

Reversal of Allergen-induced Airway Remodeling by CysLT₁ Receptor Blockade

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Rationale: Airway inflammation in asthma is accompanied by structural changes, including goblet cell metaplasia, smooth muscle cell layer thickening, and subepithelial fibrosis. This allergen-induced airway remodeling can be replicated in a mouse asthma model.

Objectives: The study goal was to determine whether established airway remodeling in a mouse asthma model is reversible by administration of the cysteinyl leukotriene (CysLT)₁ receptor antagonist montelukast, the corticosteroid dexamethasone, or the combination montelukast + dexamethasone.

Methods: BALB/c mice, sensitized by intraperitoneal ovalbumin (OVA) as allergen, received intranasal OVA periodically Days 14–73 and montelukast or dexamethasone or placebo from Days 73–163.

Measurements and Main Results: Allergen-induced trafficking of eosinophils into the bronchoalveolar lavage fluid and lung interstitium and airway goblet cell metaplasia, smooth muscle cell layer thickening, and subepithelial fibrosis present on Day 73 persisted at Day 163, 3 mo after the last allergen challenge. Airway hyperreactivity to methacholine observed on Day 73 in OVA-treated mice was absent on Day 163. In OVA-treated mice, airway eosinophil infiltration and goblet cell metaplasia were reduced by either montelukast or dexamethasone alone. Montelukast, but not dexamethasone, reversed the established increase in airway smooth muscle mass and subepithelial collagen deposition. By immunocytochemistry, CysLT₁ receptor expression was significantly increased in airway smooth muscle cells in allergen-treated mice compared with saline-treated controls and was reduced by montelukast, but not dexamethasone, administration.

Conclusions: These data indicate that established airway smooth muscle cell layer thickening and subepithelial fibrosis, key allergen-induced airway structural changes not modulated by corticosteroids, are reversible by CysLT₁ receptor blockade therapy.

Keywords: eosinophils; fibrosis; mucus; smooth muscle

In patients with asthma, persistent allergen-induced inflammation is accompanied by structural changes in the airways. These airway remodeling changes observed in both children and adults include increased airway goblet cells, smooth muscle mass, and blood vessels (1–3). The increased smooth muscle mass present in the airways of patients with asthma (4) may result from hyperplasia and hypertrophy of smooth muscle cells and myofibroblasts (5, 6). Prominent in the remodeling process is thickening of the airway wall with development of subepithelial fibrosis from deposition of extracellular matrix (ECM) proteins

(e.g., collagen, laminin, fibronectin, tenascin) in the lamina reticularis beneath the basement membrane (1, 7). The increased subepithelial layer thickness and vascularity are seen in both patients with newly diagnosed asthma and patients with long-standing disease duration (3, 8).

A key question is whether the airway structural changes in patients with asthma are reversible by drug intervention. Current therapeutic approaches to reverse established structural airway changes have had limited effect in both animal asthma models and patients with asthma (9, 10). In mouse (11, 12) and rat (13) asthma models, although concomitant treatment of corticosteroids during the allergen challenge period blocked the airway inflammatory response and structural changes including subepithelial fibrosis, dexamethasone treatment initiated after allergen challenge had only limited (11) or no effect (13) on reversing the established airway remodeling changes. In transgenic mice, transforming growth factor (TGF)- β_1 -induced lung fibrosis is reversible after cessation of transgene expression indicating that the fibrotic response in the airways is not inevitably irreversible (14).

Using an *in vivo* model of allergen-induced airway remodeling in mice, we have found cysteinyl leukotrienes (CysLTs) to be important in the development of the airway structural changes. In this asthma model, mice sensitized by intraperitoneal administration of ovalbumin (OVA), when challenged over a 2-mo period with intranasal OVA, develop airway eosinophilia, goblet cell metaplasia, smooth muscle cell layer thickening, and subepithelial fibrosis with deposition of collagen and laminin ECM proteins (12, 15). Administration of the specific CysLT₁ receptor antagonist montelukast (MK) during the intranasal OVA challenge period blocked eosinophil trafficking/degranulation, Th2 cytokine release, and structural changes in the lungs (15).

In the present study, we determined the effect of the CysLT₁ receptor antagonist MK, in comparison to the corticosteroid dexamethasone, on airway remodeling when administered after development of structural airway changes in a chronic mouse asthma model. MK and dexamethasone each partially reduced established airway inflammatory cell infiltration and goblet cell metaplasia, but only MK reversed the established increase in airway smooth muscle mass and subepithelial fibrosis. These data indicate that important structural features of allergen-induced airway remodeling resistant to corticosteroid treatment are reversible by CysLT₁ receptor blockade.

METHODS

A detailed description of these methods can be found in the online supplement. Animal use procedures were approved by the University of Washington Animal Care Committee. Female BALB/c mice (6–8 wk; Jackson Laboratory, Bar Harbor, ME) received intraperitoneal injections of 100 μ g OVA (0.2 ml of 0.5 mg/ml) complexed with alum on Days 0 and 14 and intranasal doses of 100 μ g OVA (0.05 ml of 2 mg/ml) on Day 14 and 50 μ g OVA (0.05 ml of 1 mg/ml) on Days (\pm 1–2 d) 25, 27, 29, 46, 60, 69, 71, and 73 (Figure 1). On Day 73, randomized treatment groups of mice had subcutaneous placement of miniosmotic pumps (200- μ l Alzet Model 2004 osmotic pumps; 6 μ l/d delivery rate; Durect Corporation, Cupertino, CA) containing MK sodium (1 mg/kg; Merck and Co., Inc., Rahway, NJ), dexamethasone (1 mg/kg;

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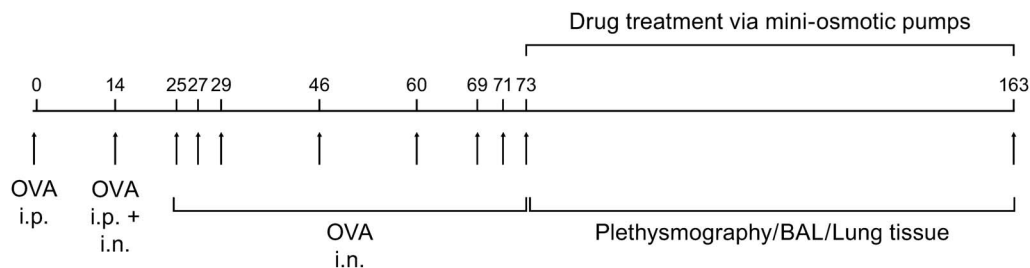
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dexamethasone–water soluble; Sigma Chemical Co., St. Louis, MO), MK (1 mg/kg) + dexamethasone (1 mg/kg), or saline control (four to eight mice per study group) with pump replacement on Days 103 and 133. The control group received intraperitoneal saline with alum on Days 0 and 14 and intranasal saline without alum on Days ($\pm 1-2$ d) 14, 25, 27, 29, 46, 60, 69, 71, and 73. In preliminary studies (see RESULTS), the 1 mg/kg dexamethasone dose inhibited airway eosinophil trafficking. OVA-sensitized mice challenged with intranasal OVA on Days 14, 25, 27, and 29 received either intraperitoneal dexamethasone (1 mg/kg) or saline 30 min before each intranasal OVA challenge on Days 25, 27, and 29 with bronchoalveolar lavage (BAL) performed on Day 30; controls were saline-treated mice.

On Days 73 (30 min after OVA) and 163 (3 mo after the last allergen challenge), airway hyperreactivity to aerosolized methacholine was performed in conscious, freely moving, spontaneously breathing

mice using whole-body plethysmography (Model PLY 3211; Buxco Electronics, Inc., Sharon, CT) (15, 16). The degree of bronchoconstriction was expressed as enhanced pause (Penh), a calculated dimensionless value that correlates with measurements of airway resistance, impedance, and intrapleural pressure. After plethysmography, mice were exsanguinated by cardiac puncture, BAL was performed on the right lung for cell counts (17), and left lung tissue was obtained for light microscopy and morphometry (15).

The lung sections were stained with hematoxylin and eosin to determine the airway inflammatory cell infiltrate (0–4+ scale) (17), eosinophils per unit airway ($2,200 \mu\text{m}^2$) (17), and smooth muscle thickness in μm (15), Alcian blue, pH 2.5, with nuclear fast red counterstaining to identify goblet cells (as percent of total airway epithelial cells) (17), and Masson's trichrome to determine collagen deposition/fibrosis (0–4+ scale) (15); 10 airways ($\sim 0.4-0.7$ mm in diameter and surrounded

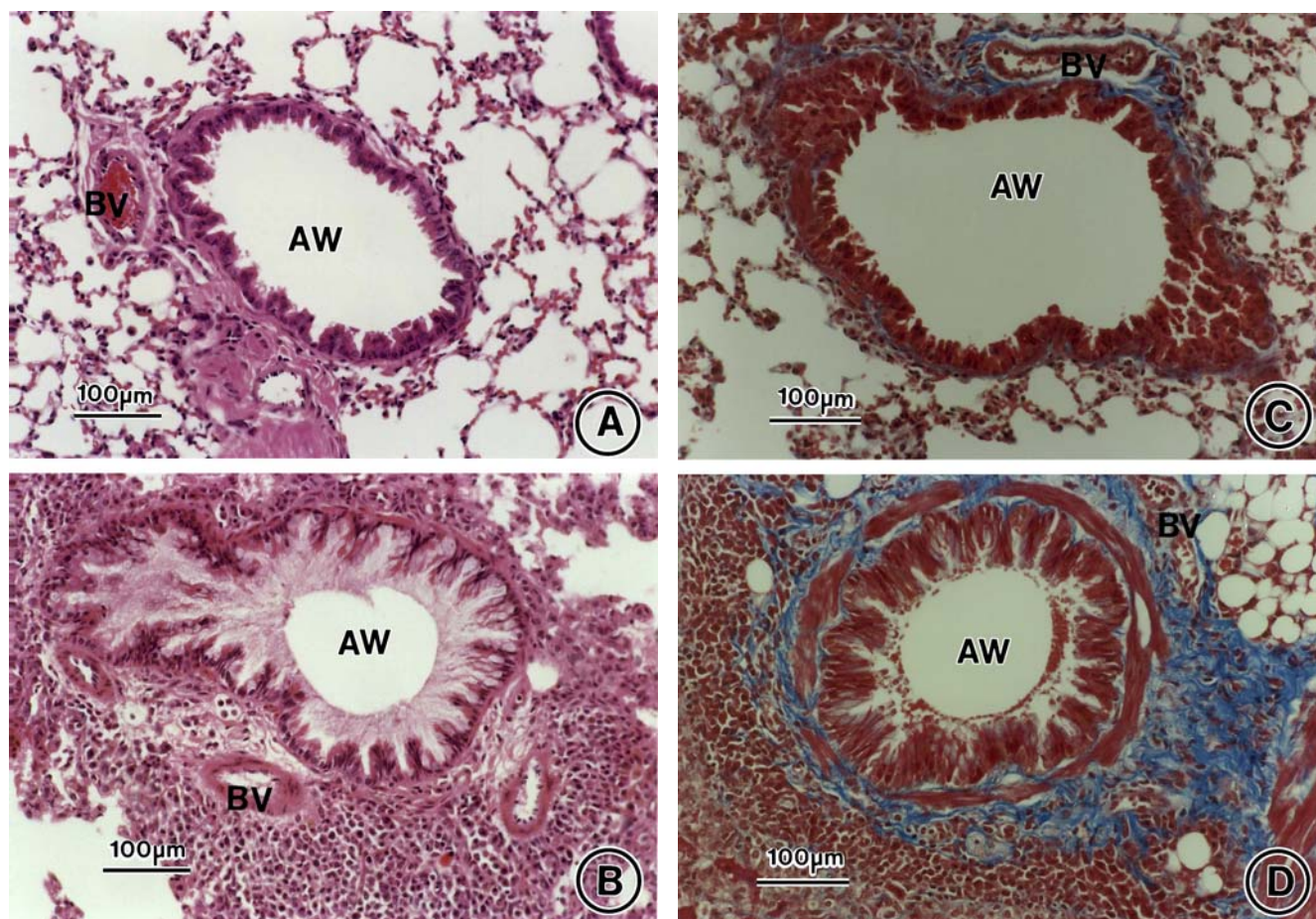


Figure 2. Allergen-induced airway inflammation and remodeling. Lung tissue was obtained on Day 73 from saline control animals (A, C) and OVA-sensitized/challenged mice (B, D) and stained with hematoxylin and eosin (A, B) or Masson's trichrome stain (C, D). AW = airway; BV = blood vessel. Bars = 100 μm .

by smooth muscle cells) per mouse were examined. Airway CysLT₁ receptor expression was localized by immunocytochemistry (18) using polyclonal goat anti-CysLT₁ receptor-specific primary antibody (19) (provided by Jilly F. Evans, Merck and Co., Inc.), followed by rabbit anti-goat secondary antibody conjugated to horseradish peroxidase (Vector Laboratories, Burlingame, CA).

The data are reported as the mean \pm SE of the combined experiments. Differences were analyzed for significance ($p < 0.05$) by analysis of variance using the protected least significance difference method.

RESULTS

Effect of CysLT₁ Receptor Antagonist and Corticosteroid Treatment on Reversal of Allergen-induced Airway Inflammation and Remodeling

The study design is shown in Figure 1. On (1) Day 73 after the final intranasal OVA or saline challenge and before initiation of randomized drug therapy and (2) Day 163, after a 3-mo treatment period with MK (1 mg/kg), dexamethasone (1 mg/kg), MK (1 mg/kg) + dexamethasone (1 mg/kg), or saline, BAL was performed, and lung tissue obtained to determine airway inflammation and remodeling. The 1 mg/kg/d dose of MK was based on our prior study (15) using the same chronic asthma model in which this dose significantly inhibited airway eosinophil infiltration and degranulation, production of Th2 cytokines interleukin 4 (IL-4) and IL-13, goblet cell metaplasia, smooth muscle cell layer thickening, and subepithelial collagen deposition/fibrosis when administered during the period of intranasal OVA challenge in sensitized mice (15). The 1-mg/kg/d dose of dexamethasone was chosen because it abrogated the influx of

eosinophils into the airways by Day 30. Compared with the saline group, OVA-treated mice had a 349-fold increase in total eosinophils recovered in BAL fluid to $2.4 \pm 0.6 \times 10^5$ ($p = 0.003$, OVA vs. saline; $n = 5$ each group) on Day 30. Dexamethasone given by intraperitoneal administration (1 mg/kg/day) before OVA on Days 25, 27, and 29 reduced the total number of eosinophils by 93.0% to $0.2 \pm 0.03 \times 10^5$ ($p = 0.01$, OVA/dexamethasone vs. OVA; $n = 5$ each group). The 1-mg/kg/d dexamethasone dose is the same or higher dose as shown effective in other chronic mouse asthma models in blocking allergen-induced airway inflammation and remodeling when administered during the allergen challenge period (11, 20–26).

Airway inflammatory cell infiltration. On Day 73, an extensive infiltrate of eosinophils, macrophages, and other inflammatory cells around the airways and blood vessels and influx of eosinophils into the BAL fluid occurred in the lungs of OVA-sensitized/challenged mice that was not seen in saline controls (Figures 2B vs. 2A and 3) in agreement with our previous work using this chronic asthma model (15). In OVA-treated mice, the (1) inflammatory cell and (2) eosinophil infiltrate in the lung interstitium and (3) eosinophil influx in BAL fluid persisted on Day 163 (Figures 4B vs. 4A), although each was significantly reduced by 33.5, 35.8, and 68.7%, respectively, compared with Day 73 (Figure 3). By morphometry, the number of inflammatory cells in the lung interstitium of the OVA-treated mice was significantly increased compared with the saline-treated controls on Day 163 (Figure 3A; $p = 0.0004$, OVA/saline vs. saline/saline). Infiltration of the airway interstitium by eosinophils increased approximately 68-fold from 0.1 ± 0.1 eosinophils/ $2,200 \mu^2$ lung tissue

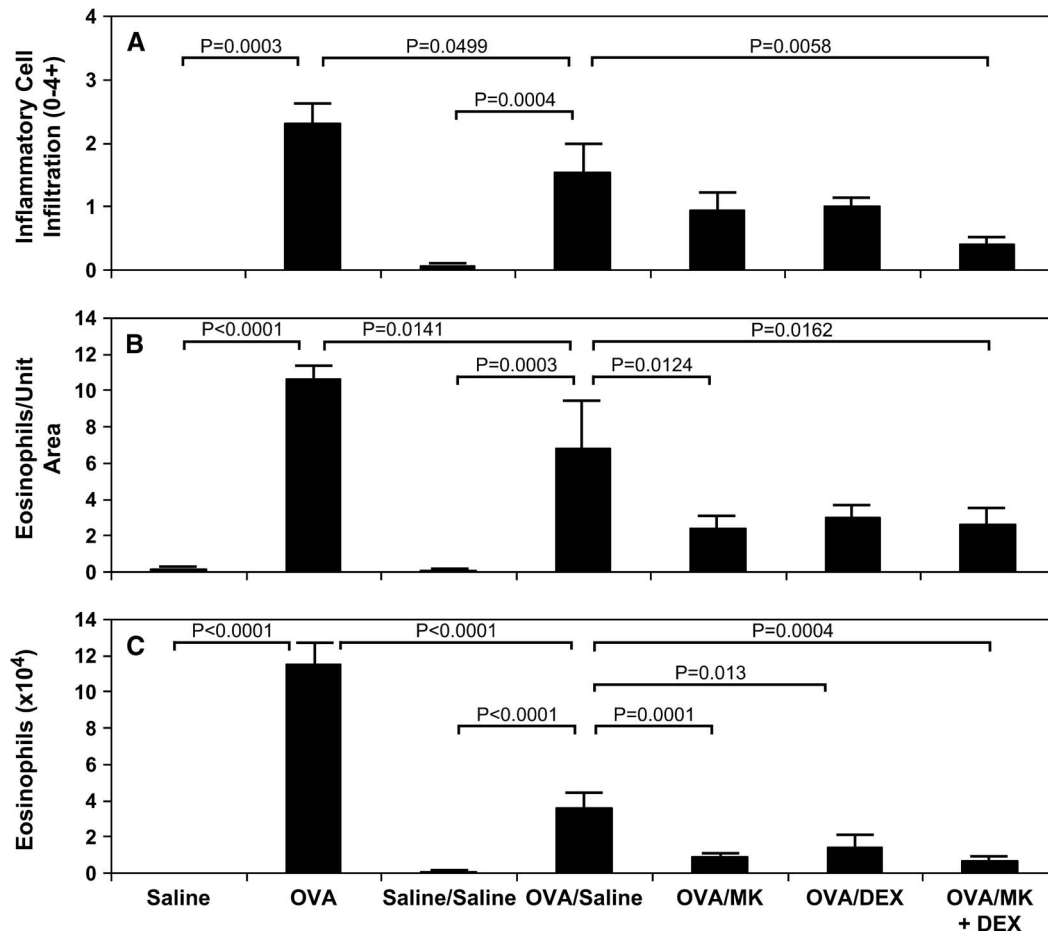


Figure 3. Effect of CysLT₁ receptor blockade and corticosteroid treatment on airway inflammatory cell infiltration in OVA-treated mice. Lung tissue (A, B) and BAL fluid (C) were obtained on Day 73 from saline-treated mice (saline; $n = 4$) and OVA-sensitized/challenged mice (OVA; $n = 4$) and Day 163 from saline-treated mice (saline/saline; $n = 7$) and OVA-treated mice in the absence (OVA/saline; $n = 6$) or presence of treatment from Days 73–163 with 1 mg/kg of montelukast (OVA/MK; $n = 8$), 1 mg/kg of dexamethasone (OVA/DEX; $n = 8$), or 1 mg/kg of montelukast + 1 mg/kg of dexamethasone (OVA/MK + DEX; $n = 4$). The total inflammatory cell infiltrate (0–4+ scale; A) and number of eosinophils per unit area ($2,200 \mu^2$; B) in the lung interstitium, and number of eosinophils ($\times 10^4$) per ml of BAL fluid (C) were determined by morphometry; p values are shown where significant ($p < 0.05$).

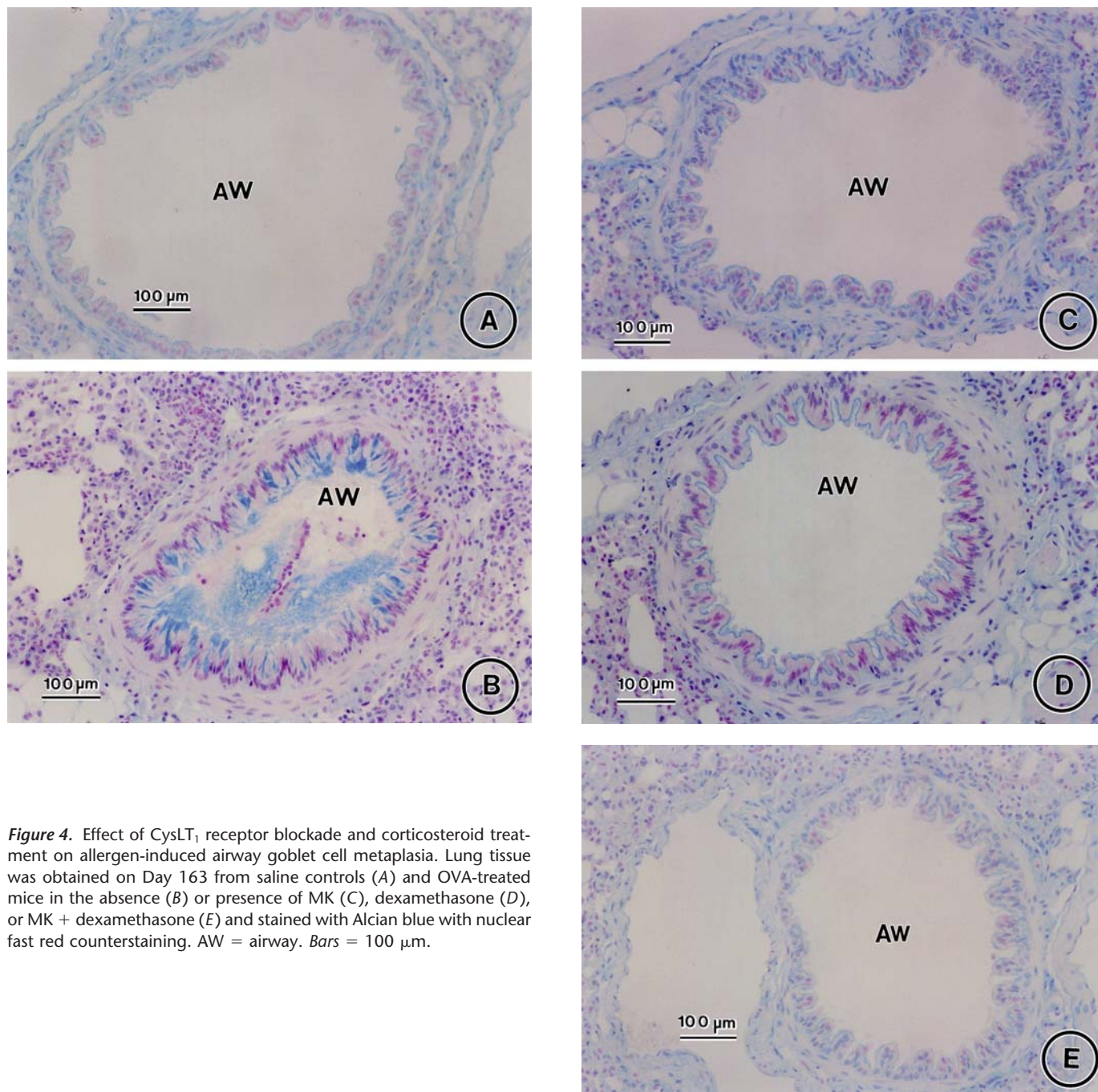


Figure 4. Effect of CysLT₁ receptor blockade and corticosteroid treatment on allergen-induced airway goblet cell metaplasia. Lung tissue was obtained on Day 163 from saline controls (A) and OVA-treated mice in the absence (B) or presence of MK (C), dexamethasone (D), or MK + dexamethasone (E) and stained with Alcian blue with nuclear fast red counterstaining. AW = airway. Bars = 100 μ m.

in control animals to 6.8 ± 2.8 eosinophils/ $2,200 \mu\text{m}^2$ lung tissue in the OVA-sensitized/challenged mice on Day 163 (Figure 3B; $p = 0.0003$, OVA/saline vs. saline/saline). Compared with the saline-treated control animals on Day 163, the OVA-treated mice had an approximate 40-fold increase in eosinophils recovered in the BAL fluid to $3.6 \pm 0.1 \times 10^4$ eosinophils/ml (Figure 3C; $p < 0.0001$, OVA/saline vs. saline/saline).

In OVA-treated mice on Day 163, MK significantly decreased the eosinophil infiltrate into the lung interstitium (Figure 3B; $p = 0.0124$, OVA/MK vs. OVA/saline and Figures 4C vs. 4B) and BAL fluid (Figure 3C; $p = 0.0001$, OVA/MK vs. OVA/saline); dexamethasone significantly reduced the number of BAL fluid eosinophils in OVA-treated mice (Figure 3C; $p = 0.013$, OVA/dexamethasone vs. OVA/saline). In OVA-treated mice,

the combination MK + dexamethasone significantly reduced the total inflammatory cell (Figure 3A; $p = 0.0058$, OVA/MK + dexamethasone vs. OVA/saline) and eosinophil (Figure 3B; $p = 0.0162$, OVA/MK + dexamethasone vs. OVA/saline) infiltration of the lung tissue (Figures 4E vs. 4B) and eosinophil influx into BAL fluid (Figure 3C; $p = 0.0004$, OVA/MK + dexamethasone vs. OVA/saline).

Airway goblet cell metaplasia. Airway goblet cell metaplasia seen in OVA-treated mice compared with saline controls on Day 73 (Figure 5; $p < 0.0001$, OVA vs. saline) persisted at Day 163, 3 mo after the last allergen challenge (Figures 4B vs. 4C and 5; $p < 0.0001$, OVA/saline vs. saline/saline). However, partial resolution of the allergen-induced mucus metaplasia observed on Day 73 occurred by Day 163 with goblet cells $8.1 \pm 1.0\%$ of

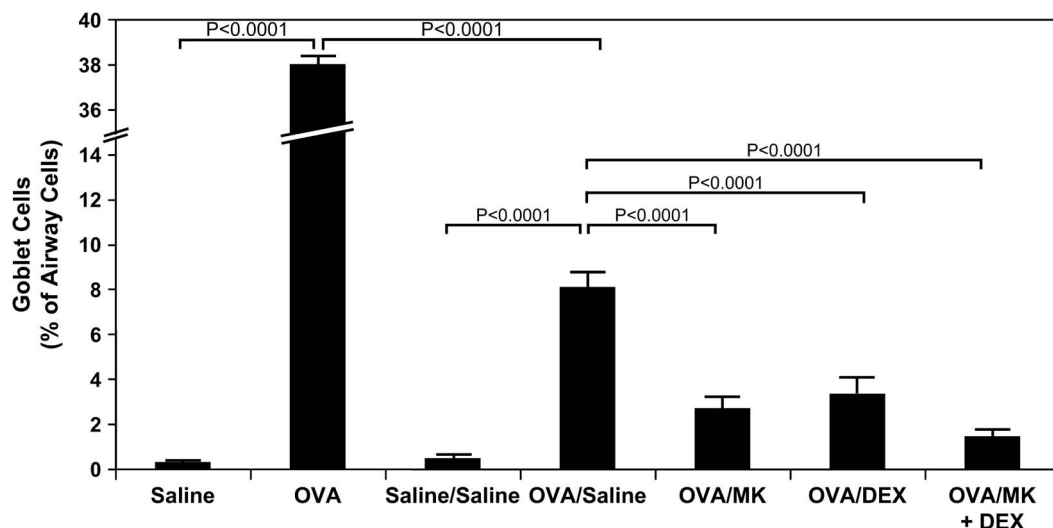


Figure 5. Reduction in allergen-induced airway goblet cell metaplasia by CysLT₁ receptor blockade and corticosteroid treatment. Lung tissue was obtained on Day 73 from saline-treated mice (saline; n = 4) and OVA-treated mice (n = 4) and on Day 163 from saline-treated mice (saline/saline; n = 7) and OVA-treated mice in the absence (OVA/saline; n = 6) or presence of MK (OVA/MK; n = 8), dexamethasone (OVA/DEX; n = 8), or MK + dexamethasone (OVA/MK + DEX; n = 4). The percentage of airway cells positive for mucus glycoproteins was determined by morphometry; p values are shown where significant (p < 0.05).

airway epithelial cells on Day 163 vs. $38.0 \pm 0.4\%$ of airway epithelial cells on Day 73 (Figure 5; $p < 0.0001$, OVA/saline vs. OVA).

Administration of MK alone, dexamethasone alone, and the combination MK + dexamethasone for a 3-mo period after the last allergen challenge each significantly reduced airway goblet cell metaplasia (Figures 4C, 4D, and 4E, respectively, vs. Figures 4B and 5; 66.5% decrease, $p < 0.0001$, OVA/MK vs. OVA/saline; 58.9% decrease, $p < 0.0001$, OVA/dexamethasone vs. OVA/Saline; 82.0% decrease, $p < 0.0001$, OVA/MK + dexamethasone vs. OVA/saline). However, the goblet cell metaplasia observed in the OVA-sensitized/challenged mice was not completely reversed by any treatment because the number of goblet cells in the MK, dexamethasone, and MK + dexamethasone groups were each significantly greater than in the saline controls (Figure 5).

Airway smooth muscle cell layer thickening. Increased airway smooth muscle thickness was seen on Day 73 in OVA-sensitized/challenged mice compared with saline controls (Figures 2B vs. 2A). This increase in airway smooth muscle mass persisted without diminution at Day 163, 3 mo after the last OVA challenge (Figures 6B vs. 6A, Figures E1B vs. E1A of the online supplement, and Figure 7A; $p < 0.0001$, OVA vs. saline). On both Days 73 and 163, the thickness of the airway smooth muscle cell layer was approximately twofold greater in the OVA-treated mice compared with control animals (Figure 7A; $p < 0.0001$, OVA vs. saline and $p < 0.0001$, OVA/saline vs. saline/saline).

MK alone (Figures 6C vs. 6B) and the combination MK + dexamethasone (Figures 6E vs. 6B) treatment for a 3-mo period after the final OVA challenge each significantly reduced the increased smooth muscle mass by 99.2% (Figure 7A; $p < 0.0001$, OVA/MK vs. OVA/saline; $p < 0.0001$, OVA/MK + dexamethasone vs. OVA/saline). There was no statistical difference observed in airway smooth muscle thickness between the saline group and the OVA-treated groups administered either MK or the combination MK + dexamethasone (Figure 7A). Dexamethasone alone had no significant effect on the increased airway smooth muscle mass observed in OVA-treated mice on Day 163 (Figures 6D vs. 6B and 7A).

Airway collagen deposition/fibrosis. On Day 73, collagen deposition markedly increased in the lung interstitium around airways and blood vessels in OVA-treated mice compared with saline-treated control animals as assessed by Masson's trichrome staining (Figures 2B vs. 2A and 7B; fourfold increase, $p < 0.0001$,

OVA vs. saline). This increase in airway collagen deposition persisted without reduction at Day 163, 3 mo after the last allergen challenge (Figures 6B vs. 6B, Figures E1B vs. E1A, and Figure 7B). As determined by morphometry, the mean airway collagen deposition/fibrosis score remained approximately fourfold greater in OVA-treated mice compared with controls at Day 163 (Figure 7B; $p < 0.0001$, OVA/saline vs. saline/saline).

Administration of MK alone (Figures 6C vs. 6B, and Figures E1C vs. E1B) or in combination with dexamethasone (Figures 6E vs. 6B, Figures E1E vs. E1B) significantly decreased collagen deposition beneath the airway epithelial cell layer (Figure 7B; 79.6% decrease, $p < 0.0001$, OVA/MK vs. OVA/saline; 90.2% decrease, $p < 0.0001$, OVA/MK + dexamethasone vs. OVA/saline). No statistical difference in airway collagen was observed between the saline group and the OVA-treated group administered MK + dexamethasone. Corticosteroid treatment with dexamethasone alone had no significant effect on subepithelial airway fibrosis in OVA-sensitized/challenged mice (Figures 6D vs. 6B, Figures E1D vs. E1B, and Figure 7B).

Airway CysLT₁ receptor expression. CysLT₁ receptor expression was significantly increased in airway smooth muscle cells in the lungs of OVA-treated mice compared with saline-treated controls on Day 163 (Figures 8C vs. 8A). Eosinophils and other inflammatory cells infiltrating the airways of OVA-treated mice had marked expression of CysLT₁ receptor (Figure 8C). The smooth muscle cells and inflammatory cells in the airways of OVA-treated mice did not exhibit immunostaining for CysLT₁ receptor when phosphate-buffered saline (Figure 8E) or nonimmunized goat IgG (Figure 8F) was substituted for goat anti-CysLT₁ receptor antibody as controls. In the OVA-treated mice, administration of MK significantly reduced the increased CysLT₁ receptor expression in airway smooth muscle cells (Figures 8B vs. 8C). In contrast, dexamethasone treatment had no significant effect on the increased CysLT₁ receptor expression in airway smooth muscle cells of OVA-treated mice (Figures 8D vs. 8C). Both MK (Figures 8B vs. 8C) and dexamethasone (Figures 8D vs. 8C) decreased the influx of CysLT₁ receptor-positive eosinophils into the airways of OVA-sensitized/challenged mice.

Effect of CysLT₁ Receptor Antagonist and Corticosteroid Treatment on Allergen-induced Airway Hyperreactivity to Methacholine

Noninvasive in vivo plethysmography. Airway hyperreactivity to aerosolized methacholine was evaluated on Days 73 and 163 by

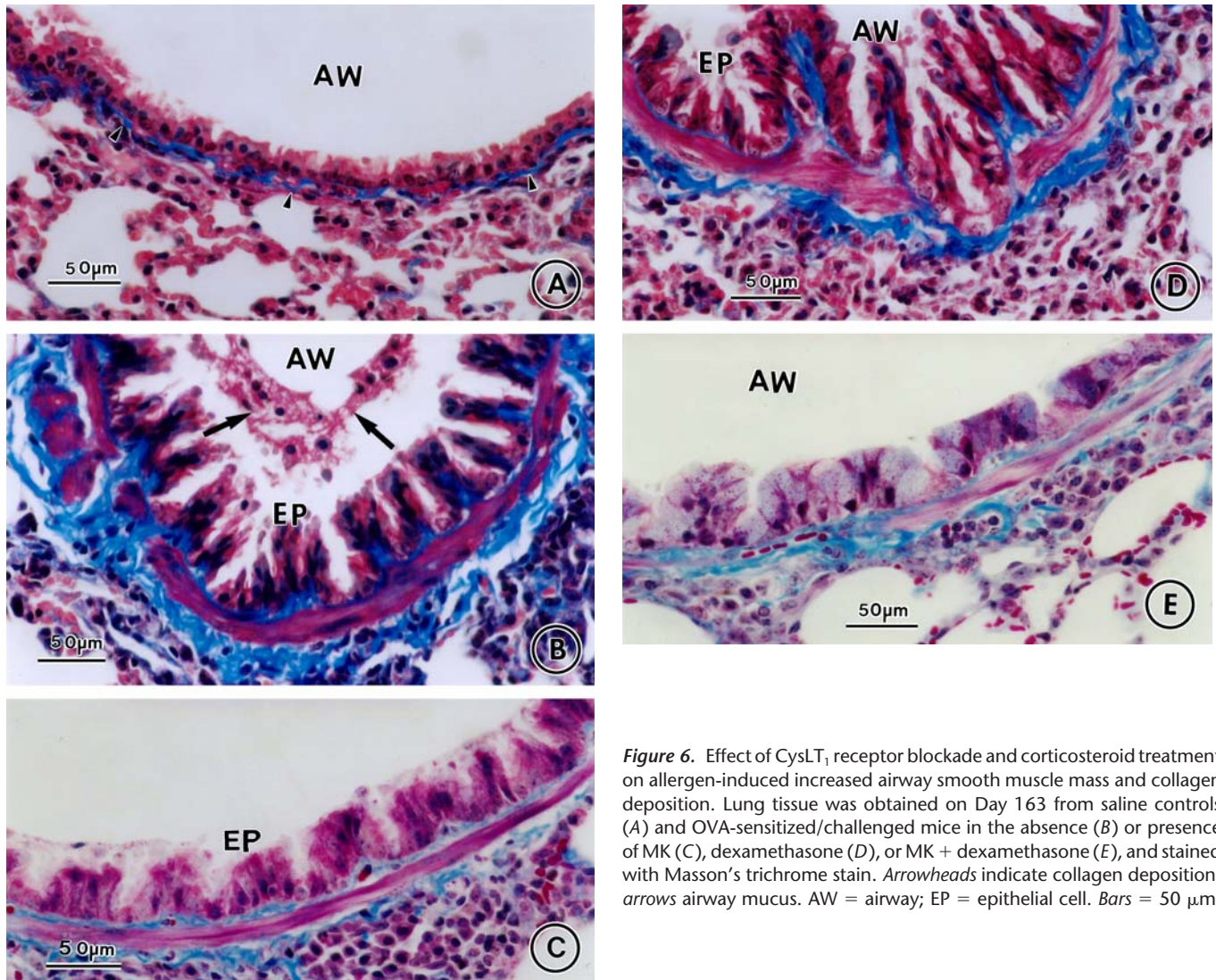


Figure 6. Effect of CysLT₁ receptor blockade and corticosteroid treatment on allergen-induced increased airway smooth muscle mass and collagen deposition. Lung tissue was obtained on Day 163 from saline controls (A) and OVA-sensitized/challenged mice in the absence (B) or presence of MK (C), dexamethasone (D), or MK + dexamethasone (E), and stained with Masson's trichrome. Arrowheads indicate collagen deposition, arrows airway mucus. AW = airway; EP = epithelial cell. Bars = 50 μ m.

noninvasive plethysmography (Figure 9). On Day 73, Penh (% of air) was significantly increased in the OVA-treated mice compared with saline controls after challenge with methacholine at 5 and 20 mg/ml (Figure 9A). However, 3 mo after the final allergen challenge on Day 163, no significant increase was seen in Penh in OVA-treated mice compared with the saline group (Figure 9B). Administration of MK, dexamethasone, or MK + dexamethasone did not significantly change the airway responses to methacholine on Day 163 in OVA-sensitized/challenged animals (Figure 9B).

DISCUSSION

In this chronic asthma model, we found spontaneous partial resolution of the influx of eosinophils into the lung interstitium and BAL fluid and airway goblet cell metaplasia and loss of the airway hyperreactivity to methacholine in OVA-treated mice 3 mo after the last intranasal allergen challenge. In contrast, the established increase in airway smooth muscle mass and subepithelial collagen deposition established by Day 73 in this model persisted without diminution for the 3-mo period after the last OVA administration. Treatment with the CysLT₁ receptor antagonist MK or the corticosteroid dexamethasone for the 3-mo period after the final allergen challenge each partially reduced

the allergen-induced airway eosinophil trafficking and goblet cell metaplasia. MK, but not corticosteroid, treatment reversed the established airway smooth muscle cell layer thickening and subepithelial collagen deposition/fibrosis suggesting that cysLTs are important in the maintenance of these allergen-induced airway structural changes.

In other chronic asthma models in mice, a progressive diminution in the increased levels of BAL fluid eosinophils from 6 d to 8 wk after final OVA challenge has been observed (24, 27, 28). The rate and extent of attenuation of the airway inflammatory response after allergen challenge likely reflect differences in mouse strains and airway challenge procedures employed (29, 30). In a mouse asthma model without systemic immunization, persistent airway eosinophilia develops in A/J mice, to a lesser extent in BALB/c mice, but not in C57BL/6 or C3H/HeJ mice (31). In these studies, chronic allergen challenge by intranasal delivery led to airway remodeling changes but by aerosol delivery did not (31). In our model, using repeated intranasal allergen challenge over a 73-d period in systemically immunized mice, total BAL fluid eosinophils decreased 68.7% from Day 73 to Day 163, 3 mo after the last allergen challenge. Similarly, eosinophils infiltrating the lung interstitium decreased 35.8% from Day 73 to Day 163. Despite this spontaneous attenuation in airway

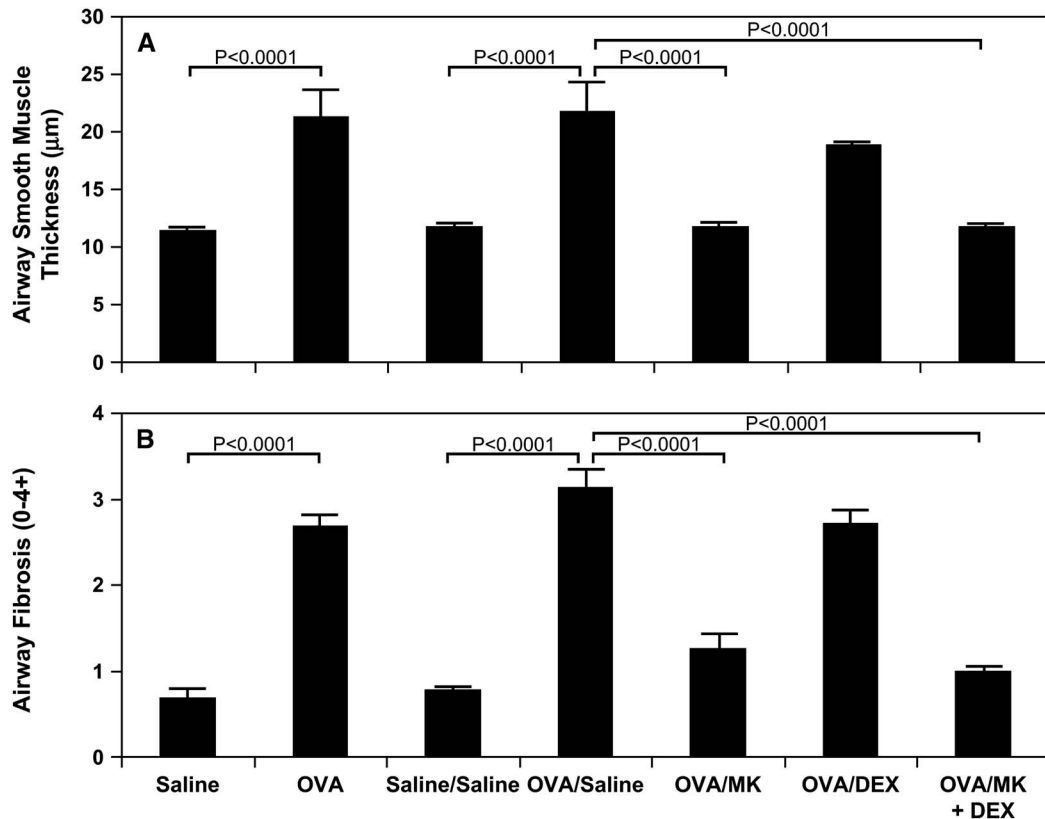


Figure 7. Reduction in allergen-induced increased airway smooth muscle mass and subepithelial collagen deposition by CysLT₁ receptor blockade but not by corticosteroid treatment. Lung tissue was obtained on Day 73 from saline-treated mice (saline; n = 4) and OVA-treated mice (OVA; n = 4) and Day 163 from saline-treated mice (saline/saline; n = 7) and OVA-treated mice in the absence (OVA/saline; n = 6) or presence of MK (OVA/MK; n = 8), dexamethasone (OVA/DEX; n = 6), or MK + dexamethasone (OVA/MK + DEX; n = 4), and thickness of the airway smooth muscle cell layer in μm (A) and collagen deposition/fibrosis (0–4+ scale; B determined; p values are shown where significant (p < 0.05).

eosinophilia, the levels of eosinophils in the BAL fluid and lung interstitium remained significantly greater in OVA-treated mice than saline control animals.

MK administered for a 3-mo period after the last OVA challenge significantly reduced the persistent eosinophil influx into the BAL fluid and decreased the infiltration of CysLT₁ receptor-positive eosinophils into the lungs of OVA-treated mice. CysLTs promote eosinophil trafficking and retention in the airways by effects on eosinophil differentiation (32), adhesion proteins (33), locomotion (34, 35), and survival (36). Corticosteroids exert their well-documented effect of decreasing influx of eosinophils and other inflammatory cells into the blood (28), lung tissue (13, 26, 37, 38), and BAL fluid (13, 20, 21, 37) when given either before (13, 20, 21, 26, 28, 37, 39–42) or after allergen challenge in animal asthma models (11). In our study, although MK and dexamethasone produced similar reductions in airway eosinophils, only CysLT₁ receptor antagonist administration significantly decreased established airway smooth muscle cell layer thickening and subepithelial fibrosis consistent with the hypothesis that airway wall remodeling and inflammation may occur in part as parallel rather than sequential pathways (43).

Spontaneous regression in allergen-induced airway goblet cell metaplasia has been observed 14 d after the last OVA challenge in a mouse asthma model (20). We found a nearly fivefold reduction in airway goblet cells at Day 163, compared with the last allergen challenge day on Day 73. Although spontaneously diminished, the percentage of airway goblet cells in OVA-treated mice remained significantly greater than in saline-treated controls. Administration of MK or dexamethasone separately or in combination for the 3-mo period after the final OVA challenge caused significant reductions in the persistent goblet cell metaplasia. We have previously shown that airway mucus release is blocked by specific inhibitors of 5-lipoxygenase and 5-lipoxygenase-activating pro-

tein that prevent leukotriene formation when administered before allergen challenge in a short-term mouse asthma model (17). We have also demonstrated in a long-term mouse asthma model in which airway remodeling occurs that MK, administered during the period of chronic allergen challenge, significantly reduces the airway goblet cell metaplasia (15). When administered after the last allergen challenge, dexamethasone decreases airway mucus-producing epithelial cell proliferation but does not completely reverse the mucus hypersecretory phenotype, despite blocking the airway inflammatory cell response in a mouse asthma model (41). Both CysLT₁ receptor blockade and corticosteroids inhibit production of Th2 cytokines IL-4 and IL-13 that are key regulators of airway mucus release (15, 44). Overexpression of IL-4 in transgenic mice results in mucus hypersecretion and increased expression of the mucin gene MUC5AC (45). IL-4, in the absence of IL-5, IL-9, and IL-13, can induce goblet cell metaplasia in mice after allergen challenge (46). IL-4 blockade by administration of soluble IL-4 receptor after development of airway goblet cell metaplasia inhibits mucus hypersecretion in OVA-sensitized/challenged mice (47). IL-13, a key mediator of mucus metaplasia in mouse asthma models (48, 49), increases expression of MUC5AC (50) and Gob-5 (51) and induces goblet cell differentiation of tracheal epithelium (50). However, IL-13-induced airway MUC5AC overexpression and goblet cell metaplasia in mouse airways are unaffected by dexamethasone in a mouse asthma model (25).

In our chronic asthma model, we found that the increase in airway smooth muscle mass and collagen deposition that develops by Day 73 persists without resolution by Day 163, 3 mo after the last allergen challenge. In this chronic asthma model, hypertrophy and hyperplasia of smooth muscle cells and myofibroblasts likely contribute to the increased airway smooth muscle mass. We previously demonstrated that MK, when

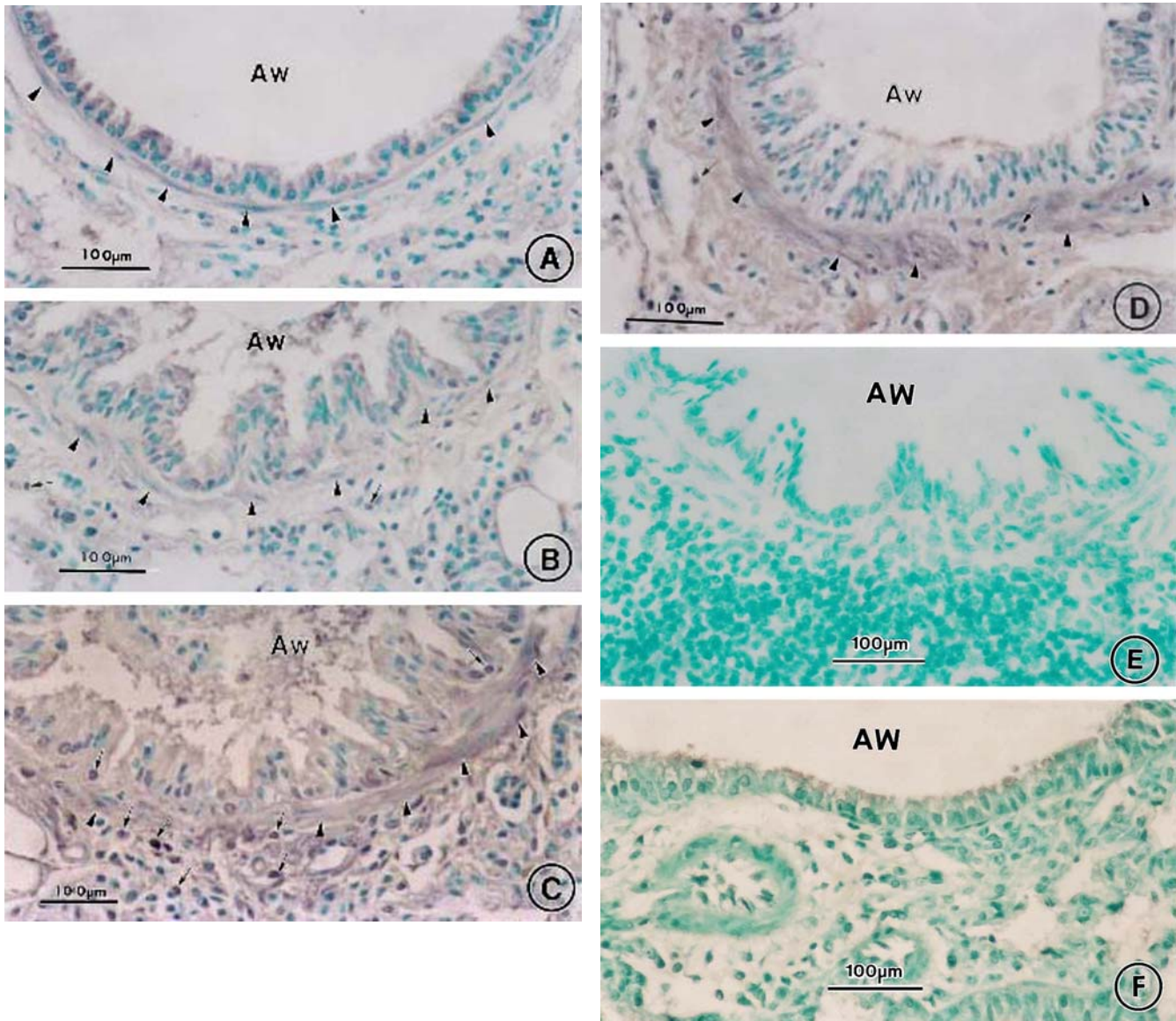


Figure 8. Effect of CysLT₁ receptor blockade on CysLT₁ receptor expression in OVA-treated mice. Lung tissue obtained on Day 163 from saline-treated mice (A) and OVA-treated mice in the absence (C, E, F) or presence of MK (B) or dexamethasone (D) underwent immunocytochemistry for CysLT₁ receptor expression using goat anti-CysLT₁ receptor antibody (A–D) or for controls, incubation with either phosphate-buffered saline (E) or nonimmunized goat IgG (F) instead of incubation with goat anti-CysLT₁ receptor primary antibody. Arrowheads indicate smooth muscle cell layer, arrows indicate inflammatory cells. Bars = 100 μm.

administered during the period of airway allergen challenge, markedly reduced the increased airway smooth muscle cell layer (15). We now show that CysLT₁ receptor blockade administered for the 3-mo period after the last allergen challenge reverses the persistent established increase in airway smooth muscle mass. CysLTs promote human airway smooth muscle hyperplasia increasing epidermal growth factor-induced proliferation of these cells (52). Transforming growth factor (TGF)- β and IL-13 each increase expression of the CysLT₁ receptor in human airway smooth muscle cells (52, 53). Our data suggest that CysLT₁ receptor blockade treatment may reverse the established increase in airway smooth muscle mass in part by reducing the increased CysLT₁ receptor expression on the smooth muscle cells in the lungs. Prior work has shown that concomitant treatment of dexamethasone during the period of allergen challenge in sensitized mice inhibits thickening of the airway smooth muscle

layer without affecting epithelial thickening or subepithelial fibrosis (28). In a rat chronic asthma model with development of airway wall thickening, the increased fibronectin deposition persisted after the final OVA challenge and was not reversed by fluticasone treatment (13). In this report, we found that dexamethasone did not reduce either the increased airway smooth muscle cell CysLT₁ receptor expression or the established increase in airway smooth muscle mass.

We have previously shown that MK prevents subepithelial collagen deposition/fibrosis in a long-term mouse asthma model when given during OVA challenge in sensitized mice (15). We now report a marked reversal in this fibrotic process when the CysLT₁ receptor antagonist is given after the last airway allergen challenge. *In vitro* studies indicate that CysLTs induce release of collagen (54) and fibroblast growth factor from mouse alveolar macrophages (55). In human fetal lung fibroblasts transformed

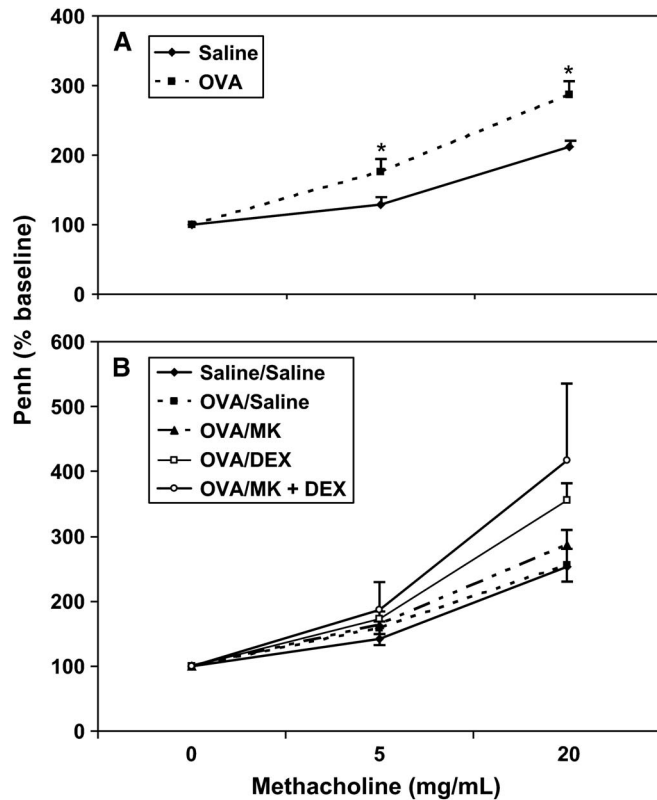


Figure 9. Effect of CysLT₁ receptor blockade and corticosteroid treatment on pulmonary mechanics to aerosolized methacholine in OVA-treated mice. The degree of bronchoconstriction (expressed as Penh [% of air] to aerosolized methacholine (0, 5, and 20 mg/ml) was determined on Day 73 in saline-treated mice (saline; n = 4) and OVA-treated mice (OVA; n = 4; *p < 0.05, OVA vs. saline) (A) and Day 163 in saline-treated mice (saline/saline; n = 7) and OVA-treated mice in the absence (OVA/saline; n = 8) or presence of MK (OVA/MK; n = 8), dexamethasone (OVA/DEX; n = 8), or montelukast + dexamethasone (OVA/MK + DEX; n = 4; B).

into myofibroblasts by TGF- β , CysLT₁ receptor expression is increased (56). LTD₄ alone stimulates collagen production by the TGF- β -transformed cells, and this LTD₄-induced augmentation of collagen release is blocked by MK (56) suggesting a potential role of CysLTs in the deposition of ECM proteins *in vivo*.

In the present study, allergen-induced airway hyperresponsiveness demonstrated on Day 73 after the final airway allergen challenge was absent on Day 163, 3 mo after the final OVA challenge in OVA-sensitized/challenged mice, despite persistent airway inflammation and structural changes. In other models, with shorter periods after final allergen challenge before assessment of pulmonary mechanics, airway hyperreactivity persisted at the end of 2-wk (13) and 8-wk (27) allergen-free intervals, despite resolution of the airway eosinophilic response. In these models, the airway hyperreactivity correlated with increased airway thickness (13) and airway contractile tissue (27). However, our data suggest that airway hyperreactivity can be dissociated from the allergen-induced airway structural changes because the hyperresponsiveness to methacholine resolved despite the persistent increased airway smooth muscle mass and subepithelial collagen deposition seen 3 mo after the final allergen challenge. The relationship of airway wall thickness and other features of remodeling to airway reactivity in patients with asthma

remains unclear (9). Whereas a positive correlation of airway wall thickness with airway hyperresponsiveness has been reported (57, 58), other studies have found either no significant relationship (59) or a negative correlation of airway reactivity with airway wall thickness (60, 61). A bronchial biopsy study to determine airway structural changes associated with severe asthma demonstrated that increases in collagen type III deposition, airway smooth muscle cell size, fibroblasts, and mucus glands selectively correlated with severe asthma, whereas mucosal eosinophilia and subepithelial basement membrane thickness did not (4). Thus the relationship of airway inflammation and remodeling with asthma severity and lung function is complex and in need of future research (for review, *see* Reference 9) to determine the clinical relevance of remodeling and benefit of therapeutic modalities controlling particular features of the airway injury/repair/remodeling response in asthma.

In summary, our studies indicate that CysLT₁ receptor antagonism has a significant anti-airway remodeling effect in an animal model of human asthma. The reversal of established airway structural changes (i.e., increased smooth muscle mass and subepithelial fibrosis) by MK, but not dexamethasone, suggests an important role for cysLTs in the immunopathogenesis of the remodeling process that is not modulated by corticosteroids. Clinical studies in patients with asthma to determine the alterability of established airway structural changes by CysLT₁ receptor blockade will be of great interest.

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