is needed to determine whether AIBP inhibits other epithelial or non-epithelial cell pathways that contribute to eosinophilic inflammation and AHR in asthma, or whether it could inhibit epithelial pathways important to T2 low asthma. In this study, we have tested RFT1081 administered in a prophylactic mode prior to allergen exposure. Further study is needed to determine whether RFT1081 would be as effective when administered post-allergen exposure. Although post-mortem studies of human lungs allow for valuable translation of findings from mouse to humans,⁸ it is important that viable lung without evidence of infection be used as was done in this study. The lungs we used are collected by the same transplant teams and under the same conditions as used for transplant. Lung tissue derived from these lungs retain important physiological properties including ciliary motility, airway contractility and secretory responses in explant culture.

As inhaled corticosteroids (ICS) are the cornerstone of treatment for asthma of all severities, further studies in pre-clinical models (i.e. human bronchial epithelial cells in vitro, human airways in precision cut lung slices) and subsequently in human subjects with asthma are needed to determine whether RFT1081 may be an alternative anti-inflammatory to ICS in asthma subjects who do not respond well to ICS, and/or have an additive anti-inflammatory effect on asthma control when combined with ICS, which warrants further study.

1 | MATERIALS AND METHODS

1.1 | Human lung specimens

Post-mortem human lungs from asthmatics and non-asthmatics were procured by the Arkansas Regional Organ Recovery Agency and by the National Disease Research Interchange and delivered to the Lung Cell Biology Laboratory at the Arkansas Children's Research Institute. Immunohistochemistry was conducted at UC San Diego. Subjects were categorized as asthmatic if they had a physician diagnosis of asthma listed in the hospital medical record and used asthma medications at the time of death. Subjects were categorized as non-asthmatic if they had no physician diagnosis of asthma as well as no asthma medication use listed in the hospital medical record at the time of death. The allergic status or the peripheral blood eosinophil counts of the asthmatic individuals whose post-mortem lungs we studied is not known as it was not documented in their

Key Messages

- Bronchial epithelium is the primary lung site of AIBP expression in non-asthmatic subjects and control mice.
- Bronchial AIBP expression is significantly reduced in asthmatic patients and in a mouse model of acute asthma.
- Intranasal administration of recombinant AIBP prevents lung eosinophilic inflammation and airway hyperresponsiveness in female and male mice.

anonymized medical records. The acquisition of deceased donor tissue was reviewed by the University of Arkansas for Medical Sciences Institutional Review Board and determined not to be human subject research. This study was approved by the University of California, San Diego Human Research Protections Program.

1.2 | Human bronchial epithelial cells

Primary bronchial epithelial cells were isolated from bronchi of post-mortem lungs. In brief, bronchi were dissected, and the interior of each bronchus was scraped with a Cell Lifter (Corning, Inc.) to obtain bronchial epithelial cells. The bronchial epithelial cells were collected and cultured in CnT-17 media (CellIntec, Bern, Switzerland). These primary bronchial epithelial cells were >95% pure as assessed by E-cadherin expression by flow cytometry.

1.3 | Human and mouse lung immunohistochemistry

Paraffin-embedded lung sections were stained using a cocktail of mouse anti-human and anti-mouse AIBP monoclonal antibodies A7 and BE-1 developed in our lab^{6,7} and mixed at 1:2 ratio. Due to close homology of mouse and human AIBP, both antibodies recognize the mouse and the human protein. Quantification of AIBP-positive staining in epithelial cells was performed for each lung section using an image analysis system (Image-Pro plus, Media Cybernetics), and results were expressed as AIBP-positive area of bronchial epithelium per μm length of the bronchial basal membrane in human specimens. AIBP expression in the mouse lung was measured using a mean grey value tool in Image J (NIH), and the values in the cytosol of bronchial epithelium of bronchiole with a 150–200 μm internal diameter were normalized to that in adjacent alveolae. The operators were blinded to the identity of samples.

1.4 | mRNA quantification

To quantify *APOA1BP* mRNA in human bronchial epithelial cells and the mRNA for *Il4*, *Il13*, *Il33* and *Muc5ac* in mouse lung homogenates, total RNA from each sample was processed for RT-qPCR as

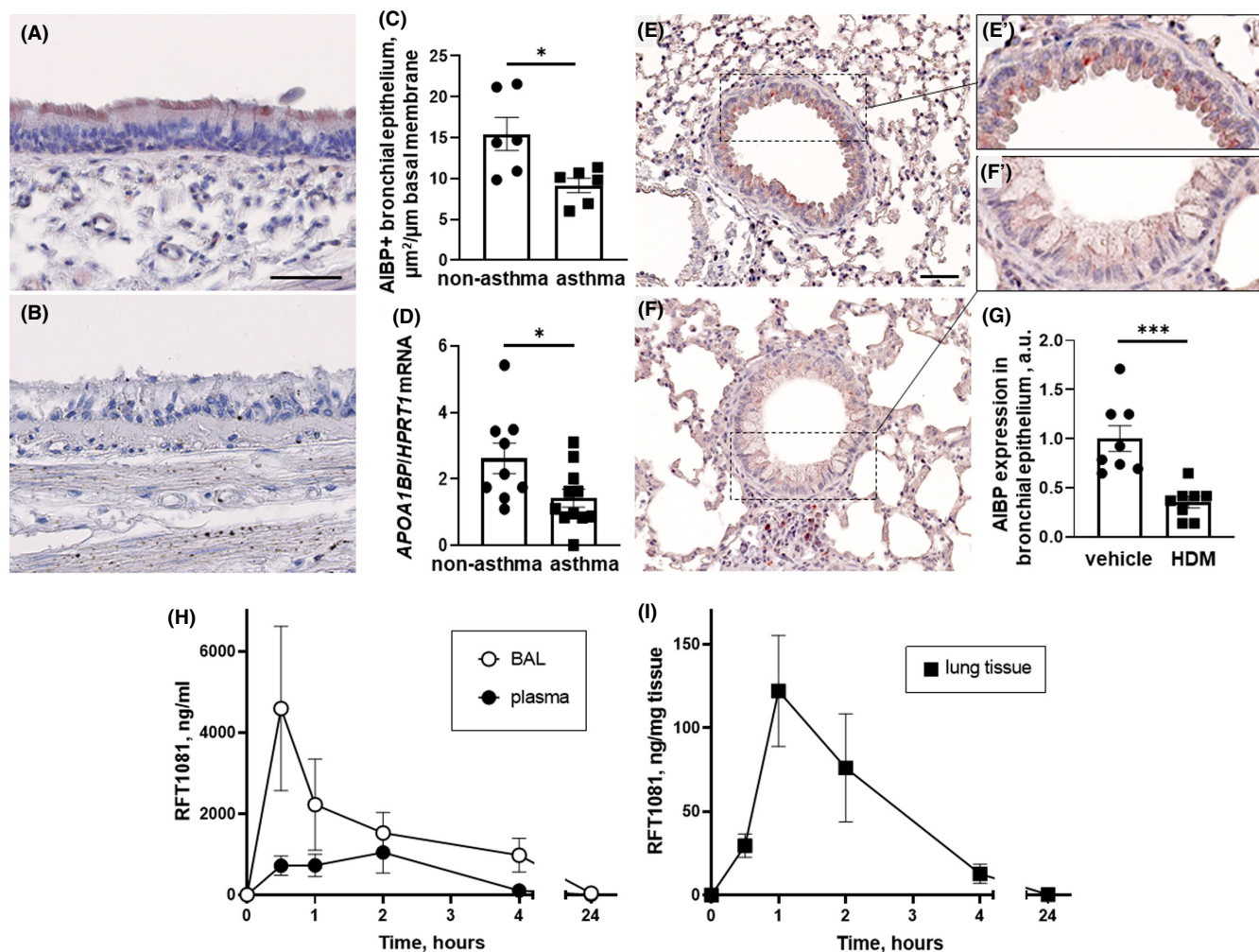


FIGURE 1 Reduced AIBP expression in bronchial epithelium and intranasal RFT1081 pharmacokinetics. A–B, Post-mortem lung specimens from human subjects without asthma (A) and with asthma (B) stained with anti-AIBP antibodies. C, Quantification of AIBP-positive bronchial epithelium ($n = 6$). D, *APOA1BP* mRNA in bronchial epithelial cells isolated from non-asthmatics ($n = 9$) and asthmatics ($n = 11$) normalized to *HPRT1*. E–F, Lungs from mice that received 4 weekly intranasal doses of vehicle (E, E') or 100 μ g HDM (F, F') were harvested 3 days after the last HDM challenge and stained with anti-AIBP antibodies. G, Quantification of AIBP staining in bronchial epithelium ($n = 8$). H–I, *Apoa1bp*^{−/−} mice received 25 μ g of intranasal RFT1081 and were sacrificed at the specified time points to collect BAL, blood (H) and lung tissue (I). RFT1081 levels were measured using a sandwich ELISA as described in Methods ($n = 4$ –5 mice per time point; 2 males and 2–3 females). Scale bar, 50 μ m. Mean $\pm$ SEM; *, $p < 0.05$; ***, $p < 0.001$

previously described.⁸ In brief, samples were treated with RNA-STAT-60 (TelTest), and reverse-transcribed with Oligo-dT and SuperScript II kit (Life Technologies). qPCR was performed with TaqMan PCR Master Mix and TaqMan primers obtained from Integrated DNA Technologies, Coralville, IA. The relative amounts of mRNA were normalized to those of human or mouse housekeeping gene hypoxanthine phosphoribosyltransferase-1 (*HPRT1* or *Hprt1*).

1.5 | Production of RFT1081

Modified, His-tagged AIBP,⁷ designated as RFT1081, was developed by Raft Pharmaceuticals and custom produced by Selvita, Inc. (Cambridge, MA). In brief, RFT1081 contains an N-terminal extension, including His tag, which augments anti-inflammatory properties

of AIBP. It was expressed in a baculovirus/insect cell system to ensure post-translational modification and endotoxin-free preparation and purified by affinity chromatography using a Ni-NTA agarose column, followed by ion exchange chromatography and buffer replacement. The product was >90% pure, with no detectable aggregates (HPLC-SEC) and residual endotoxin <0.2 EU/mg. Storage-stability study of RFT1081 for up to 6 months at -80°C or for 1 week at 4°C did not show any loss of its titre or purity.

1.6 | RFT1081 pharmacokinetics

Similar to measuring pharmacokinetics of intrathecally delivered AIBP in spinal cord and CSF,⁷ to measure RFT1081 exposure in lung and BAL, we used *Apoa1bp*^{−/−} mice in order to avoid cross-reactivity

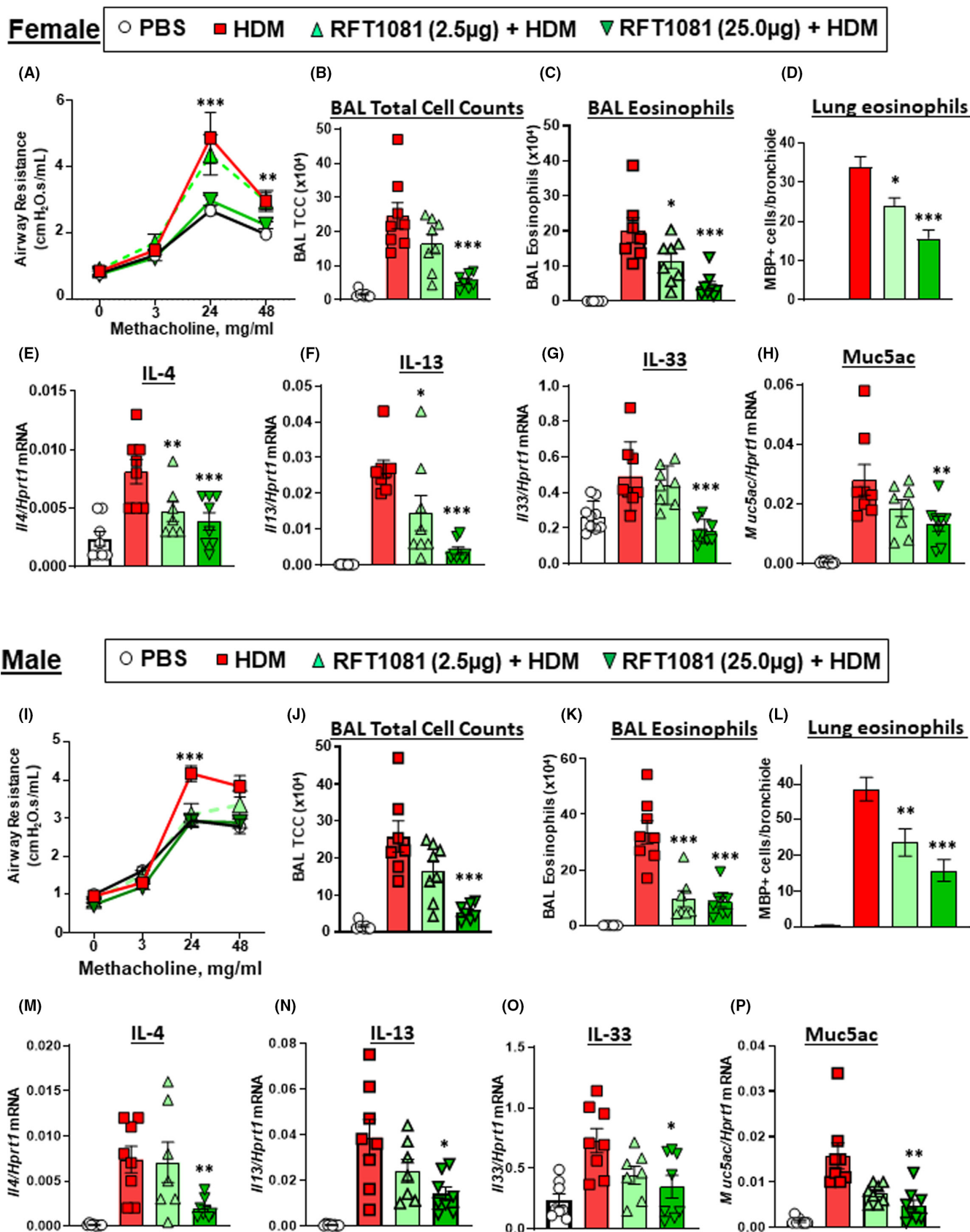


FIGURE 2 RFT1081 reduces airway hyperresponsiveness, eosinophilic pulmonary inflammation, IL33, Muc5ac and lung T2 response in an HDM model of asthma in female and male mice. Female (A–H) and male (I–P) C57BL/6J mice received 4 weekly intranasal instillations of 2.5µg or 25.0µg of RFT1081 or PBS. Two hours later, the mice received intranasal instillations of 100µg HDM or vehicle. Three days after last challenge, mice were tested for airway resistance to methacholine (A, I), and lung (D–H, L–P) and BAL (B–C, J–K) were collected for analyses as indicated. Mean ± SEM; $n = 8$ mice per group. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. One-way ANOVA with Tukey's multiple comparisons test

of the antibodies we use with endogenous mouse AIBP in the lung tissue. Plates were coated with BE-1 anti-AIBP monoclonal antibody (5 µg/mL), incubated for 3h with BAL, plasma or lung extract samples and detected with a rabbit polyclonal anti-AIBP antibody (gift from Dr. Longhou Fang, Houston Methodist Research Institute), followed by a goat-anti-rabbit-ALP antibody (Sigma Aldrich) and LumiPhos 530 (Lumigen); luminescence was measured using a plate reader (BioTek).

1.7 | HDM mouse model of asthma

All experiments were conducted according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, San Diego. Wild-type C57BL/6J mice (male and female) aged 8 weeks were administered 100 µg of intranasal HDM (*Dermatophagoides pteronyssinus*) extract (Greer Laboratories) on days 0, 7, 14 and 21 as previously described.⁸ Two hours prior to each HDM administration, 50 µl of PBS control or RFT1081 solution, either 2.5 µg or 25 µg dose, were given intranasally. Control group received intranasal PBS instead of HDM. On day 24, airway hyperresponsiveness to methacholine was assessed as described,⁸ and the mice were sacrificed to collect the BAL and the lungs. BAL was collected by lavage of 1 ml PBS via tracheal catheter, centrifuged and the pellet was resuspended in 1 ml PBS. After determining BAL total cell count, differential cell counts were quantified in Wright-Giemsa-stained slides.⁸ Lung eosinophil counts were quantified in the peribronchial space in lung paraffin-embedded sections stained with an anti-mouse major basic protein (MBP) rabbit polyclonal antibody (kindly provided by Mayo Foundation for Medical Education and Research). Results are expressed as the number of peribronchial cells staining positive per bronchiole with a 150–200 µm internal diameter. At least five bronchioles were counted in each slide. The operator was blinded to the identity of samples.

1.8 | Statistics

All results are presented as Mean ± SEM. A statistical software package (GraphPad Prism) was used for the analysis. Mann–Whitney U-test was used for analysis of two groups. Two-way or one-way ANOVA with post hoc Tukey's multiple comparisons test was used when more than two groups were compared. *p*-values of less than 0.05 were considered statistically significant.

KEYWORDS

airway hyperresponsiveness, apolipoprotein A-I binding protein, asthma, bronchial epithelium, eosinophil, inflammation

CONFLICT OF INTEREST

YIM is an inventor listed in patent applications related to the topic of this paper and scientific founder of Raft Pharmaceuticals LLC. The terms of this arrangement have been reviewed and approved by the

University of California, San Diego in accordance with its conflict of interest policies. ALO is a consultant for Raft Pharmaceuticals. Other authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

YIM, DHB, KB and MM designed and planned studies. MM, AKP, AG, JMN-P, KX and SP conducted experiments and analysed data. RCK provided human specimens. GB and AOL contributed to production of RFT1081. YIM, DHB and MM wrote first draft of the manuscript. GB, ALO, KB and RCK contributed to study discussions and manuscript revisions.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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