

ASTHMA

DP₂ antagonism reduces airway smooth muscle mass in asthma by decreasing eosinophilia and myofibroblast recruitment

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Increased airway smooth muscle mass, a feature of airway remodeling in asthma, is the strongest predictor of airflow limitation and contributes to asthma-associated morbidity and mortality. No current drug therapy for asthma is known to affect airway smooth muscle mass. Although there is increasing evidence that prostaglandin D₂ type 2 receptor (DP₂) is expressed in airway structural and inflammatory cells, few studies have addressed the expression and function of DP₂ in airway smooth muscle cells. We report that the DP₂ antagonist fevipiprant reduced airway smooth muscle mass in bronchial biopsies from patients with asthma who had participated in a previous randomized placebo-controlled trial. We developed a computational model to capture airway remodeling. Our model predicted that a reduction in airway eosinophilia alone was insufficient to explain the clinically observed decrease in airway smooth muscle mass without a concomitant reduction in the recruitment of airway smooth muscle cells or their precursors to airway smooth muscle bundles that comprise the airway smooth muscle layer. We experimentally confirmed that airway smooth muscle migration could be inhibited in vitro using DP₂-specific antagonists in an airway smooth muscle cell culture model. Our analyses suggest that fevipiprant, through antagonism of DP₂, reduced airway smooth muscle mass in patients with asthma by decreasing airway eosinophilia in concert with reduced recruitment of myofibroblasts and fibrocytes to the airway smooth muscle bundle. Fevipiprant may thus represent a potential therapy to ameliorate airway remodeling in asthma.

INTRODUCTION

Asthma affects over 300 million people worldwide, and its prevalence is increasing (1) despite currently available therapies (2). Asthma is characterized by variable airflow limitation that becomes more persistent in severe disease. Increased airway smooth muscle (ASM) mass is an important component of airway remodeling in asthma, contributing substantially to symptoms and disordered airway physiology (3–5). To date, no drug has affected the increased ASM mass observed in asthma in randomized placebo-controlled trials (3, 4). However, bronchial thermoplasty has demonstrated a potential reduction in ASM mass in asthma in uncontrolled studies (6, 7).

The prostaglandin D₂ (PGD₂) type 2 receptor [DP₂, also known as chemoattractant receptor-homologous molecule expressed on T helper 2 (T_H2) cells (CRT_H2)] is expressed by inflammatory cells critical in the immunopathogenesis of asthma, including eosinophils, T_H2 lymphocytes, type 2 innate lymphoid cells, and mast cells. DP₂ activation promotes cellular release of cytokines, inflammatory cell migration, and cell survival (8–10). Its archetypal ligand PGD₂ is predominantly released by mast cells localized to the ASM bundle (11). The DP₂ antagonist fevipiprant has been shown to improve asthma symptoms, lung function, airway eosinophilia, and epithelial

integrity (4). However, the role of the PGD₂/DP₂ axis in ASM dysfunction in asthma has not been extensively studied. We hypothesized that the PGD₂/DP₂ axis may contribute to increased ASM mass in asthma and that antagonism of DP₂ with fevipiprant might result in a decrease in ASM mass.

Here, we analyzed bronchial biopsies from patients with asthma treated with the DP₂ antagonist fevipiprant in a previous phase 2a randomized placebo-controlled trial undertaken to determine the impact of drug on airway inflammation, remodeling, and asthma control (4). Using an agent-based computational model representing an asthmatic airway in human patients and supported by in vitro ASM cell-based observations, we propose that the reduced ASM mass observed in the bronchial biopsies after fevipiprant treatment may be a consequence of inhibition of eosinophilic airway inflammation together with reduced recruitment of myofibroblasts and fibrocytes to the ASM bundle.

RESULTS

Fevipiprant reduces ASM mass in patients with asthma in a randomized placebo-controlled trial

We obtained bronchial biopsies from moderate to severe asthmatics with airway eosinophilic inflammation, as evidenced by increased sputum eosinophil counts. These individuals had participated in a 12-week single-center (University of Leicester), randomized, double-blind, parallel-group, placebo-controlled trial of the DP₂ antagonist fevipiprant (4). We then performed an a priori quantification of ASM mass (percentage of total bronchial biopsy area) (11). A representative photomicrograph of a bronchial biopsy from a subject showing disrupted epithelium and increased ASM mass is shown in

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Fig. 1A. The absolute ASM mass percentage (mean $\pm$ SEM) observed decreased significantly after treatment with fevipiprant ($-13 \pm 5\%$; $P = 0.022$, $n = 14$) versus placebo ($4 \pm 5\%$; $P = 0.52$, $n = 13$) [mean difference [95% confidence interval (CI)], 16.2% (-1.4 to -31.1%); $P = 0.034$] (Fig. 1B). In view of the above data, we performed a post hoc quantification of ASM mass (percentage of total biopsy area) in bronchial biopsies derived from a subgroup of moderate to severe eosinophilic asthmatics who had participated in a 50-week single-center (University of Leicester), randomized, double-blind, parallel-group, placebo-controlled trial of the anti-interleukin-5 (anti-IL-5)-neutralizing antibody mepolizumab (12). Although the sample size was small, in contrast to fevipiprant, we observed no significant effect of mepolizumab on ASM mass [absolute ASM mass percentage increase after treatment with mepolizumab ($2.9 \pm 4.0\%$; $n = 7$) versus placebo ($1.5 \pm 2.2\%$; $n = 5$) [mean difference (95% CI), 1.4% (-9.9 to 12.7%); $P = 0.79$]. For these and other data, see data file S1.

An agent-based computational model recapitulates the features of airway remodeling observed in asthma

To interrogate the mechanisms governing the pathogenesis of asthma, we developed an agent-based computational model of airway remodeling comprising epithelial, mesenchymal, and inflammatory parameters. In agent-based modeling, a system is divided into agents (here, airway cells; table S1) capable of interacting with each other and their environment based on defined rule sets (13–15). The initial state of the model is illustrated in fig. S1. Our model considered interactions between epithelial (columnar and goblet cells), mesenchymal (fibroblast, myofibroblast, and ASM cells), and inflammatory (eosinophil) cell types. The various cell types, depending on their phenotype, displayed behaviors ranging from proliferation, migration, (de)differentiation, apoptosis, and synthesis of extracellular matrix proteins and cytokines (table S1). These virtual cells were simulated within a Strahler order 3 virtual airway with a lumen diameter of 1.21 mm and a wall area of 1.79 mm². The number of each cell type within the model was based on geometrical constraints and published data. The rule set governing agent behaviors and interactions was derived from existing in vitro, animal, and clinical studies (Table 1). The underpinning agent interactions and rule sets attributed to the agents are summarized in Table 1 and represented schematically in fig. S2.

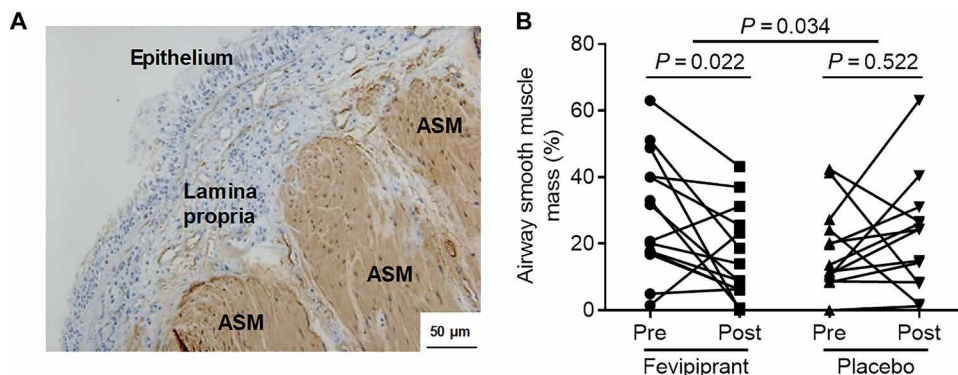


Fig. 1. Increased ASM mass in asthma is reduced by fevipiprant. (A) Representative photomicrograph of a bronchial biopsy from a participant with severe asthma in the fevipiprant (DP₂ antagonist) trial, showing increased ASM [brown-stained α -smooth muscle actin (α -SMA)], disrupted epithelium, and lamina propria. (B) ASM mass, as measured by percentage α -SMA-positive area, in bronchial biopsies from asthmatic subjects before treatment (Pre) and 12 weeks after treatment (Post) with fevipiprant ($n = 14$) or placebo ($n = 13$). A two-tailed paired *t* test was used for within group comparisons ($P = 0.022$ and $P = 0.522$), and a two-tailed unpaired *t* test was used to compare the difference in ASM mass observed after treatment with fevipiprant to that seen in the placebo group ($P = 0.034$).

In the model, we initially damaged the epithelium to cause 50% epithelial denudation. We then simulated the consequent normal injury repair and pathological airway remodeling over 180 days by introducing alterations to model parameters (tables S2 to S4). The following pathological markers and value ranges were considered necessary in the model to reflect the key hallmarks of severe asthma (1, 2): eosinophilic inflammation (eosinophils/mm² submucosa) of >10 , epithelial integrity of $<70\%$, and ASM mass of ≥ 10 and $\leq 50\%$. We conducted parametric testing by varying, both individually and collectively, parameters from all agent categories and observing which conditions best captured the above hallmarks of asthma. We defined the most parsimonious set of parameters to capture these three hallmarks (tables S2 to S4). The response to epithelial injury in this model displayed significantly increased eosinophilic inflammation ($P < 0.001$), ASM mass ($P = 0.002$), and persistent epithelial damage ($P < 0.001$) compared to the model of healthy control individuals over the 180-day time course (Fig. 2A).

Computational modeling predicts that a reduction in eosinophil recruitment is not sufficient to decrease ASM mass

To predict the impact of reducing eosinophil number in our model of airway remodeling, we incorporated proapoptotic or antirecruitment elements into the model. These variables were chosen to represent the major respective effects of neutralizing IL-5 (12, 16), an obligate cytokine for eosinophil survival and maturation, and of blocking the activation of DP₂, which promotes eosinophil recruitment (4). We tested an increasing range of intervention doses and found that they resulted in a progressive reduction in airway eosinophilia and ASM mass in our model of airway remodeling (Fig. 2, B and C).

We then used our computational model of a remodeled asthmatic airway to determine the predicted percentage increase in eosinophil apoptosis and the percentage reduction in eosinophil recruitment required to reduce the number of bronchial wall eosinophils to that seen in vivo in clinical trials of mepolizumab (17) and fevipiprant (4), which reduced the eosinophil count in patients by 55 and 80%, respectively, compared to placebo control. To attain the reduction in airway eosinophil number clinically observed with mepolizumab, the model predicted that 15% of the eosinophil population must be induced to undergo apoptosis (reduction in airway eosinophilia versus control of $54.1 \pm 4.1\%$; Fig. 2B, checked bar). To attain the reduction in airway eosinophil number clinically observed for fevipiprant, the model predicted that a 40% reduction in eosinophil recruitment was required (reduction in airway eosinophilia versus control of $81 \pm 0.6\%$; Fig. 2B, hatched bar).

We subsequently used the proapoptotic (15%) and antirecruitment (40%) models resulting in a reduction in eosinophil number equivalent to that seen in the mepolizumab and fevipiprant clinical trials to predict the impact of each intervention on ASM mass. When assuming an increase in eosinophil apoptosis of 15%, the proapoptosis model predicted a small mean $\pm$ SEM decrease in ASM mass (absolute reduction of $4.0 \pm 0.6\%$

Table 1. Agents, rules, and model. The computational airway model rule set, parameters, and which parameters were altered to observe airway remodeling. N/A, not applicable.

Category	Activity	Rules	Parameters	Baseline value	Comments
Epithelial	Migration	Dedifferentiated epithelial cells migrate to repair the damaged epithelium and then undergo proliferation.	Migration rate	5 μm/hour	Migration rate was consistent with in vitro studies (41–43).
			Migration delay	5 hours	Dedifferentiated cells migrate after 5 hours, doubled to 10 hours to represent slowly recovering epithelium.
	Differentiation	Cells at the edge dedifferentiate into a flattened phenotype.	Dedifferentiation probability	50%	A 50% probability per iteration was applied to determine whether an epithelial cell dedifferentiates. This was altered to 5% per iteration for the defective epithelium.
			Delay in proliferation	2 to 8 hours	Edge cells require 2 to 8 hours to flatten out (41); this was increased by three times (6 to 24 hours) for slow epithelial recovery.
	Proliferation	Dedifferentiated epithelial cells proliferate after their migration to the opposite edge of a compromised epithelium.	Ciliated:goblet ratio	0.7:0.3 and 0.1:0.9	The number of goblet cells increase by two to three times in severe asthma (44–47) given the inflammation status in disease. The probability of differentiating to a ciliated:goblet cell fate was either 0.7:0.3 (less inflamed) or 0.1:0.9 (more inflamed).
Inflammatory	Apoptosis	This feature was not included in the baseline model.	Auto-denudation	N/A	No apoptosis rate was applied to the baseline model. In “mild” shedding every 25 hours, cells underwent apoptosis with 50% probability, “moderate” apoptosis with the probability of 1% at each iteration, “extreme” 10% at each iteration.
	Cell migration and activation	Inflammatory cells migrate into the airway in response to epithelial denudation, and their activation promotes eosinophil recruitment.	Frequency of recruitment	120 hours	Inflammatory cells were added every 120 hours to the model with recruitment frequency increased to 60 and 50 hours in disease.
			Inflammatory cells recruited	30	Inflammatory cell number was chosen to reflect previous reports (11), and in disease, this was increased by 1.5 to 2 times (48).
			Eosinophil recruitment	45	Eosinophil recruitment was triggered by increased inflammatory cell number (45), based on previous reports (11), and in disease, this threshold was halved.
	Degranulation	Inflammatory cell activation promotes cell recruitment and fibroblast differentiation.	Inflammatory cytokine release	25/75/105 hours	The “universal” inflammatory cell was assigned 150 hours life, releasing cytokines for 25 hours (or 75 and 105 hours for epithelial integrity of <55 and 30%, respectively). In disease, inflammatory cell life was reduced to 65, 40, and 25 hours (releasing cytokines for 85, 110, and 125 hours, respectively).
	Apoptosis	Inflammatory cells promote eosinophil survival.	Eosinophil life in the airway	10/30/45 hours	Eosinophils survival is ~10 hours within a normal airway (49). This was increased to 30 and 45 hours for epithelial integrity of <55 and 30%, respectively. In disease, the life of eosinophils was doubled to 20, 60, and 90 hours.
Mesenchymal	Differentiation	Inflammatory cells promote increased ASM mass.	ASM activation	25 cells	The threshold number of inflammatory cells above which myofibroblast-ASM differentiation is induced (11), which was reduced by 50% in disease.
	Differentiation	Inflammatory cells promote fibroblast-myofibroblast differentiation.	(Myo)fibroblast differentiation probability	30%	Increased fibroblast differentiation or myofibroblast recruitment in the presence of activated inflammatory cells increased to 50% in disease.
	Proliferation	Fibroblast proliferation increased by inflammatory and epithelial cells.	Fibroblast growth rate	132 iterations	Animal models indicate that lung fibroblasts divide every 5.5 days (132 iterations in model), which reduces to 2 days (48 iterations in model) in the animal model of asthma (50).
	Apoptosis	Myofibroblast survival supported by activated inflammatory cells.	Myofibroblast apoptosis	5 cells	The threshold number of inflammatory cells below which myofibroblast apoptosis was induced, which was reduced to two in disease.

and relative reduction of 12 ± 2% versus control; Fig. 2C, checked bar). This is consistent with the mepolizumab clinical trial (12) in which no significant change in ASM mass was observed. When assuming a decrease in eosinophil recruitment of 40%, the antirecruitment

model predicted a modest reduction in ASM mass (absolute reduction of 8.1 ± 0.5% and relative reduction of 25 ± 1% versus control; Fig. 2C, hatched bar), which was not sufficient to result in the observed response to fevipiprant (13% absolute reduction and 44% relative reduction

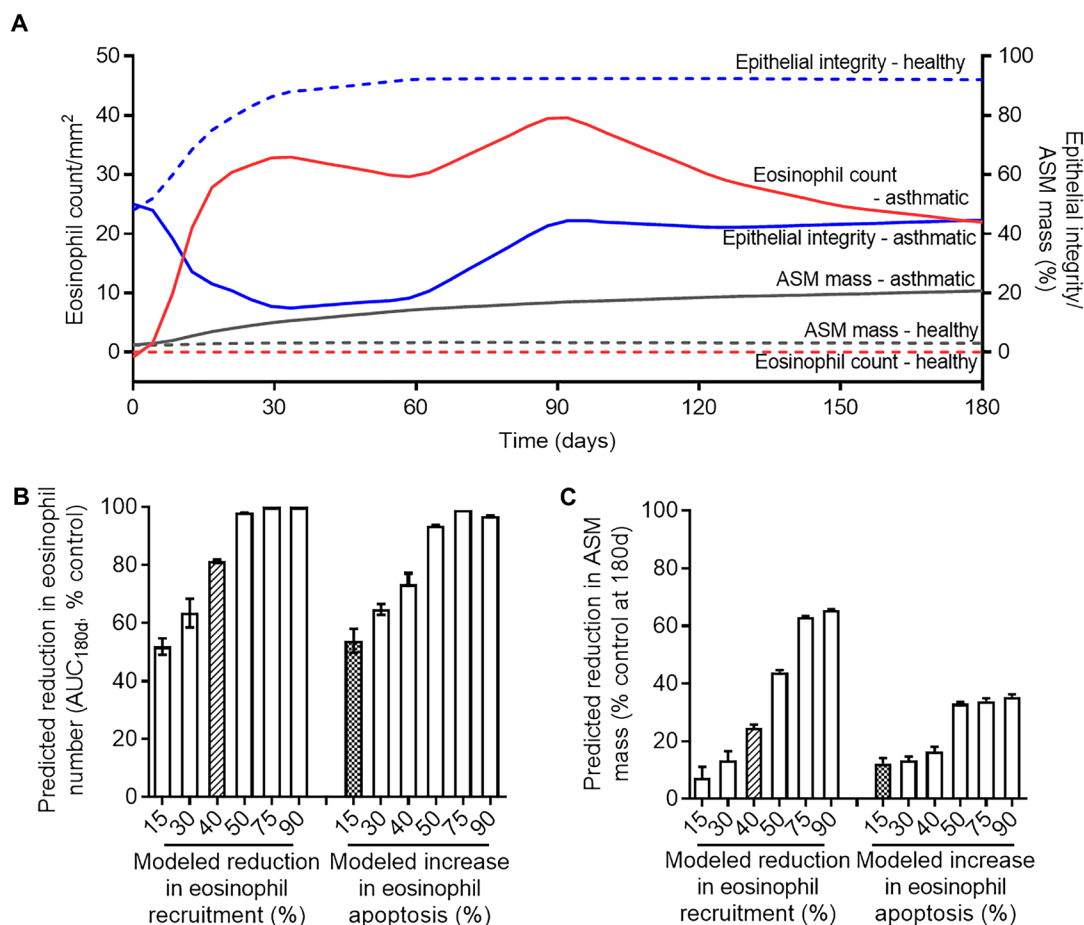


Fig. 2. Computational model-based investigation of interactions between airway inflammation and ASM mass. (A) The mean time course from six simulations of the response to epithelial injury (50% denudation at time zero) over 180 days showing increased eosinophil numbers, ASM mass, and persistent epithelial damage in the model of airway remodeling in asthma versus resolution of the epithelial injury, eosinophil numbers, and persistently low ASM mass in the healthy control model ($P < 0.01$ for comparisons of each parameter over time between the patient model versus healthy control model, two-tailed unpaired t tests). (B) Predicted reduction in eosinophil number over 180 days (180d) after reduction in eosinophil recruitment or increase in eosinophil apoptosis in the computational model ($n = 5$ simulations). (C) Relative change in the ASM mass percentage at 180 days, predicted as a consequence of results in (B) ($n = 5$ simulations).

in ASM mass). The model therefore suggested the existence of additional mechanisms that, along with a reduction in airway eosinophilia, mediated the reduction in ASM mass after treatment with fevipiprant.

The ASM PGD₂/DP₂ axis mediates ASM migration

To explore the mechanism by which DP₂ antagonism resulted in a decrease in ASM mass, we assessed the expression and function of DP₂ in ASM. We found that DP₂ was expressed in the ASM bundle in bronchial biopsies from patients recruited for research bronchoscopies (Fig. 3A), in line with the previous finding that PGD₂ primes the migration of ASM cells toward platelet-derived growth factor via DP₂ (18). However, DP₂ expression was not significantly different between patients with severe asthma (60 ± 1 ; $n = 8$) and healthy controls (57 ± 5 ; $n = 11$) [mean difference (95% CI), 2.6 (−9.9 to 15.0); $P = 0.67$]. We also confirmed DP₂ expression in primary human ASM cells at the mRNA (Fig. 3B) and protein levels (Fig. 3, C and D, and fig. S3).

PGD₂ can activate PGD₂ type 1 (DP₁), DP₂, and thromboxane receptors (9). Therefore, we investigated the effect of the selective DP₂ agonist 13,14-dihydro-15-keto-PGD₂ (DK-PGD₂) and selective

DP₂ antagonists (fevipiprant, CAY10471, and OC000459) on DP₂ receptor activation, phenotype, and behavior of primary human ASM cells. DK-PGD₂ (10 to 100 nM) stimulated a small but significant increase in filamentous actin (F-actin) polymerization and intracellular calcium elevation [area under curve of the dose response (AUC DR), $P = 0.01$ and $P = 0.002$, respectively; Fig. 3, E and F]. Although this did not translate to an effect of DK-PGD₂ on ASM cell migration in vitro (Fig. 4A), blocking the activation of DP₂ by endogenous PGD₂ with the DP₂ antagonist fevipiprant significantly inhibited ASM cell migration in vitro at the highest drug concentrations [percentage reduction in cells migrating into the wound after 24 hours versus vehicle control: 10 nM, $8.8 \pm 7.8\%$ ($P = 0.294$); 50 nM, $7.7 \pm 7.4\%$ ($P = 0.332$); 100 nM, $12.8 \pm 4.9\%$ ($P = 0.034$); and 500 nM, $17.4 \pm 6.6\%$ ($P = 0.034$); Fig. 4A]. We confirmed this effect using other DP₂ antagonists including CAY10471 [percentage reduction in cells migrating into the wound after 24 hours versus vehicle control: 10 nM, $11.6 \pm 2.2\%$ ($P = 0.010$); 50 nM, $9.3 \pm 3.7\%$ ($P = 0.038$); and 100 nM, $13.6 \pm 4.4\%$ ($P = 0.027$); Fig. 4A] and OC000459 [percentage reduction in cells migrating into the wound after 24 hours versus vehicle control: 10 nM, $8.4 \pm 3.3\%$ ($P = 0.043$); 50 nM, $6.7 \pm 4.2\%$

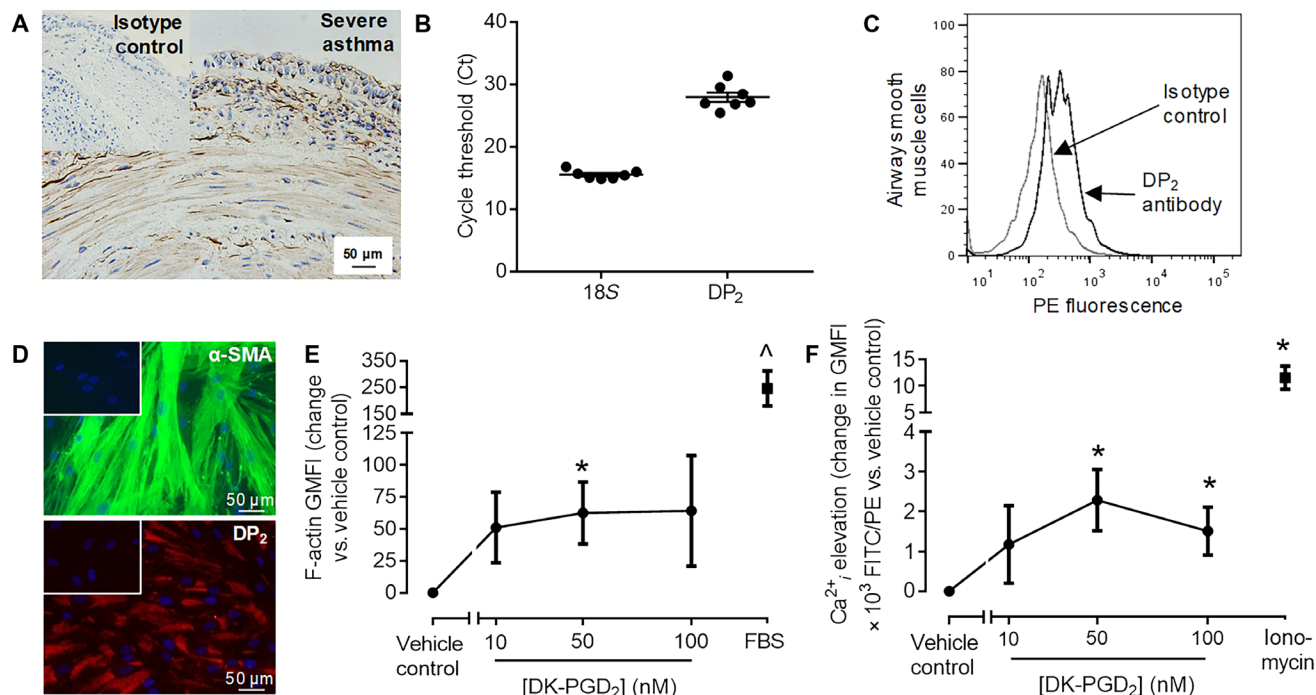


Fig. 3. ASM cells express functional DP₂. (A) Representative photomicrograph of DP₂ staining in bronchial biopsies from a subject with severe asthma (inset: isotype control). (B) Quantitative polymerase chain reaction cycle threshold (Ct) values for expression of ASM DP₂ mRNA versus the 18S ribosomal RNA housekeeping gene *RNA18S5* [mean DP₂ Ct (95% CI), 27.9 (26.1 to 29.8); $n = 7$]. (C) Example histogram of DP₂ expression (black trace) in ASM cells by flow cytometry versus isotype control antibody (gray trace); fold increase in geometric mean fluorescence intensity (GMFI) of anti-DP₂ antibody/isotype control antibody (95% CI), 1.3 (1.2 to 1.4) [$n = 15$ donors, $P < 0.001$, two-tailed paired t test against isotype control]. (D) Representative photomicrographs ($\times 20$ magnification) showing ASM α -SMA expression (green, left; isotype control antibody, inset) and ASM DP₂ expression (red, right; isotype control antibody, inset) by immunofluorescence staining. Nuclei are stained with 4',6-diamidino-2-phenylindole (blue). (E) F-actin polymerization in primary human ASM cells ($n = 9$ donors) in response to DK-PGD₂ treatment or Dulbecco's modified Eagle's medium (DMEM) containing 50% fetal bovine serum (FBS) as a positive control [geometric mean AUC DR of DK-PGD₂ (95% CI), 46 (25 to 104) $\times 10^2$; $P = 0.01$, one sample t test against a hypothetical value of zero]. (F) Intracellular calcium (Ca^{2+}) elevation in primary human ASM cells ($n = 6$ to 9 donors) in response to DK-PGD₂ treatment or ionomycin (1.5 $\mu\text{g/ml}$) as a positive control [geometric mean AUC DR of DK-PGD₂ (95% CI), 130 (78 to 230) $\times 10^3$; $P = 0.002$, one sample t test against a hypothetical value of zero]. FITC, fluorescein isothiocyanate; PE, phycoerythrin. Data are plotted as means $\pm$ SEM. Two-tailed paired t tests were performed to compare each condition with its vehicle control; * $P < 0.05$, except FBS, where Wilcoxon matched-pairs signed-rank test was used, denoted by $\wedge P < 0.05$.

($P = 0.157$); and 100 nM, $8.8 \pm 1.9\%$ ($P = 0.003$); Fig. 4A]. Representative photomicrographs of primary ASM monolayer cultures wounded by scratching, followed by incubation with different treatments for 24 hours are shown in Fig. 4B. Thus, we hypothesized that PGD₂ was released into the extracellular milieu by ASM cells to affect ASM behavior in an autocrine manner. Genes involved in PGD₂ biosynthesis and metabolism, including PGD₂ synthase, were expressed by ASM cells from subjects with and without asthma (tables S5 and S6). Consistent with previous reports (19), PGD₂ was released by ASM, albeit at a low concentration compared with mast cells (20, 21), and this PGD₂ release increased after wounding [129 ± 18 PGD₂ pg/ml per 10^5 ASM versus 176 ± 22 PGD₂ pg/ml per 10^5 ASM; mean difference (95% CI), 52.0 (4.8 to 99.2); $P = 0.02$; Fig. 4C]. In addition to myofibroblasts, we demonstrated that the ASM progenitor fibrocytes expressed DP₂ (Fig. 4D). The correlation between the change in ASM percentage observed in those treated with fevipiprant or placebo and the change in lamina propria myofibroblast or fibrocyte number supported the view that the effects of fevipiprant on ASM mass and lamina propria mesenchymal cells may have occurred in parallel (Fig. 4, E and F). These findings suggested that anti-DP₂ might, in part, reduce ASM mass via a direct and concomitant effect upon ASM and myofibroblast or fibrocyte recruitment to the ASM bundle.

The effects on ASM cell migration were not due to cytotoxic effects on the ASM cells because there was no effect on cell number after treatment for 24 hours with DK-PGD₂ (100 nM), fevipiprant (500 nM), CAY10471 (100 nM), or OC000459 (100 nM) (fig. S4A). DK-PGD₂ (100 nM), fevipiprant (500 nM), and CAY10471 (100 nM) had no effect on apoptosis or necrosis (fig. S4B). This was supported by a lack of effect of DK-PGD₂ (100 nM), fevipiprant (500 nM), or CAY10471 (100 nM) on cell size or granularity, which is known to change during apoptosis and necrosis (fig. S4, C and D). Furthermore, DK-PGD₂ (10 to 100 nM; fig. S5A), CAY10471 (10 to 100 nM; fig. S5B), and fevipiprant (10 to 500 nM; fig. S5C) neither induced the proliferation of ASM in the presence of serum-free media nor inhibited the ASM cell proliferation induced by FBS over 3 days, as assessed by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium inner salt assay and carboxyfluorescein succinimidyl ester fluorescence (fig. S5, D and E). In the fevipiprant clinical trial, there was no change in proliferating cell nuclear antigen (PCNA) staining in bronchial biopsies before versus after treatment with fevipiprant or placebo after 12 weeks of treatment. In addition, DK-PGD₂ (10 to 100 nM), fevipiprant (10 to 500 nM), or CAY10471 (10 to 100 nM) had no effect on ASM α -SMA expression (fig. S6A). This was supported by a lack of effect of DK-PGD₂ (100 nM), fevipiprant

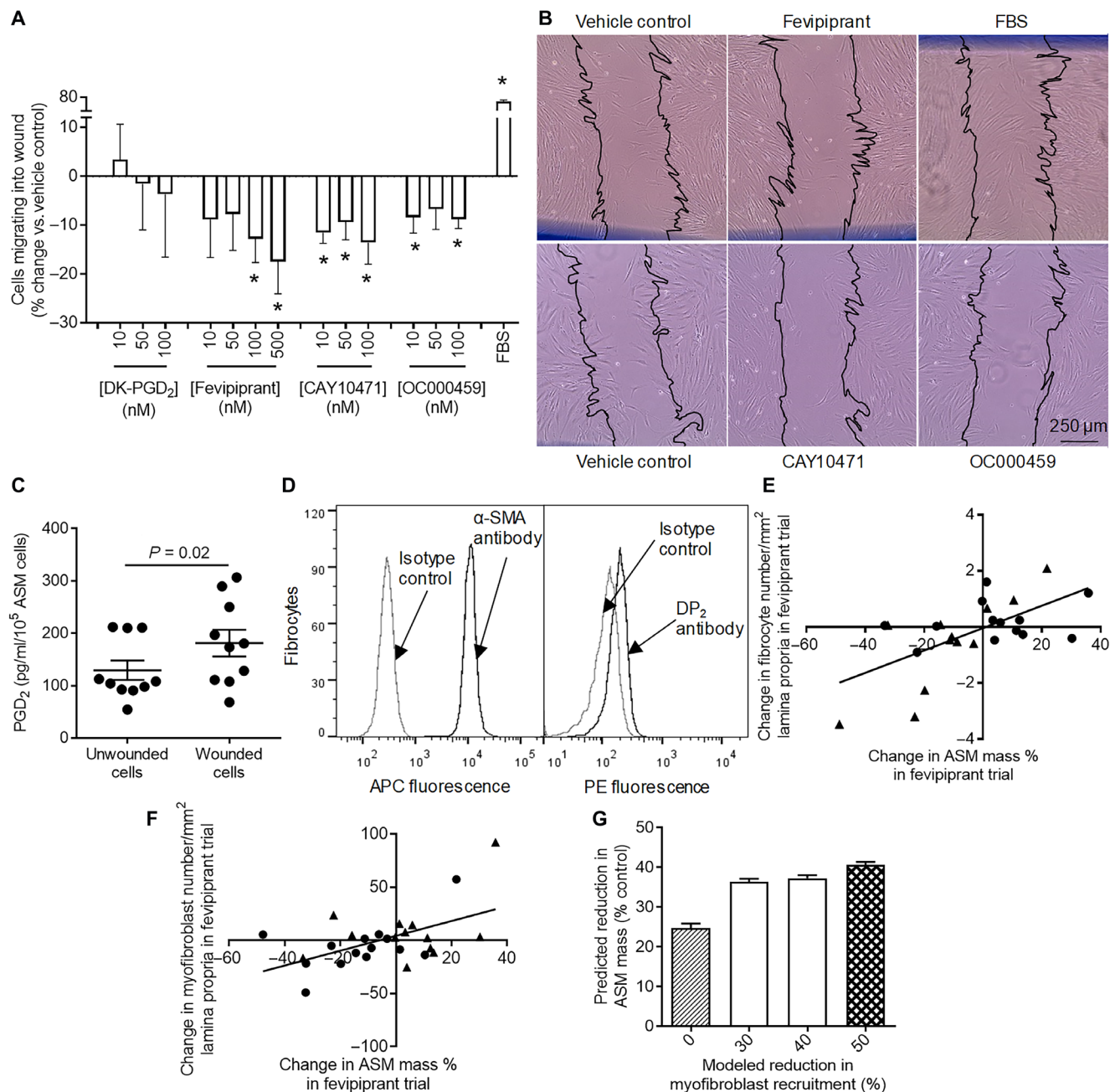


Fig. 4. A DP₂ antagonist reduces ASM migration and recruitment of myofibroblasts and fibrocytes. All experiments were carried out in serum-free media. **(A)** Data for the wound closure after 24 hours of ASM cells that had been grown in monolayers and then wounded by scratching with a pipette tip, followed by incubation with different treatments for 24 hours are shown: DP₂ agonist DK-PGD₂ ($n = 4$ to 5 donors), the DP₂ antagonists fevipirant ($n = 8$ donors), CAY10471 ($n = 6$ to 8 donors), and OC000459 ($n = 7$ donors) or DMEM culture medium containing 10% FBS as a positive control. Two-tailed paired t tests were performed to compare each condition with its vehicle control; $*P < 0.05$ versus vehicle control. Data are expressed as means $\pm$ SEM. **(B)** Representative photographs of ASM monolayers wounded by scratching with a pipette tip after 24 hours [vehicle control for fevipirant (500 nM), and DMEM containing 10% FBS control (top); vehicle control for CAY10471 (100 nM) and OC000459 (100 nM) (bottom)]; black lines represent the wound edge at 0 hours. **(C)** PGD₂ release by unwounded and wounded ASM cells after 24 hours; $P = 0.02$, Wilcoxon matched-pairs signed-rank test ($n = 10$ donors). Data are expressed as means $\pm$ SEM. **(D)** Representative flow cytometry traces of isotype control antibodies (gray traces) versus α -SMA expression [left, black trace; mean percentage fibrocyte population positive for α -SMA expression (95% CI), 97% (93 to 100%); $n = 4$ donors] and DP₂ expression [right, black trace; GMFI fold difference DP₂ antibody/isotype control antibody (95% CI), 1.6 (1.3 to 2); $P = 0.0064$, two-tailed paired t test against isotype control antibody ($n = 6$ donors)] by fibrocytes. **(E)** Correlation between change in myofibroblast number in the lamina propria and absolute change in ASM mass as a percentage of the total biopsy area [fevipirant, black circle ($n = 14$); placebo, black triangle ($n = 13$); Spearman (95% CI) $r = 0.347$ (–0.050 to 0.649), $P = 0.076$]. **(F)** Correlation between change in fibrocyte number in the lamina propria and absolute change in ASM mass as a percentage of the total biopsy area [fevipirant, black circle ($n = 12$); placebo, black triangle ($n = 13$); Spearman (95% CI) $r = 0.538$ (0.169 to 0.774), $P = 0.006$]. **(G)** Predicted relative reduction in percent ASM mass at 180 days in the computational model as a consequence of reduced myofibroblast recruitment (30 to 50%) in combination with a 40% reduction in eosinophil recruitment in the computational model ($n = 5$ simulations).

(500 nM), or CAY10471 (100 nM) on basal or bradykinin-stimulated ASM contraction (fig. S6, B and C).

Modeling predicts that reductions in myofibroblast and eosinophil recruitment are required for fevipiprant to decrease ASM mass

To support our *in vitro* findings, we reduced the myofibroblast recruitment (0 to 50%) in the computational model together with the 40% reduction in eosinophil recruitment required to reflect the observed reduction in bronchial biopsy eosinophils after fevipiprant treatment in patients with asthma as described above. The resulting model predicted that a 50% reduction in myofibroblast recruitment in concert with reduced eosinophil recruitment would result in a decrease in ASM mass equivalent to that seen after fevipiprant treatment (Fig. 4G, cross hatched bars). In contrast, a reduction in myofibroblast recruitment alone was predicted to result in minimal effects on ASM mass ($1.8 \pm 1.2\%$ relative reduction). A comparison of the computational model and fevipiprant trial findings is summarized in fig. S7.

DISCUSSION

We report that a drug intervention in asthma, namely, fevipiprant (a DP₂ antagonist), reduced ASM mass in bronchial biopsies from patients with asthma who had participated in a previous randomized placebo-controlled trial (4). This is in contrast to the lack of an effect on ASM mass in response to mepolizumab that we report here, corticosteroids, or the anti-IL-13 monoclonal antibody tralokinumab (3, 22, 23). Our computational model and *in vitro* work supported the view that the reduction in ASM mass in response to fevipiprant was a consequence of inhibiting eosinophilic inflammation in concert with a direct reduction in the recruitment of myofibroblasts to the ASM bundle. Thus, fevipiprant may be a potential therapy to target airway remodeling in asthma, and its clinical benefits observed previously could be in part due to its effects on ASM.

One limitation of our study is that the number of paired biopsies collected in the fevipiprant trial included modest numbers of subjects despite the trial being one of the largest biopsy studies undertaken in subjects with asthma. Therefore, it is important to extend and confirm these findings in future studies. Likewise, it is possible that the lack of effect observed with other anti-inflammatory interventions is due to the lack of statistical power conferred by the small sample sizes. However, our computational model data suggest that these anti-inflammatory approaches are unlikely to be effective unless they have additional direct effects on ASM. A small reduction in ASM mass was previously reported after treatment with the calcium channel blocker gallopamil, which had been proposed to have direct effects on ASM activation, but the reduction in ASM mass was no different from placebo (24).

Another limitation of our study is that we cannot completely exclude the possibility that the effect of fevipiprant on ASM mass both *in vivo* and *in vitro* is an off-target effect. However, we used three selective and specific DP₂ antagonists, including fevipiprant, for the *in vitro* experiments, and therefore, we consider it unlikely that the findings we report on ASM activation and migration are due to off-target effects. Our *in vitro* findings also imply that the major effect of DP₂ antagonism on ASM function was the inhibition of migration of ASM progenitors to the airway, either from the blood or via attenuation of epithelial-mesenchymal transition, rather than through

effects on proliferation or apoptosis. This is consistent with the concept that mesenchymal cells exhibit plasticity in phenotype (25). In keeping with our *in vitro* observations, we did not identify any changes in PCNA staining in the ASM bundle *in vivo*, suggesting that there was no active proliferation of ASM. Together, these results suggest that neither proliferation nor apoptosis contribute to the effects of DP₂ antagonism on ASM mass, although we cannot completely exclude some contribution from these processes.

The strength of our study is the integration of findings from *in vivo* clinical trials and *in vitro* and computational models. We developed a comprehensive agent-based model of airway remodeling during asthma. Previous computational approaches have been applied to uncover mechanisms driving unresolved allergic inflammation and airway hyperresponsiveness in asthma (15, 26) but not airway remodeling. Our agent-based model was created to represent the airway in three dimensions. A possible limitation is that our model was used for one layer of agents and simulated a distal airway to balance model resolution and computational complexity. However, we believe that our model is a representative because it captures the features of the normal and pathological workings of the entire airway. Specifically, our computational model displayed features consistent with moderate to severe asthma including damaged bronchial epithelium, eosinophilic inflammation, and increased ASM mass. This model responded to perturbations reflective of changes in eosinophil survival and trafficking and provided new insights into possible mechanisms of action of DP₂ antagonists versus anti-IL-5 upon airway remodeling. This model has also given us insights into the effects of DP₂ antagonism, which would not be possible *in vitro* because of the limitations of studying multiple cell-cell interactions within a complex airway structure. Although our computational model is not patient specific, it represents an average patient with asthma and airway remodeling. This “virtual patient” represents a step toward patient-specific modeling in respiratory medicine. We anticipate our integrated approach combining agent-based modeling with *in vivo* clinical data, and *in vitro* findings will provide further insights into asthma in future studies.

MATERIALS AND METHODS

Study design

The objective of the study was to use an integrated strategy encompassing samples from a randomized placebo-controlled trial in patients with asthma evaluating fevipiprant (DP₂ antagonist) and mepolizumab (anti-IL-5 antibody), *in vitro* experiments, and predictive computational agent-based models simulating asthma pathogenesis to investigate the impact of DP₂ antagonism on ASM mass and determine the mechanisms driving this effect.

Patients with persistent moderate to severe asthma and an elevated sputum eosinophil count ($n = 61$) participated in a single-center (University of Leicester), randomized placebo-controlled trial of the DP₂ antagonist fevipiprant (225 mg twice per day orally) in addition to standard of care (4). In an independent study, patients ($n = 61$) who had refractory eosinophilic asthma participated in a single-center (University of Leicester), randomized placebo-controlled trial of an anti-IL-5–neutralizing antibody mepolizumab (750 mg intravenous infusions every 4 weeks for 50 weeks) in addition to standard of care (12). A subgroup of patients underwent bronchoscopy and bronchial biopsy in each independent study before and after administration of drug or placebo. The studies were approved by the

Leicester and Northamptonshire Ethics Committee (05/Q2502/98 and 11/EM/0402, respectively) and registered with ClinicalTrials.gov [ISRCTN75169762 and NCT01545726, with EudraCT (no. 2011-004966-13)]. The studies were carried out in accordance with CONSORT (Consolidated Standards of Reporting Trials) guidelines (4, 12).

The sample size for the fevipiprant and mepolizumab randomized controlled trials were determined on the basis of change in the sputum eosinophil count as the primary outcome as described in Gonem *et al.* (4) and on the number of exacerbations of asthma per patient as the primary outcome as described in Halder *et al.* (12). Assessing change in ASM mass was in the prespecified exploratory analysis plan for the fevipiprant clinical trial and was performed post hoc for mepolizumab. The inclusion and exclusion criteria for the fevipiprant and mepolizumab trials, randomization, and blinding procedures are described in Gonem *et al.* (4) and Halder *et al.* (12), respectively. For the in vitro experiments, the experiment was analyzed by the observers blinded to conditions.

Additional asthmatic patients and healthy controls were recruited from a single center (University of Leicester) for research bronchoscopies from which tissue sections and primary ASM cells could be derived. Those with asthma gave an appropriate history and had objective evidence of variable airflow obstruction or airway hyper-responsiveness, as described previously (27). Healthy controls had no history of asthma and had normal lung function. The study was approved by the Leicestershire and Northamptonshire Ethics Committee (08/H0406/189).

Immunohistochemistry

To determine DP₂ expression by ASM, bronchial biopsies from healthy controls ($n = 11$ donors) and asthmatic patients ($n = 8$ donors) were embedded in glycomethacrylate (GMA) (11). For each patient, sequential 2- μ m sections were cut and stained using polyclonal anti-DP₂ antibody (Thermo Fisher Scientific) or rabbit immunoglobulin G (IgG) isotype control (Immunostep) and an α -SMA antibody (clone 1A4, Dako) or mouse IgG2a isotype control (clone DAK-GO5, Dako). Antibody binding was detected using the EnVision FLEX Kit (Dako). For determining ASM mass before and after treatment with fevipiprant ($n = 14$) or placebo ($n = 13$) and before and after treatment with mepolizumab ($n = 7$) or placebo ($n = 5$), bronchial biopsies from asthmatic patients were embedded in GMA and stained for α -SMA as above. ASM mass was determined as the percentage of the total assessable biopsy area as previously described by a single observer (R.B.). The repeatability of ASM mass assessment was tested and was excellent within and between observers with intraclass correlations of 0.95 and 0.96, respectively. Myofibroblasts were identified as α -SMA-positive stained cells in the lamina propria that were neither located as part of the ASM bundle nor as vascular smooth muscle cells adjacent to vessels per square millimeter of submucosa. To identify fibrocytes in bronchial biopsies before and after treatment with fevipiprant ($n = 12$) or placebo ($n = 13$), for each subject, sequential 2- μ m sections were cut and stained using an mouse monoclonal anti-cluster of differentiation 34 (CD34) antibody (Dako) or mouse IgG1 isotype control (Dako) and α -SMA as above. Fibrocytes were identified as the subset of α -SMA-positive cells per square millimeter of lamina propria that also stained positive for CD34 in sequential sections. The intensity of DP₂ stain was quantified as reciprocal intensity (28) on a scale of 250, assessed by a single observer. Assessors were blind to clinical characteristics, treatment allocation, and order of bronchial biopsy in the clinical trial.

Cell culture

ASM bundles were isolated from bronchial biopsies ($n = 27$ asthmatic and 2 nonasthmatic) and lung resection material ($n = 4$ nonasthmatic). The clinical characteristics of subjects that underwent bronchoscopy to provide primary ASM cultures are shown in table S5. Primary ASM cells were cultured in DMEM with glutamax-1 supplemented with 10% FBS, penicillin (100 U/ml), streptomycin (100 μ g/ml), amphotericin (0.25 μ g/ml), 100 μ M nonessential amino acids, and 1 mM sodium pyruvate (Gibco). Cells were characterized for α -SMA expression using a mouse monoclonal anti- α -SMA antibody (clone 1A4, Dako) or mouse IgG2a isotype control (clone DAK-GO5, Dako) by flow cytometry and used between passages 2 to 6.

Fibrocytes ($n = 6$) were isolated from peripheral blood mononuclear cells (PBMCs) as described previously (29). PBMCs were washed twice with Hanks' balanced salt solution and cultured in tissue culture flasks coated with fibronectin (40 μ g/ml) for 5 to 10 days before experimentation.

Before experimentation, ASM cells from each individual donor were incubated in media in the presence of a selective DP₂ agonist [DK-PGD₂; Cayman Chemical Company] (30) or selective DP₂ antagonists [CAY10471, OC000459, and fevipiprant, Cayman Chemical Company and Novartis (31–33) versus appropriate vehicle controls: dimethyl sulfoxide (DMSO) for DK-PGD₂, CAY10471, and OC000459 and 10% dH₂O in DMSO for fevipiprant].

Wound healing assay

ASM cells from individual donors were seeded onto six-well plates coated with fibronectin (10 μ g/ml) at a density of 2×10^5 cells and allowed to adhere and reach 90 to 100% confluence. Cells were then serum deprived for 24 hours. Cells were wounded by scratching using a sterile 200- μ l pipette tip in a predetermined grid pattern (34). After wounding, ASM cells were washed four times with serum-free media before addition of serum-free media with DK-PGD₂ (10 to 100 nM), fevipiprant (10 to 500 nM), CAY10471 (10 to 100 nM), or OC000459 (10 to 100 nM) or vehicle control for 24 hours. Photographs of four different wounded areas per condition were then photographed at baseline and after 24 hours using an EVOS XL Core Cell Imaging System (Thermo Fisher Scientific), and the outline of the wound at time zero was transposed onto the corresponding 24-hour photograph. The number of cells that had moved into the wounds was analyzed by a blinded observer.

Computational model approach and framework

The computational model capturing airway remodeling was developed via the agent-oriented approach (13), which charts the spatiotemporal evolution of a system as a result of flexible, high-level interactions between agents and agents and their environment (14). The Flexible Large-scale Agent-based Modelling Environment (www.flame.ac.uk), an agent-based platform using stream communicating X-machines (35) as agents, was used to develop the model.

The baseline model and computational iterations

On the basis of the agents, set of rules, and initial conditions (Table 1, table S1, figs. S1 and S2, and Supplementary Materials), a baseline model of airway remodeling that captures trends observed during normal airway remodeling, comprising epithelial, mesenchymal, and inflammatory parameters, was developed, with the implication that introducing abnormal levels of variation in model parameters will lead to the emergence of patterns observed during pathological remodeling and,

as such, the key hallmarks of asthma—this approach is referred to as pattern-oriented modeling (13). After review of clinical literature, the following clinical markers were considered to appropriately reflect the key hallmarks of asthma: (i) eosinophilic inflammation (defined as eosinophils/mm² submucosal area) of >10 (5, 11, 36), (ii) epithelial integrity of <70% (4, 5, 37–40), and (iii) airway muscle mass of ≥10 and ≤50% (4, 5, 37, 39).

The model starts by assessing the epithelial integrity, the number and location of inflammatory cells (both the universal inflammatory cells and eosinophils), and the status of muscle cells. Remodeling was initiated in case of a compromised epithelium or increased inflammation within the system, resulting in a cascade of events, which, depending on the relevant boundary conditions, lead to further inflammation, fibrosis, goblet cell hyperplasia, recruitment of muscle, and increased collagen deposition. Furthermore, remodeling could be exacerbated or prolonged by the nature of initial or secondary conditions assigned to the computation.

More specifically, a normal or “healthy” set of conditions triggered remodeling in the absence of an intact epithelium (or a challenge that resulted in epithelial denudation) by initiating fibrosis and recruiting the universal inflammatory cell. The inflammatory cell further “released” proinflammatory cytokines to recruit eosinophils and muscle—the latter was accounted for in the model by the differentiation of fibroblasts into myofibroblasts. The eosinophils, moreover, caused further damage by degranulating and releasing cytotoxic proteins, which, if close to the epithelium, resulted in necrosis of the epithelial cells, thereby prolonging remodeling. We worked with the hypothesis that any set of conditions that perpetuate these interactions will result in pathological remodeling, thereby capturing the hallmarks of asthma. The various parameters and their quantitative values, derived from existing literature, have been, along with the relevant references, listed in Table 1, with a schematic interlinking the various elements of the model shown in fig. S2.

Although the model does not explicitly consider cytokine activity, i.e., their release, diffusion, and half-life, it implicitly accounts for it by requiring that those cells affected by the cytokine molecules share a localized region with the effector cell. For example, only ASM cells within close proximity of the universal inflammatory cells will undergo hypertrophy or contraction (refer to the Supplementary Materials for more details).

Last, the simulations progressed in a number of time steps, with each time step matching 1 hour of real time. Time intervals of 30 min and 2 hours were also tested on the baseline case (case I) and yielded results indistinguishable from simulations conducted with 1-hour time steps. Thus, we opted for the 1-hour interval to strike a balance between computational costs and ensuring adequate resolution regarding activities we wished to capture via the model. The total simulated time for all simulations, including remodeling and intervention, was ~6 months (4350 iterations). This time period allowed the investigation of both the short- and long-term response after either a challenge or intervention. Each model was simulated five times ($n = 5$) to assess the sensitivity of the model to inherent stochastic elements (such as cell cycle, new coordinates of the daughter cells, and migration of the universal inflammatory cells). The internal random elements accounted for intracellular and intercellular biological stochasticity. Testing the model for insensitivity to these random elements also served to provide an indicator for model precision. A detailed description of the model, its development, and its validation is provided in the Supplementary Materials.

Statistical analysis

Statistical analysis was performed using with SAS/STAT software and GraphPad Prism. Data were tested for normality using the Shapiro-Wilk test. For normally distributed data, two-tailed paired t tests, one sample t tests, or one-way analysis of variance (ANOVA) were used as appropriate. For nonparametric data, Wilcoxon matched-pairs signed-rank test or Kruskal-Wallis test were used as appropriate. Correlations were performed using Spearman's correlation. Details of statistical tests used are provided in figure legends. $P < 0.05$ was considered statistically significant.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/11/479/eaao6451/DC1

Materials and Methods

Fig. S1. The virtual airway at baseline.

Fig. S2. Model parameters and agent interactions.

Fig. S3. Flow cytometric analysis of ASM cells.

Fig. S4. DK-PGD₂, fevipiprant, CAY10471, and OC000459 had no effect on ASM cell number, apoptosis, or necrosis after 24 hours.

Fig. S5. DK-PGD₂, fevipiprant, and CAY10471 had no effect on ASM proliferation after 72 hours.

Fig. S6. DK-PGD₂, fevipiprant, and CAY10471 had no effect on basal or BK-induced ASM contraction.

Fig. S7. Conceptual summary.

Table S1. Description of agents used in computational model.

Table S2. Alterations made to epithelial parameters in the computational model.

Table S3. Alterations made to mesenchymal parameters in the computational model.

Table S4. Alterations made to inflammatory parameters in the computational model.

Table S5. Clinical characteristics of subjects that provided additional bronchial biopsies for primary ASM cultures.

Table S6. Analysis of expression of genes involved in PGD₂ biosynthesis and metabolism in ASM cells.

Table S7. Output of computations simulating pathological airway remodeling.

Data file S1. Data values for individual experiments.

References (51–70)

REFERENCES AND NOTES

1. A. Papi, C. Brightling, S. E. Pedersen, H. K. Reddel, Asthma. *Lancet* **391**, 783–800 (2018).
2. K. F. Chung, S. E. Wenzel, J. L. Brozek, A. Bush, M. Castro, P. J. Sterk, I. M. Adcock, E. D. Bateman, E. H. Bel, E. R. Bleecker, L. P. Boulet, C. Brightling, P. Chaney, S. E. Dahlen, R. Djukanovic, U. Frey, M. Gaga, P. Gibson, Q. Hamid, N. N. Jajour, T. Mauad, R. L. Sorkness, W. G. Teague, International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur. Respir. J.* **43**, 343–373 (2014).
3. R. Berair, C. E. Brightling, Asthma therapy and its effect on airway remodelling. *Drugs* **74**, 1345–1369 (2014).
4. S. Gonem, R. Berair, A. Singapuri, R. Hartley, M. F. M. Laurencin, G. Bacher, B. Holzhauser, M. Bourne, V. Mistry, I. D. Pavord, A. H. Mansur, A. J. Wardlaw, S. H. Siddiqui, R. A. Kay, C. E. Brightling, Fevipiprant, a prostaglandin D₂ receptor 2 antagonist, in patients with persistent eosinophilic asthma: A single-Centre, randomised, double-blind, parallel-group, placebo-controlled trial. *Lancet Respir. Med.* **4**, 699–707 (2016).
5. L. Benayoun, A. Druilhe, M.-C. Dombret, M. Aubier, M. Pretolani, Airway structural alterations selectively associated with severe asthma. *Am. J. Respir. Crit. Care Med.* **167**, 1360–1368 (2003).
6. M. Pretolani, A. Bergqvist, G. Thabut, M. C. Dombret, D. Knapp, F. Hamidi, L. Alavoine, C. Taillé, P. Chaney, J. S. Erjefält, M. Aubier, Effectiveness of bronchial thermoplasty in patients with severe refractory asthma: Clinical and histopathologic correlations. *J. Allergy Clin. Immunol.* **139**, 1176–1185 (2017).
7. I. L. Chernyavsky, R. J. Russell, R. M. Saunders, G. E. Morris, R. Berair, A. Singapuri, L. Chachi, A. H. Mansur, P. H. Howarth, P. Dennison, R. Chaudhuri, S. Bicknell, F. R. A. J. Rose, S. Siddiqui, B. S. Brook, C. E. Brightling, In vitro, in silico and in vivo study challenges the impact of bronchial thermoplasty on acute airway smooth muscle mass loss. *Eur. Respir. J.* **51**, 1701680 (2018).
8. S. E. Stinson, Y. Amrani, C. E. Brightling, D. prostanoide receptor 2 (chemoattractant receptor-homologous molecule expressed on T_H2 cells) protein expression in asthmatic patients and its effects on bronchial epithelial cells. *J. Allergy Clin. Immunol.* **135**, 395–406.e7 (2015).
9. R. Schuligoi, E. Sturm, P. Luschnig, V. Konya, S. Philipose, M. Sedej, M. Waldhoer, B. A. Peskar, CRTH2 and D-type prostanoide receptor antagonists as novel therapeutic agents for inflammatory diseases. *Pharmacology* **85**, 372–382 (2010).

10. L. Xue, M. Salimi, I. Panse, J. M. Mjösberg, A. N. J. McKenzie, H. Spits, P. Klenerman, G. Ogg, Prostaglandin D₂ activates group 2 innate lymphoid cells through chemoattractant receptor-homologous molecule expressed on T_H2 cells. *J. Allergy Clin. Immunol.* **133**, 1184–1194.e7 (2014).
11. C. E. Brightling, P. Bradding, F. A. Symon, S. T. Holgate, A. J. Wardlaw, I. D. Pavord, Mast-cell infiltration of airway smooth muscle in asthma. *N. Engl. J. Med.* **346**, 1699–1705 (2002).
12. P. Haldar, C. E. Brightling, B. Hargadon, S. Gupta, W. Monteiro, A. Sousa, R. P. Marshall, P. Bradding, R. H. Green, A. J. Wardlaw, I. D. Pavord, Mepolizumab and exacerbations of refractory eosinophilic asthma. *N. Engl. J. Med.* **360**, 973–984 (2009).
13. H. Kaul, Y. Ventikos, Investigating biocomplexity through the agent-based paradigm. *Brief. Bioinform.* **16**, 137–152 (2015).
14. H. Kaul, B. K. Hall, C. Newby, Y. Ventikos, Synergistic activity of polarised osteoblasts inside condensations cause their differentiation. *Sci. Rep.* **5**, 11838 (2015).
15. J. J. Pothén, M. E. Poynter, J. H. T. Bates, A computational model of unresolved allergic inflammation in chronic asthma. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **308**, L384–L390 (2015).
16. R. Russell, C. Brightling, Mepolizumab for the reduction of exacerbations in severe eosinophilic asthma. *Expert Rev. Respir. Med.* **10**, 607–617 (2016).
17. P. T. Flood-Page, A. N. Menzies-Gow, A. B. Kay, D. S. Robinson, Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am. J. Respir. Crit. Care Med.* **167**, 199–204 (2003).
18. K. Parameswaran, K. Radford, A. Fanat, J. Stephen, C. Bonnans, B. D. Levy, L. J. Janssen, P. G. Cox, Modulation of human airway smooth muscle migration by lipid mediators and Th-2 cytokines. *Am. J. Respir. Cell Mol. Biol.* **37**, 240–247 (2007).
19. S. R. Johnson, A. J. Knox, Synthetic functions of airway smooth muscle in asthma. *Trends Pharmacol. Sci.* **18**, 288–292 (1997).
20. A. Lewis, J. Wan, B. Baotman, P. N. Monk, S. K. Suvarna, P. T. Peachell, Heterogeneity in the responses of human lung mast cells to stem cell factor. *Clin. Exp. Allergy* **43**, 50–59 (2013).
21. M. C. Weston, N. Anderson, P. T. Peachell, Effects of phosphodiesterase inhibitors on human lung mast cell and basophil function. *Br. J. Pharmacol.* **121**, 287–295 (1997).
22. A. R. Sousa, R. P. Marshall, L. C. Warnock, S. Bolton, A. Hastie, F. Symon, B. Hargadon, H. Marshall, M. Richardson, C. E. Brightling, P. Haldar, R. Milone, P. Chalk, R. Williamson, R. Panettieri Jr., R. Knowles, E. R. Bleeker, A. J. Wardlaw, Responsiveness to oral prednisolone in severe asthma is related to the degree of eosinophilic airway inflammation. *Clin. Exp. Allergy* **47**, 890–899 (2017).
23. R. J. Russell, L. Chachi, J. M. FitzGerald, V. Backer, R. Olivenstein, I. L. Titlestad, C. S. Ulrik, T. Harrison, D. Singh, R. Chaudhuri, B. Leaker, L. McGarvey, S. Siddiqui, M. Wang, M. Braddock, L. H. Nordenmark, D. Cohen, H. Parikh, G. Colice, C. E. Brightling; MESOS study investigators, Effect of tralokinumab, an interleukin-13 neutralising monoclonal antibody, on eosinophilic airway inflammation in uncontrolled moderate-to-severe asthma MESOS: A multicentre, double-blind, randomised, placebo-controlled phase 2 trial. *Lancet Respir. Med.* **6**, 499–510 (2018).
24. P.-O. Girodet, G. Dournes, M. Thumerel, H. Begueret, P. Dos Santos, A. Ozier, I. Dupin, T. Trian, M. Montaudon, F. Laurent, R. Marthan, P. Berger, Calcium channel blocker reduces airway remodeling in severe asthma. A proof-of-concept study. *Am. J. Respir. Crit. Care Med.* **191**, 876–883 (2015).
25. A. J. Halayko, H. Salari, X. MA, N. L. Stephens, Markers of airway smooth muscle cell phenotype. *Am. J. Physiol.* **270**, L1040–L1051 (1996).
26. A. Z. Politi, G. M. Donovan, M. H. Tawhai, M. J. Sanderson, A.-M. Lauzon, J. H. T. Bates, J. Sneyd, A multiscale, spatially-distributed model of asthmatic airway hyper-responsiveness. *J. Theor. Biol.* **266**, 614–624 (2010).
27. C. E. Brightling, A. J. Ammit, D. Kaur, J. L. Black, A. J. Wardlaw, J. M. Hughes, P. Bradding, The CXCL10/CXCR3 axis mediates human lung mast cell migration to asthmatic airway smooth muscle. *Am. J. Respir. Crit. Care Med.* **171**, 1103–1108 (2005).
28. D. H. Nguyen, T. Zhou, J. Shu, J. H. Mao, Quantifying chromogen intensity in immunohistochemistry via reciprocal intensity. *Cancer In Cytos* **2**, 1–4 (2013).
29. R. M. Saunders, S. Siddiqui, D. Kaur, C. Doe, A. Sutcliffe, F. Hollins, P. Bradding, A. Wardlaw, C. E. Brightling, Fibrocyte localization to the airway smooth muscle is a feature of asthma. *J. Allergy Clin. Immunol.* **123**, 376–384 (2009).
30. H. Hirai, K. Tanaka, O. Yoshie, K. Ogawa, K. Kenmotsu, Y. Takamori, M. Ichimasa, K. Sugamura, M. Nakamura, S. Takano, K. Nagata, Prostaglandin D₂ selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor Crth2. *J. Exp. Med.* **193**, 255–262 (2001).
31. T. Ulven, E. Kostenis, Minor structural modifications convert the dual TP/CRTH2 antagonist ramatroban into a highly selective and potent CRTH2 antagonist. *J. Med. Chem.* **48**, 897–900 (2005).
32. R. Pettipher, S. L. Vinall, L. Xue, G. Speight, E. R. Townsend, L. Gazi, C. J. Whelan, R. E. Armer, M. A. Payton, M. G. Hunter, Pharmacologic profile of OC000459, a potent, selective, and orally active D prostanoid receptor 2 antagonist that inhibits mast cell-dependent activation of T helper 2 lymphocytes and eosinophils. *J. Pharmacol. Exp. Ther.* **340**, 473–482 (2012).
33. V. J. Erpenbeck, E. Vets, L. Gheyle, W. Osuntokun, M. Larbig, S. Neelakantham, D. Sandham, G. Dubois, W. Elbast, P. Goldsmith, M. Weiss, Pharmacokinetics, safety, and tolerability of fempiprant (QAW039), a novel CRTH2 receptor antagonist: Results from 2 randomized, phase 1, placebo-controlled studies in healthy volunteers. *Clin. Pharmacol. Drug Dev.* **5**, 306–313 (2016).
34. D. Kaur, R. Saunders, P. Berger, S. Siddiqui, L. Woodman, A. Wardlaw, P. Bradding, C. E. Brightling, Airway smooth muscle and mast cell-derived CC chemokine ligand 19 mediate airway smooth muscle migration in asthma. *Am. J. Respir. Crit. Care Med.* **174**, 1179–1188 (2006).
35. G. Laycock, The Theory and Practice of Specification Based Software Testing, thesis, Department of Computer Science, University of Sheffield, Sheffield (1993).
36. M. Hoshino, Y. Nakamura, J. J. Sim, Expression of growth factors and remodelling of the airway wall in bronchial asthma. *Thorax* **53**, 21–27 (1998).
37. H. Begueret, P. Berger, J. M. Vernejoux, L. Dubuisson, R. Marthan, J. M. Tunon-de-Lara, Inflammation of bronchial smooth muscle in allergic asthma. *Thorax* **62**, 8–15 (2007).
38. S. Pégorier, N. Arouche, M.-C. Dombret, M. Aubier, M. Pretolani, Augmented epithelial endothelin-1 expression in refractory asthma. *J. Allergy Clin. Immunol.* **120**, 1301–1307 (2007).
39. N. Carroll, J. Elliot, A. Morton, A. James, The structure of large and small airways in nonfatal and fatal asthma. *Am. Rev. Respir. Dis.* **147**, 405–410 (1993).
40. K. Amin, D. Lúdvíksdóttir, C. Janson, O. Nettelbladt, E. Björnsson, G. M. Roomans, G. Boman, L. Sevéus, P. Venge, Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. *Am. J. Respir. Crit. Care Med.* **162**, 2295–2301 (2000).
41. D. L. Wilhelm, Regeneration of tracheal epithelium. *J. Pathol. Bacteriol.* **65**, 543–550 (1953).
42. J. S. Erjefält, I. Erjefält, F. Sandler, C. G. A. Persson, In vivo restitution of airway epithelium. *Cell Tissue Res.* **281**, 305–316 (1995).
43. J.-M. Zahm, H. Kaplan, A.-L. Hérad, F. Doriot, D. Pierrot, P. Somelette, E. Puchelle, Cell migration and proliferation during the in vitro wound repair of the respiratory epithelium. *Cell Motil. Cytoskeleton* **37**, 33–43 (1997).
44. C. L. Ordoñez, R. Khashayar, H. H. Wong, R. Ferrando, R. Wu, D. M. Hyde, J. A. Hotchkiss, Y. Zhang, A. Novikov, G. Dolganov, J. V. Fahy, Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *Am. J. Respir. Crit. Care Med.* **163**, 517–523 (2001).
45. J. V. Fahy, Remodeling of the airway epithelium in asthma. *Am. J. Respir. Crit. Care Med.* **164**, S46–S51 (2001).
46. T. Aikawa, S. Shimura, H. Sasaki, M. Ebina, T. Takishima, Marked goblet cell hyperplasia with mucus accumulation in the airways of patients who died of severe acute asthma attack. *Chest* **101**, 916–921 (1992).
47. B. Thomas, A. Rutman, R. A. Hirst, P. Haldar, A. J. Wardlaw, J. Bankart, C. E. Brightling, C. O'Callaghan, Ciliary dysfunction and ultrastructural abnormalities are features of severe asthma. *J. Allergy Clin. Immunol.* **126**, 722–729.e2 (2010).
48. S. Siddiqui, A. Sutcliffe, A. Shikotra, L. Woodman, C. Doe, S. McKenna, A. Wardlaw, P. Bradding, I. Pavord, C. Brightling, Vascular remodeling is a feature of asthma and nonasthmatic eosinophilic bronchitis. *J. Allergy Clin. Immunol.* **120**, 813–819 (2007).
49. M. A. Gienbycz, M. A. Lindsay, Pharmacology of the eosinophil. *Pharmacol. Rev.* **51**, 213–340 (1999).
50. H. Sugiura, X. Liu, F. Duan, S. Kawasaki, S. Togo, K. Kamio, X. Q. Wang, L. Mao, Y. Ahn, R. F. Ertl, T. W. Bargar, A. Berro, T. B. Casale, S. I. Rennard, Cultured lung fibroblasts from ovalbumin-challenged “asthmatic” mice differ functionally from normal. *Am. J. Respir. Cell Mol. Biol.* **37**, 424–430 (2007).
51. A. Sutcliffe, F. Hollins, E. Gomez, R. Saunders, C. Doe, M. Cooke, R. A. J. Challiss, C. E. Brightling, Increased nicotinamide adenine dinucleotide phosphate oxidase 4 expression mediates intrinsic airway smooth muscle hypercontractility in asthma. *Am. J. Respir. Crit. Care Med.* **185**, 267–274 (2012).
52. E. J. Swindle, J. E. Collins, D. E. Davies, Breakdown in epithelial barrier function in patients with asthma: Identification of novel therapeutic approaches. *J. Allergy Clin. Immunol.* **124**, 23–34 (2009).
53. S. T. Holgate, D. E. Davies, S. Puddicombe, A. Richter, P. Lackie, J. Lordan, P. Howarth, Mechanisms of airway epithelial damage: Epithelial-mesenchymal interactions in the pathogenesis of asthma. *Eur. Respir. J. Suppl.* **44**, 24s–29s (2003).
54. A. Detoraki, F. Granata, S. Staibano, F. W. Rossi, G. Marone, A. Genovese, Angiogenesis and lymphangiogenesis in bronchial asthma. *Allergy* **65**, 946–958 (2010).
55. J. V. Fahy, B. F. Dickey, Airway mucus function and dysfunction. *N. Engl. J. Med.* **363**, 2233–2247 (2010).
56. N. Awadh, N. L. Müller, C. S. Park, R. T. Abboud, J. M. FitzGerald, Airway wall thickness in patients with near fatal asthma and control groups: Assessment with high resolution computed tomographic scanning. *Thorax* **53**, 248–253 (1998).

57. A. L. James, J. G. Elliot, R. L. Jones, M. L. Carroll, T. Mauad, T. R. Bai, M. J. Abramson, K. O. McKay, F. H. Green, Airway smooth muscle hypertrophy and hyperplasia in asthma. *Am. J. Respir. Crit. Care Med.* **185**, 1058–1064 (2012).
58. H. Kaul, Z. Cui, Y. Ventikos, A multi-paradigm modeling framework to simulate dynamic reciprocity in a bioreactor. *PLOS ONE* **8**, e59671 (2013).
59. O. Sacco, M. Silvestri, F. Sabatini, R. Sale, A.-C. Defilippi, G. A. Rossi, Epithelial cells and fibroblasts: Structural repair and remodelling in the airways. *Paediatr. Respir. Rev.* **5** (suppl. 1), S35–S40 (2004).
60. S. Kirkham, J. K. Sheehan, D. Knight, P. S. Richardson, D. J. Thornton, Heterogeneity of airways mucus: Variations in the amounts and glycoforms of the major oligomeric mucins MUC5AC and MUC5B. *Biochem. J.* **361**, 537–546 (2002).
61. N. G. Carroll, S. Mutavdzic, A. L. James, Increased mast cells and neutrophils in submucosal mucous glands and mucus plugging in patients with asthma. *Thorax* **57**, 677–682 (2002).
62. P. Bradding, Mast cell regulation of airway smooth muscle function in asthma. *Eur. Respir. J.* **29**, 827–830 (2007).
63. S. Oddera, M. Silvestri, A. Balbo, B. O. Jovovich, R. Penna, E. Grimi, G. A. Rossi, Airway eosinophilic inflammation, epithelial damage, and bronchial hyperresponsiveness in patients with mild-moderate, stable asthma. *Allergy* **51**, 100–107 (1996).
64. S. Y. Eum, S. Hailé, J. Lefort, M. Huerre, B. B. Vargaftig, Eosinophil recruitment into the respiratory epithelium following antigenic challenge in hyper-IgE: Mice is accompanied by interleukin 5-dependent bronchial hyperresponsiveness. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 12290–12294 (1995).
65. S. Adra, T. Sun, S. MacNeil, M. Holcombe, R. Smallwood, Development of a three dimensional multiscale computational model of the human epidermis. *PLOS ONE* **5**, e8511 (2010).
66. H. Kaul, Y. Ventikos, Dynamic reciprocity revisited. *J. Theor. Biol.* **370**, 205–208 (2015).
67. E. Shebani, S. Shahana, C. Janson, G. M. Roomans, Attachment of columnar airway epithelial cells in asthma. *Tissue Cell* **37**, 145–152 (2005).
68. E. M. Levina, M. A. Kharitonova, Y. A. Rovinsky, J. M. Vasiliev, Cytoskeletal control of fibroblast length: Experiments with linear strips of substrate. *J. Cell Sci.* **114**, 4335–4341 (2001).
69. C. Twort, C. Van Breemen, Human airway smooth muscle in culture. *Tissue Cell* **20**, 339–344 (1988).
70. E. S. Schulman, A. Kagey-Sobotka, D. W. MacGlashan Jr., N. F. Adkinson Jr., S. P. Peters, R. P. Schleimer, L. M. Lichtenstein, Heterogeneity of human mast-cells. *J. Immunol.* **131**, 1936–1941 (1983).

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DP₂ antagonism reduces airway smooth muscle mass in asthma by decreasing eosinophilia and myofibroblast recruitment

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Smoothing out muscle in asthma

Asthma is often treated with drugs that reduce airway inflammation. Saunders *et al.* now show that fevipiprant, a prostaglandin D₂ type 2 receptor antagonist, reduced smooth muscle mass in bronchial biopsies from asthma patients. Computational simulations of an asthmatic airway predicted that this decrease in airway smooth muscle mass was due to both amelioration of inflammation observed in a prior clinical trial together with direct effects on migration of myofibroblasts. Treating smooth muscle cells from bronchial biopsies from asthma patients with fevipiprant in vitro revealed that the drug-induced decrease in airway smooth muscle mass may be due to reduced migration of myofibroblasts and fibrocytes.

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