

Effect of a Cysteinyl Leukotriene Receptor Antagonist on Experimental Emphysema and Asthma Combined with Emphysema

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Abstract

The incidence of overlapping bronchial asthma and chronic obstructive pulmonary disease has increased in recent years. Cysteinyl leukotrienes (CysLTs) play an important role in asthma, and the type 1 CysLT receptor (CysLT₁R) is expressed by many inflammatory cells. We evaluated the effect of montelukast, a CysLT₁R antagonist, on mouse models of asthma, porcine pancreatic elastase (PPE)-induced emphysema, and asthma combined with emphysema. Mice were sensitized with ovalbumin (OVA) on Days 0 and 14 and subsequently challenged with OVA on Days 28, 29, and 30. Pulmonary emphysema was induced by intratracheal instillation of PPE on Day 25. Mice were treated subcutaneously with montelukast or vehicle from Day 25 to Day 31. Airway hyperresponsiveness (AHR), static compliance; the number of inflammatory cells, the levels of cytokines, chemokines, LTs, and perforin in the bronchoalveolar lavage fluid, and the quantitative morphometry of lung sections were analyzed on Day 32. Treatment with montelukast significantly attenuated the AHR and eosinophilic airway inflammation in OVA-sensitized and OVA-challenged mice. Administration of montelukast significantly reduced the AHR, static compliance, and neutrophilic airway inflammation, while attenuating emphysematous lung changes, in PPE-treated mice. In PPE-treated mice subjected to allergen sensitization and challenges, montelukast significantly suppressed the AHR, static compliance, and eosinophilic and neutrophilic airway inflammation in addition to the development of experimentally induced emphysema in the lungs. Our data suggest that CysLT₁R antagonists may be effective in

ameliorating the consequences of PPE-induced lung damage and the changes that follow allergen sensitization and challenges.

Keywords: asthma; chronic obstructive pulmonary disease; overlap of asthma and chronic obstructive pulmonary disease; cysteinyl leukotriene; cysteinyl leukotriene receptor

Clinical Relevance

In the present study, we investigated the effect of type 1 cysteinyl leukotriene receptor (CysLT₁R) antagonist (montelukast) on the development of airway hyperresponsiveness (AHR) and both eosinophilic and neutrophilic airway inflammation in porcine pancreatic elastase (PPE)-treated experimental mouse models of emphysema and ovalbumin (OVA)-sensitized and OVA-challenged asthma combined with emphysema. This study presents interesting research that aims to explore the role of montelukast in experimental emphysema and asthma combined with emphysema, and because little work has been done in this area. Our research suggests that CysLT₁R antagonist (montelukast) may be effective in ameliorating the consequences of PPE-induced lung damage and the changes that follow allergen sensitization and challenges, and especially might impact the therapy of overlap of bronchial asthma and chronic obstructive pulmonary disease.

The incidence of patients with both chronic obstructive pulmonary disease (COPD) and bronchial asthma is increasing (1), and

questions remain regarding how to treat these patients while maintaining their quality of life. These two obstructive airway

diseases are major global causes of disability and death; in particular, COPD is estimated to become the third most common cause of

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death by 2020. The chronic airflow limitation characteristic of COPD is caused by a mixture of small airway disease and parenchymal destruction (emphysema), the relative contributions of which vary among individuals. In the present study, we highlight that chronic inflammatory diseases, such as COPD and asthma, result from ongoing inflammatory exacerbations that result in changes in lung cells, inflammatory mediators found in the lung, and, at later time points, the physiologic capacity of the lungs.

Structural and pathophysiological studies performed on asthma models have shown that eosinophilic inflammation is predominant, whereas neutrophilic inflammation is characteristic of COPD. However, a significant overlap of other pathological features, such as airway wall thickening, airway inflammation, and airway hyperresponsiveness (AHR), may exist in individual patients, thus making it difficult to distinguish between COPD and asthma (2). Furthermore, compared with subjects with mild and moderate asthma, both those with severe asthma and those with asthma who smoke have higher numbers of neutrophils in the bronchoalveolar lavage (BAL) fluid and biopsies. Moreover, in patients with COPD, particularly those with acute disease exacerbation, tissue eosinophilia is common, and is associated with a favorable response to steroid therapy (3). Epithelial fragility and the thickening of the reticular basement membrane are observed even in mild asthma, whereas increased smooth muscle mass in the airway, hypertrophy of mucus-secreting glands, increased number of fibroblasts, and increased deposition of collagen are common in both severe asthma and COPD (4).

The pathological changes within the airways that are associated with asthma and COPD are complicated, and many mediators with redundant activity have been identified in asthma and COPD. Cysteinyl leukotrienes (CysLTs), including LTC₄, LTD₄, and LTE₄, play many roles in smoking-related lung diseases (5). CysLT production has been shown to be increased in smokers, which suggests that some of the adverse effects of cigarette smoke exposure on pulmonary structures may be related to enhanced LT synthesis (6). LTs are generated from the arachidonic acid present in membrane lipids by 5-lipoxygenase and play critical roles in the pathogenesis of asthma and other

pulmonary diseases because of their bronchoconstrictive and inflammatory effects (7). LTs increase vascular permeability by inducing the exudation of plasma into the airway walls and lumen, which is an important pathological feature of emphysema and bronchial asthma (7, 8). Moreover, several studies have suggested that LTs are potent chemoattractants for recruiting inflammatory cells to the lung parenchyma. Enhanced LT generation from peripheral leukocytes is observed in patients with COPD, and the presence of specific IgE antibodies against inhalant allergens enhances LTC₄ generation and bronchial hyperresponsiveness, thus suggesting a relationship between LTC₄ generation and airway obstruction (9).

Two classical pharmacologically defined G protein-coupled LT receptors, CysLT₁R and CysLT₂R, have been described, and recent studies have identified two receptors that are selective for LTE₄: P2Y₁₂ and CysLTER (10). CysLT₁R binds LTD₄ with high affinity, but does not bind to LTE₄. A recent study has shown that the number of CysLT₁R-expressing cells, including mast cells, monocytes/macrophages, neutrophils, B-lymphocytes, and eosinophils, is significantly higher in patients with COPD experiencing a severe exacerbation than in subjects with stable-phase COPD or nonsmoker control subjects (11). Pharmacological antagonism of the CysLT₁R was shown to have a significant effect in a mouse model of experimental asthma and airway remodeling (12). In contrast, the destruction of the alveolar walls during COPD is considered irreversible, and no effective anti-inflammatory treatment exists to prevent the progression of the disease. The effect of CysLT₁R antagonists on COPD or emphysema in animal models remains unknown. Montelukast, a CysLT₁R antagonist, has been reported to inhibit 5-lipoxygenase in both activated neutrophils and monocytes/macrophages via an uncharacterized mechanism that appears to be distinct from the mechanism underlying the inhibition of CysLT₁Rs (13–15).

In the present study, we hypothesized that, because LTs are a crucial component of both asthma and COPD, the use of a CysLT₁R antagonist should not only relieve both diseases, but also reduce inflammation in a combined model of both diseases. Therefore, we investigated the

effect of montelukast on the development of AHR and on eosinophilic and neutrophilic airway inflammation in a porcine pancreatic elastase (PPE)-induced mouse model of emphysema with and without ovalbumin (OVA)-sensitized and OVA-challenged asthma. Our results show that montelukast significantly suppressed the AHR, static compliance, airway inflammation, and development of emphysematous changes in a combination model of asthma and emphysema.

Materials and Methods

An expanded description of the methods can be found in the online supplement.

Animals

Female BALB/c mice at 8 to 10 weeks of age were purchased from Charles River Japan, Inc. (Yokohama, Japan). The mice were maintained on an OVA-free diet. All of the experimental animals used in the present study were housed under a protocol approved by the Institutional Animal Care and Use Committee of Okayama University Medical School (Okayama, Japan).

Sensitization and Airway Challenge

The mice were sensitized by intraperitoneal injection of 20 µg of OVA (Grade V; Sigma, St. Louis, MO) emulsified in 2.25 mg of alum (Inject Alum; Pierce Biotechnology, Rockford, IL) on Days 0 and 14. The mice were subsequently challenged with OVA (1% in saline) for 20 minutes on Days 28, 29, and 30 by ultrasonic nebulization (OVA/OVA) (Figure 1). AHR was assessed 48 hours after the last challenge, and tissues and cells were obtained for further analysis (16).

COPD Induction

For PPE (EMD Chemicals, San Diego, CA) instillation, the mice were anesthetized with pentobarbital. The experimental animals received intratracheal instillation of 25 U PPE/100 g body weight dissolved in 40 µl of normal saline, whereas the control animals received 40 µl of normal saline solution (Figure 1).

Administration of the CysLT Receptor Antagonist

The mice received subcutaneous injections of 500 µg of montelukast (Merck and Co., Inc., Whitehouse Station, NJ) daily from Day 25 to Day 31. The control mice were administered saline subcutaneously (Figure 1).

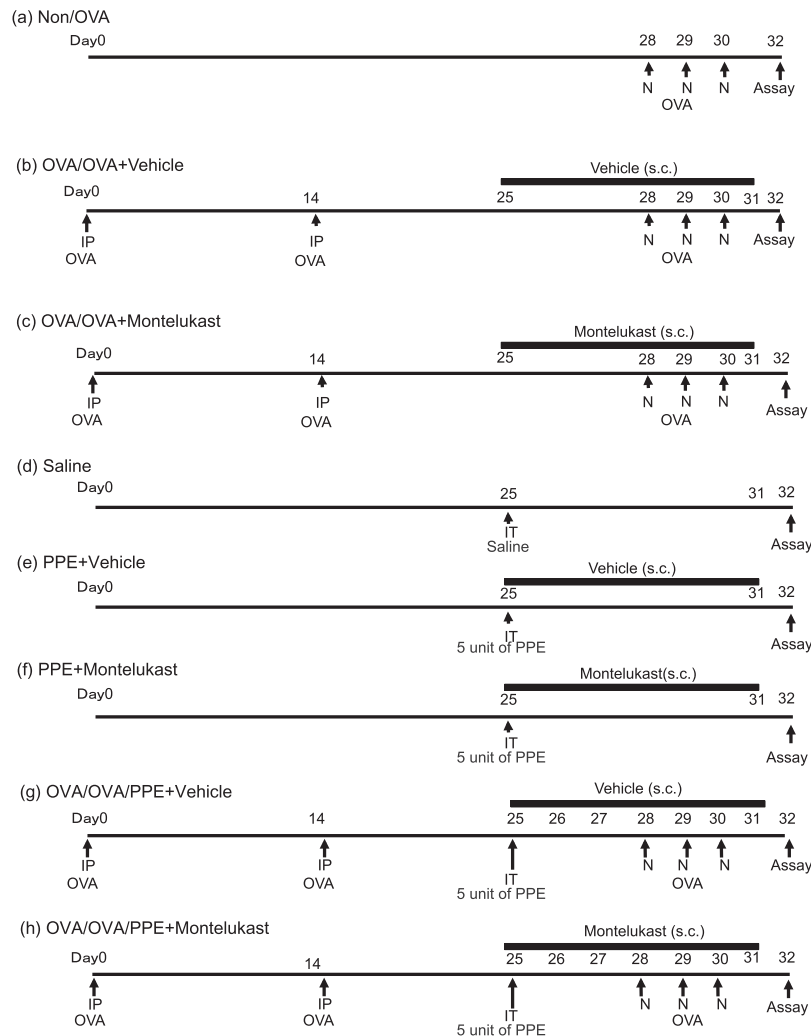


Figure 1. Experimental protocols. (a) Nonsensitized mice received 3 consecutive days of aerosolized ovalbumin (OVA) challenge (Non/OVA). (b) Sensitized mice were challenged with OVA and received subcutaneous injections of vehicle from Day 25 to Day 31 (OVA/OVA + Vehicle). (c) Sensitized mice were challenged with OVA and received subcutaneous injections of montelukast from Day 25 to Day 31 (OVA/OVA + Montelukast). (d) Control mice were administered saline intratracheally (Saline). (e) Mice were administered porcine pancreatic elastase (PPE) intratracheally and received subcutaneous injections of vehicle from Day 25 to Day 31 (PPE + Vehicle). (f) To assess the effect of montelukast, mice were administered PPE intratracheally and received subcutaneous injections of montelukast from Day 25 to Day 31 (PPE + Montelukast). (g) Sensitized mice were challenged, administered PPE intratracheally, and received subcutaneous injections of vehicle from Day 25 to Day 31 (OVA/OVA/PPE + Vehicle). (h) Sensitized mice were challenged, administered PPE intratracheally, and received subcutaneous injections of montelukast from Day 25 to Day 31 (OVA/OVA/PPE + Montelukast). IP, intraperitoneal injection; IT, intratracheal instillation; N, nebulization.

Determination of Airway Responsiveness and Static Compliance

A flexiVent small-animal ventilator (SCIREQ, Montreal, PQ, Canada) was used to assess airway function in anesthetized, mechanically ventilated animals by measuring changes in lung resistance (RL) in response to increasing doses of inhaled methacholine (MCh), as previously described

(17, 18). The static compliance, which is a measurement of the elasticity of the lung, was calculated from the pressure-volume curves using flexiVent software (19).

Lung Histology

The right lung was inflated and fixed by intratracheal instillation of 10% formalin. The transpulmonary pressure at which the lungs were inflated was 25 cm H₂O static pressure

(19). The mean linear intercept (Lm) and area-weighted mean equivalent diameter (D2) were determined for each core obtained from emphysematous lungs. For the Lm determination, 10 axial sections at the height of the hilum were randomly selected from each mouse, and the Lm was calculated by counting lines of a defined length, as previously described (20). The equivalent diameter (D) of the airspace was determined, and the mean D was calculated from a minimum of 500 values per mouse (limited to D > 5 mm). D2 was also calculated (21, 22).

Results

Treatment with Montelukast Attenuates AHR and Airway Inflammation in OVA-Sensitized and OVA-Challenged Mice

We assessed the airway responsiveness of OVA-sensitized and OVA-challenged mice to increasing doses of inhaled MCh. Administration of montelukast daily from Day 25 to Day 31 significantly prevented the increase in AHR (Figure 2A) and the number of eosinophils, lymphocytes, and CD3⁺ and CD4⁺ T cells in the BAL fluid relative to vehicle-treated mice (Figures 2C and 2D). In addition, treatment with montelukast significantly reduced the levels of IL-4, IL-5, IL-13, LTB₄, and CysLT in the BAL fluid of OVA-sensitized and OVA-challenged mice (Figure 2E). No significant difference was found in the TNF-α levels in the lungs, static compliance, Lm, or D2 among any of the groups tested (Figures 2B and 2F–2H).

Treatment with Montelukast Attenuates AHR and Static Compliance after PPE Treatment

We assessed the AHR in the recipients of PPE. Intratracheal PPE instillation led to the development of increased RL in a MCh dose-dependent manner and increased static compliance relative to the controls that received saline. Daily administration of montelukast to PPE recipients from Day 25 to Day 31 largely prevented the PPE-induced increases in AHR (Figure 3A) and static compliance (Figure 3B).

Treatment with Montelukast Reduces Inflammatory Cell and Lymphocyte Accumulation in the BAL Fluid of PPE-Treated Mice

PPE instillation resulted in a marked increase in the number of macrophages, lymphocytes, and neutrophils in the BAL

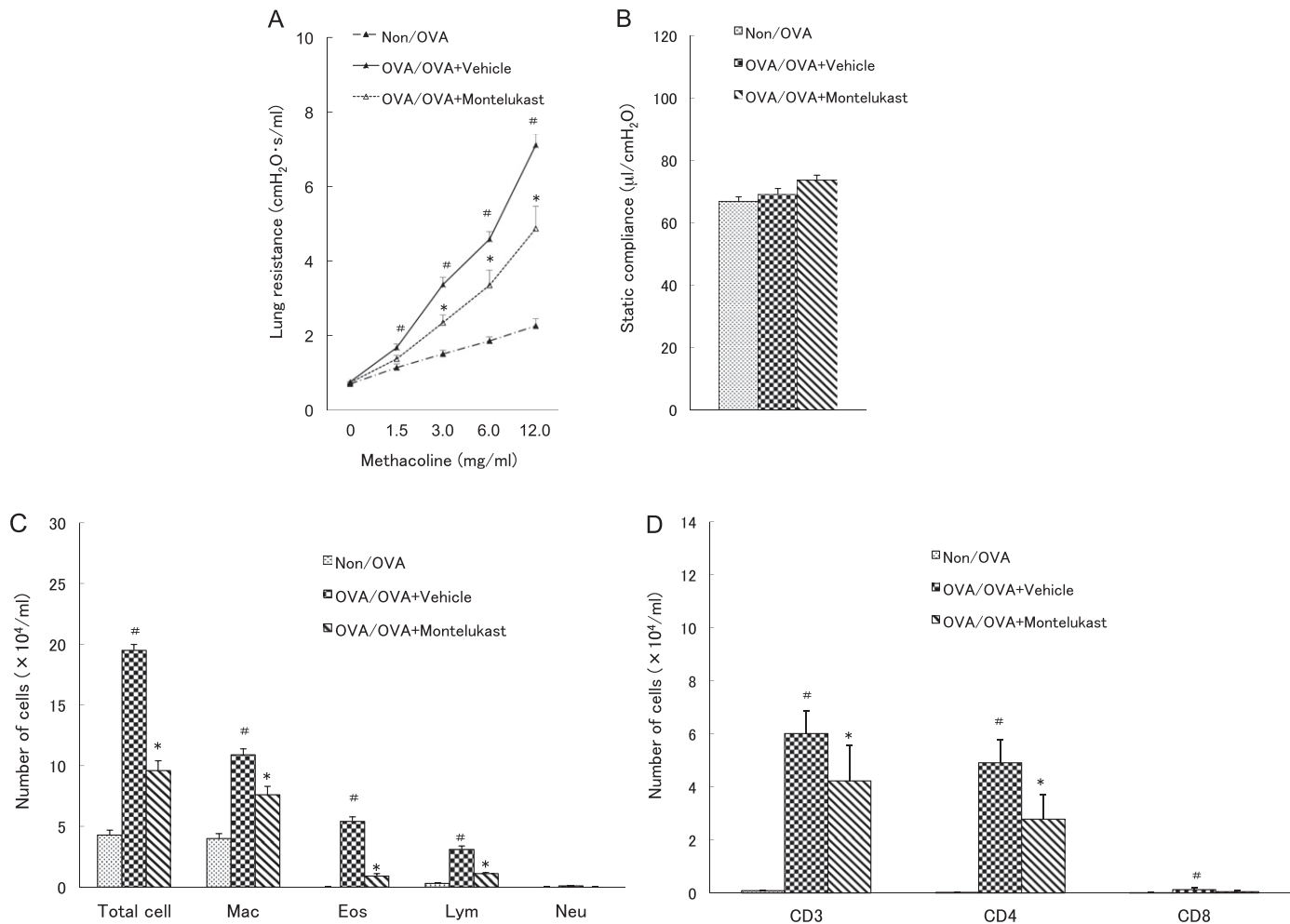


Figure 2. Treatment with montelukast attenuates airway hyperresponsiveness (AHR) and airway inflammation in OVA-sensitized and OVA-challenged mice. Lung resistance (RL) (A), static compliance (B), inflammatory cells (C), CD3⁺, CD4⁺, and CD8⁺ T cells (D), cytokine, chemokine, leukotriene (LT), and perforin levels in the bronchoalveolar lavage (BAL) fluid (E), TNF- α levels in the lungs (F), mean linear intercept (Lm) values (G), and area-weighted mean equivalent diameter (D2) (H). The results for each group are expressed as the mean ($\pm$ SEM; $n = 8-12$ in each group). #Significant difference ($P < 0.05$) between Non/OVA and OVA/OVA + Vehicle; *Significant difference ($P < 0.05$) between OVA/OVA + Vehicle and OVA/OVA + Montelukast. Eos, eosinophil; KC, keratinocyte chemoattractant; Lym, lymphocyte; Mac, macrophage; MIP, macrophage inflammatory protein; Neu, neutrophil.

fluid. Administration of montelukast to PPE-treated mice significantly reduced the total number of cells in the BAL fluid, including the number of macrophages, lymphocytes, and neutrophils (Figure 3C). The number of CD3⁺, CD4⁺, and CD8⁺ T cells was significantly higher in the PPE-treated mice than in saline-treated control mice, and administration of montelukast significantly reduced the number of these cells in the BAL fluid (Figure 3D).

Treatment with Montelukast Reduces the Levels of Chemokines, LTs, and Perforin in the BAL Fluid of PPE-Treated Mice

The levels of keratinocyte chemoattractant (KC), macrophage inflammatory protein

(MIP)-2, perforin, LTB₄, and CysLT in the BAL fluid of PPE-treated mice were significantly increased relative to control mice. In contrast, the levels of IL-4, IL-5, and IL-13 were not significantly changed. The levels of KC, MIP-2, LTB₄, CysLT, and perforin were significantly reduced in montelukast-treated mice (Figure 4A).

Treatment with Montelukast Ameliorates the Increased TNF- α Levels and Lm and D2 Values Induced by PPE Administration

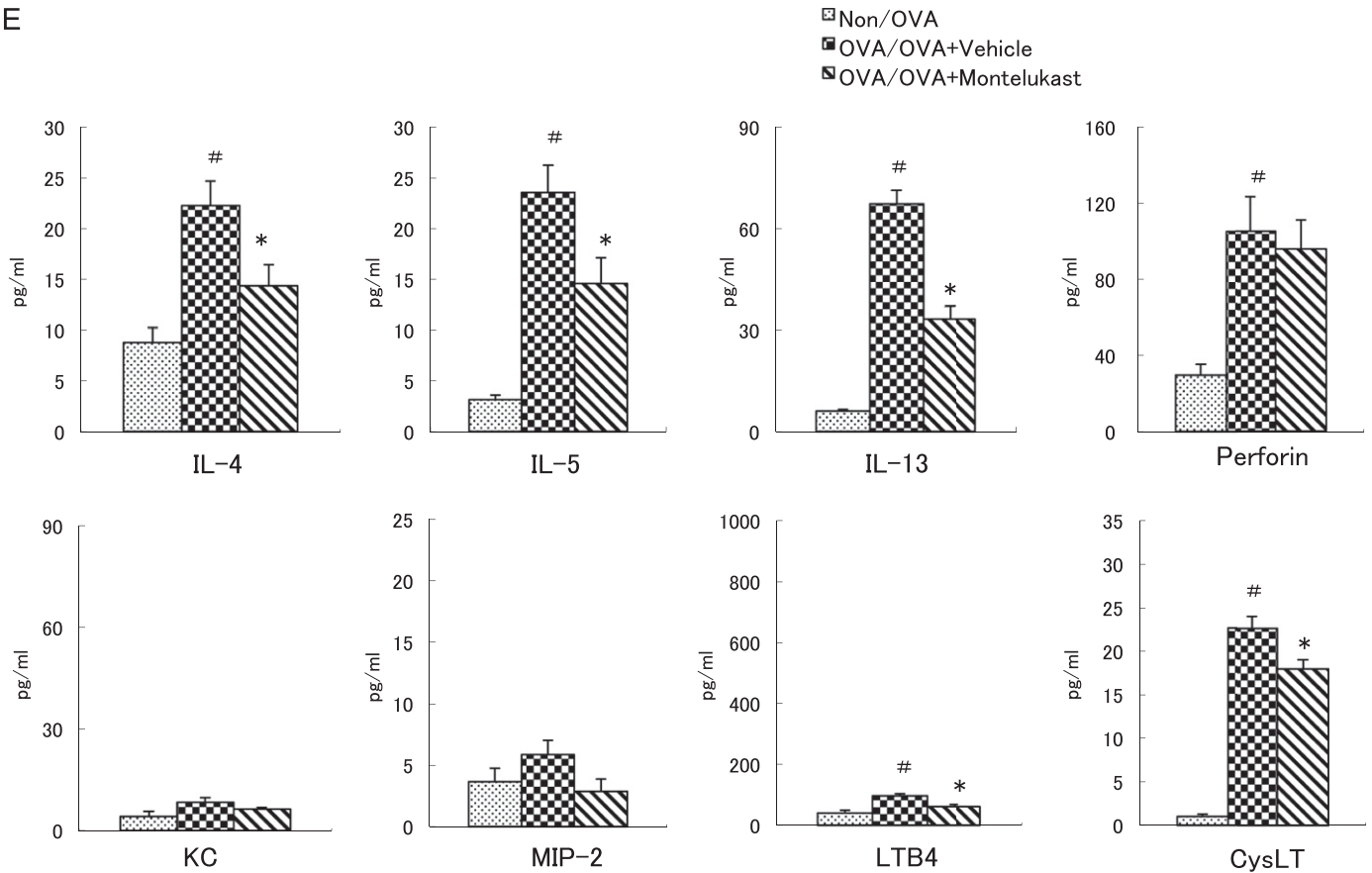
The TNF- α level in the lungs of the PPE-treated mice was significantly increased relative to saline-treated control mice, and this increase was ameliorated by montelukast administration (Figure 4B).

PPE instillation resulted in a significant increase in the Lm and D2 values, and treatment with montelukast significantly ameliorated this increase (Figures 4C and 4D). Lung histology was also examined in hematoxylin and eosin (HE)-stained slides (Figure 4E).

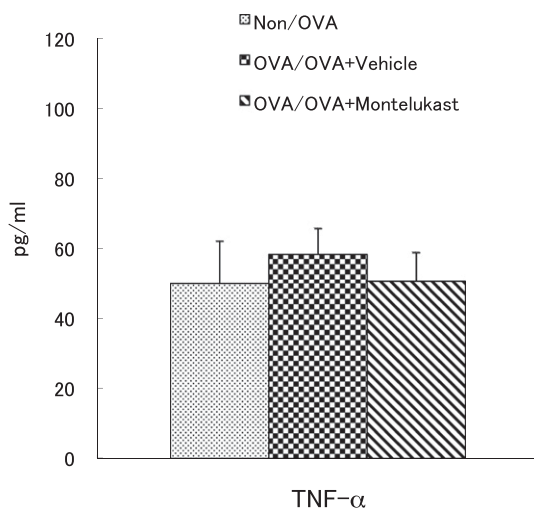
Treatment with Montelukast Attenuates AHR and Static Compliance in OVA-Sensitized and OVA-Challenged Mice that Received PPE

We monitored RL and static compliance in the combined asthma and emphysema model. Intratracheal PPE instillation into OVA-sensitized and OVA-challenged asthmatic mice increased AHR in an MCh

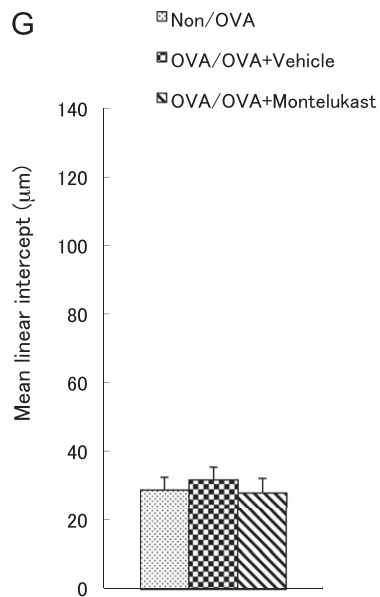
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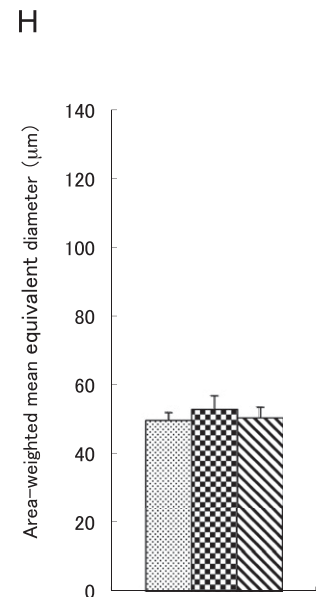


Figure 2. (Continued).

dose-dependent manner (Figures 2A, 3A, and 5A). Treatment with montelukast daily from Day 25 to Day 31 significantly

suppressed AHR relative to vehicle-treated mice (Figure 5A). Administration of montelukast significantly reduced

static compliance in sensitized and challenged mice that received PPE (Figure 5B).

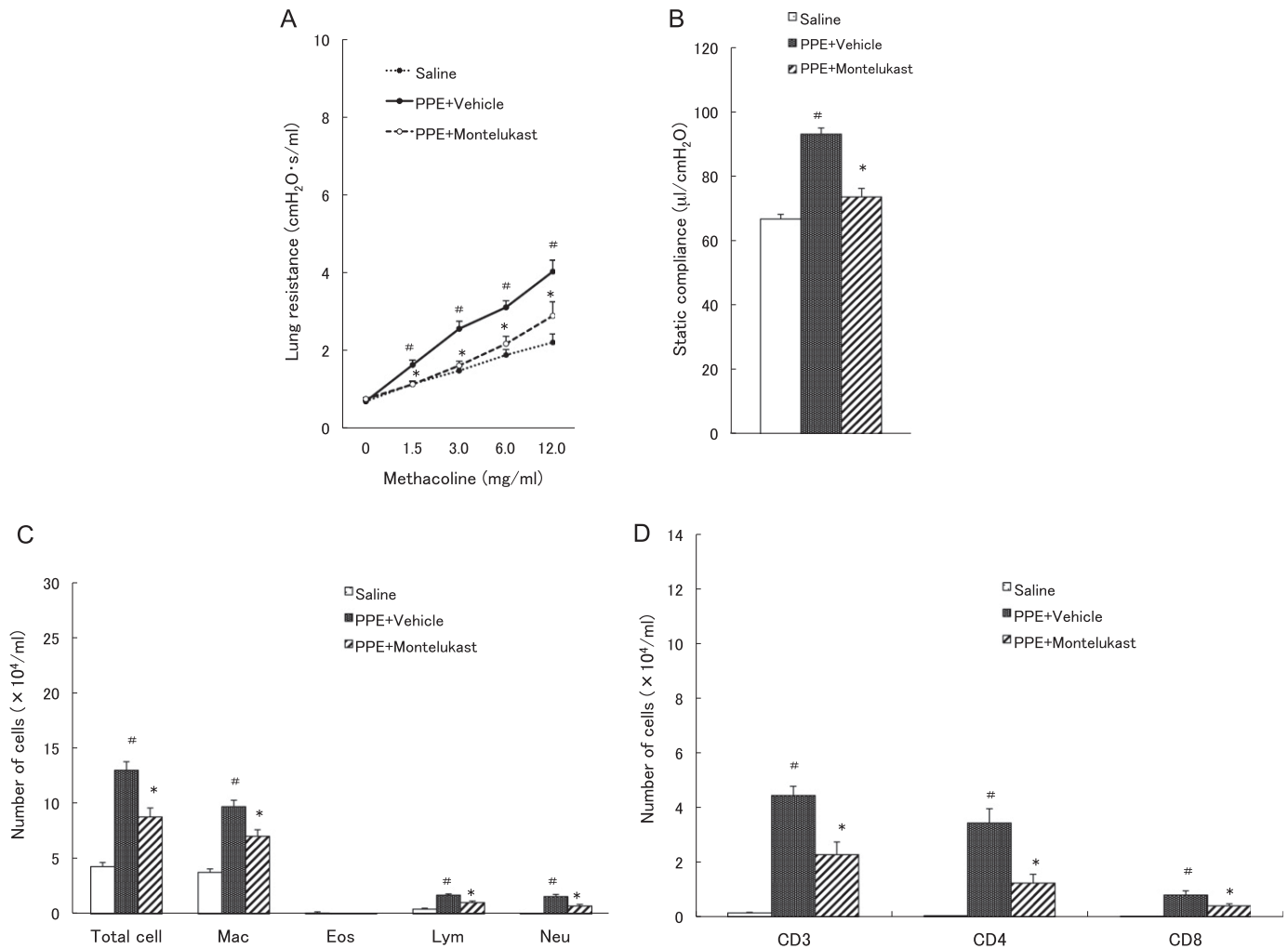


Figure 3. Treatment with montelukast prevents the increase in RL (A), static compliance (B), inflammatory cell number (C), and CD3⁺, CD4⁺, and CD8⁺ T cell numbers in the BAL fluid (D) in PPE-treated mice. The results for each group are expressed as the mean ($\pm$ SEM; $n = 8-12$ in each group). #Significant difference ($P < 0.05$) between the Saline and PPE + Vehicle mice. *Significant difference ($P < 0.05$) between PPE + Vehicle and PPE + Montelukast mice. Eos, eosinophil; Lym, lymphocyte; Mac, macrophage; Neu, neutrophil.

Treatment with Montelukast Reduces Inflammatory Cell and Lymphocyte Accumulation in the BAL Fluid in the Combined Model

Montelukast treatment significantly reduced the number of macrophages, eosinophils, lymphocytes, and neutrophils in the BAL fluid (Figure 5C). The accumulation of inflammatory cells in the peribronchial and perivascular areas was investigated through histological examination of HE-stained slides. Mice treated with montelukast presented fewer inflammatory cells than the vehicle-treated mice (Figure 5D). In addition, treatment with montelukast significantly reduced the number of CD3⁺, CD4⁺, and CD8⁺ T cells in the BAL fluid of PPE-treated, OVA-sensitized, and OVA-challenged mice (Figure 5E).

Treatment with Montelukast Reduces the Levels of Cytokines, Chemokines, LTs, and Perforin in the BAL Fluid of Mice in the Combined Model

In combination with PPE, intraperitoneal OVA sensitization and OVA airway challenge led to an increase in the levels of KC, MIP-2, LTB₄, perforin, IL-4, IL-5, IL-13, and CysLT in the BAL fluid (Figures 2E, 4A, and 6A). Treatment with montelukast significantly reduced these levels (Figure 6A).

Treatment with Montelukast Ameliorates the Increased TNF- α Level and Lm and D2 Values Induced by PPE Administration in the Combined Model

Montelukast treatment significantly reduced the TNF- α level in the lungs relative to

PPE- and vehicle-treated mice (Figure 6B). Treatment with montelukast significantly reduced the Lm and D2 values in mice with combined PPE treatment and OVA sensitization and challenge relative to vehicle-treated mice (Figures 6C and 6D). Lung histology was examined using HE-stained slides. Treatment with montelukast suppressed the emphysematous changes observed in the combined model animals treated with vehicle (Figure 6E).

Discussion

COPD is a progressive disease characterized by persistent airflow limitation that is associated with an enhanced chronic inflammatory response in the airways and

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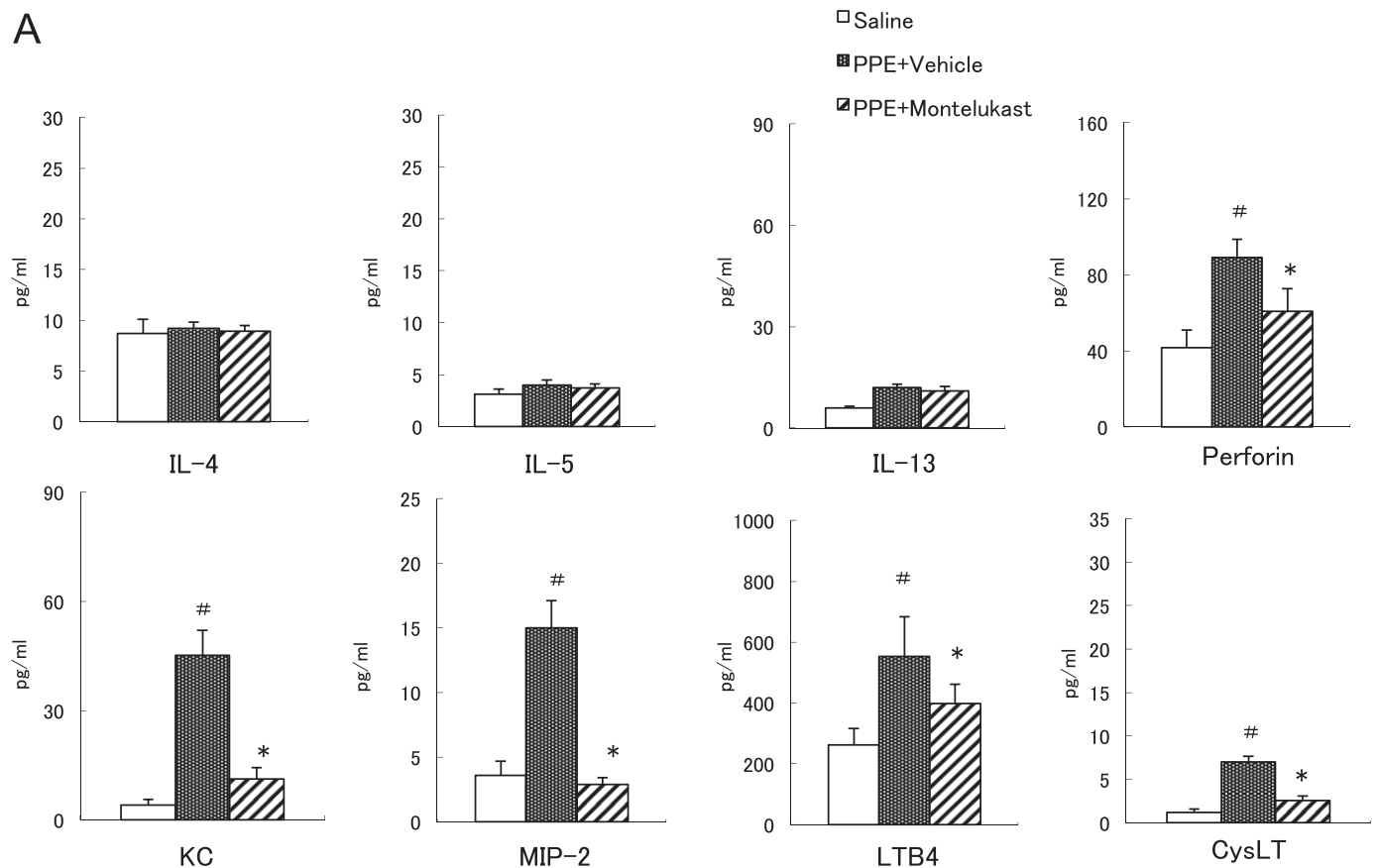


Figure 4. Treatment with montelukast prevents the increase in chemokine, LT, and perforin levels in the BAL fluid (A), TNF- α in the lung (B), Lm values (C), and D2 (D) in PPE-treated mice. Lung histology (E): (a) Saline, (b) PPE + Vehicle, and (c) PPE + Montelukast. The results for each group are expressed as the means ($\pm$ SEM; $n = 8-12$ in each group). [#]Significant difference ($P < 0.05$) between the Saline and PPE + Vehicle mice. ^{*}Significant difference ($P < 0.05$) between the PPE + Vehicle and PPE + Montelukast mice. KC, keratinocyte chemoattractant; MIP, macrophage inflammatory protein.

lungs. This chronic inflammation induces the destruction of the parenchymal tissue, leads to emphysema, and disrupts normal repair and defense mechanisms, thus resulting in small airway fibrosis. COPD is considered a disease of fixed-airflow obstruction for which no treatment exists, and the detailed mechanisms underlying COPD are not known. The overlap of asthma and COPD is becoming more prevalent, and these patients respond less favorably to guideline-recommended drug therapies. Our study showed that OVA-sensitized and OVA-challenged asthmatic mice treated with intratracheal PPE instillation displayed a significant increase in the AHR, LTB₄ levels, and CD4⁺ and CD8⁺ T cell counts in the BAL fluid relative to animals received airway challenge asthma or PPE-induced emphysema alone. In contrast, the static compliance, Lm, D2, neutrophil number, levels of KC, MIP-2, and LTB₄ in the BAL

fluid, and TNF- α levels in the lungs were significantly increased in the PPE-treated animals relative to the asthma model animals. However, the eosinophil numbers and the levels of IL-4, IL-5, IL-13, and CysLTs in the BAL fluid were significantly higher in the asthma model than in the PPE-treated emphysema model. Treatment with montelukast significantly attenuated the AHR and eosinophilic airway inflammation in the OVA-sensitized and OVA-challenged asthma model in the present study. Our data are consistent with previous studies showing that CysLT₁R antagonists can reverse the effects of asthma on airway inflammation and airway remodeling (12, 23). The effect and mechanism of action of CysLT₁R antagonists in an animal model of COPD have not been well studied, although the short-term effect of montelukast treatment in stable patients with moderate-to-severe COPD has been studied. Celik and

colleagues (24) reported significant increases in forced vital capacity, FEV₁, visual analog scores, and arterial oxygen pressure at 2 months after treatment, in addition to a significant improvement in the quality of life score. In the sputum samples of the montelukast treatment group, a decrease in neutrophilic activity was evident. In a recent small study, patients with stable COPD received montelukast over a 12-month period and experienced decreased serum levels of LTB₄, IL-8, and TNF- α , reductions in dyspnea and sputum production, and a reduction in the number and duration of hospitalizations (25). Pathologically, COPD is characterized by diffuse inflammation of the lung parenchyma and airways, and it is known that macrophages, recruited neutrophils, and CD8⁺ T cells play important roles in elastolysis and emphysema. The mobilization of these inflammatory cells to the lung and their

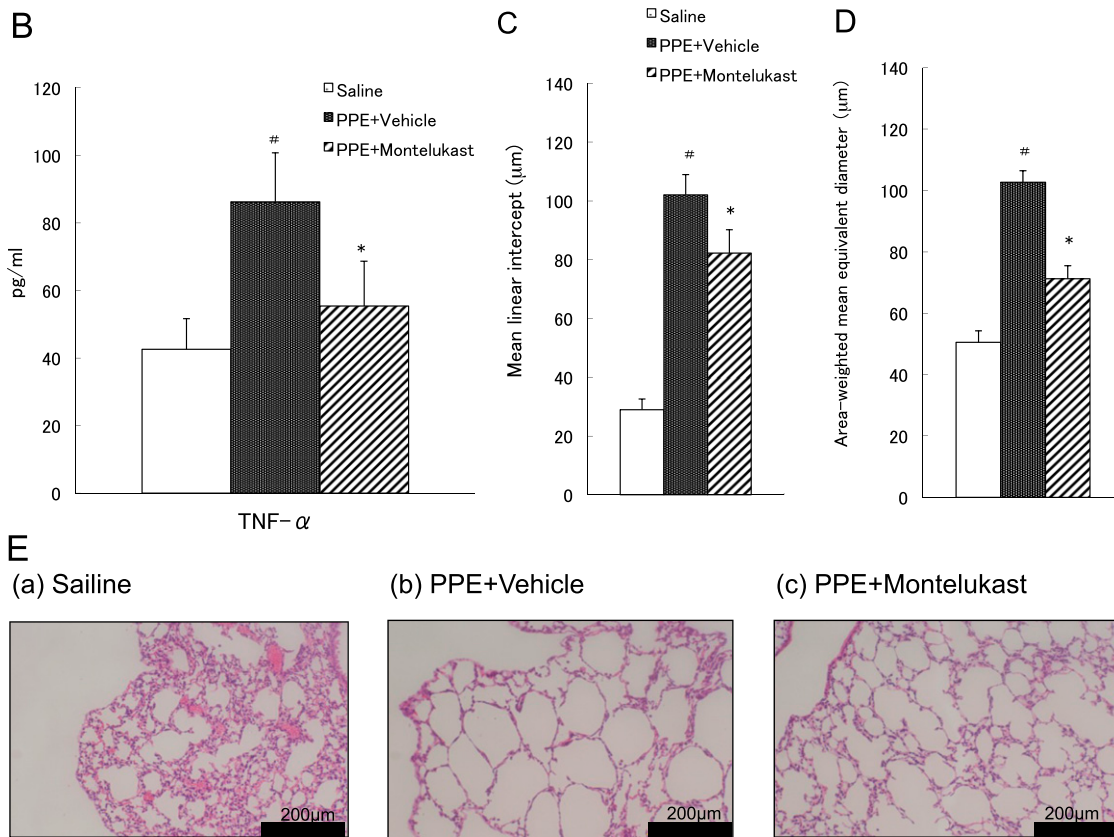


Figure 4. (Continued).

subsequent activation depend on proinflammatory cytokines, such as TNF- α , chemokines, such as IL-8 in humans and KC and MIP-2 in mice, and chemical mediators, such as LTB $_4$ (26–28). We demonstrated that montelukast treatment significantly suppressed static compliance, the number of lymphocytes and neutrophils, the levels of KC, MIP-2, and LTB $_4$ in the BAL fluid, the TNF- α levels in the lung, and the development of lung damage in PPE-treated animals. In addition, after intratracheal PPE instillation into the OVA sensitization and challenge asthma model animals, montelukast significantly suppressed the AHR, the number of neutrophils, eosinophils, and CD4 $^{+}$ and CD8 $^{+}$ T cells, and the levels of Th2 cytokines, chemokines, and LTs in the BAL fluid. Furthermore, montelukast significantly reduced the TNF- α concentration in lung homogenates and also reduced the Lm and D2 in the lungs. These data suggest that a significant reduction in the abundance of various inflammatory cell types in the airways by montelukast treatment may lead to

decreased production of CysLTs, LTB $_4$, and TNF- α in our models. Furthermore, the CysLT $_1$ R-independent mechanism of montelukast, including inhibition of 5-lipoxygenase, may play an important role in the decreased production of CysLTs and LTB $_4$. Recently, Woodruff and colleagues (29) found no evidence to suggest that treatment with a 5-lipoxygenase inhibitor shortened the hospital stay during COPD exacerbation, although this finding was probably affected by an insufficient sample size. Further study is necessary to elucidate the precise molecular mechanism underlying montelukast-mediated effects on LT levels.

Recently, we have shown that the lipid mediator, LTB $_4$, and its interaction with its high-affinity receptor, leukotriene B $_4$ receptor-1 (BLT1), are involved in the migration of antigen-sensitized IL-13-producing effector T cells to the lungs (30, 31). Specifically, the activation of the LTB $_4$ -BLT1 pathway resulted in the recruitment of antigen-specific effector CD8 $^{+}$ T cells to the lungs (32–34); thus, the chemoattraction and activation of

neutrophils through the LTB $_4$ -BLT1 pathway may contribute, at least in part, to allergic airway inflammation in established asthma (14). In patients with COPD, there was a significant correlation between the neutrophil chemotactic activity in exhaled breath condensate (EBC) samples and the number of neutrophils in sputum samples. LTB $_4$ levels were increased in EBC samples from patients with COPD relative to healthy subjects, and an LTB $_4$ receptor antagonist reduced the neutrophil chemotactic activity found in the EBC (35). Attenuation of LTB $_4$ production thus represents a potential therapeutic approach for controlling corticosteroid-insensitive, neutrophil-mediated inflammation (36, 37). In addition, Diamant and colleagues (38) and Calhoun and colleagues (39) showed that LTs induced granulocyte and basophil accumulation in the lung. Nakamura and colleagues (40) reported that LTs cause T cell and B cell infiltration into the pulmonary parenchyma. Whereas recurrent wheezing in asthma is characterized by an increase in the number of CD4 $^{+}$ T cells, mast cells, neutrophils, and activated

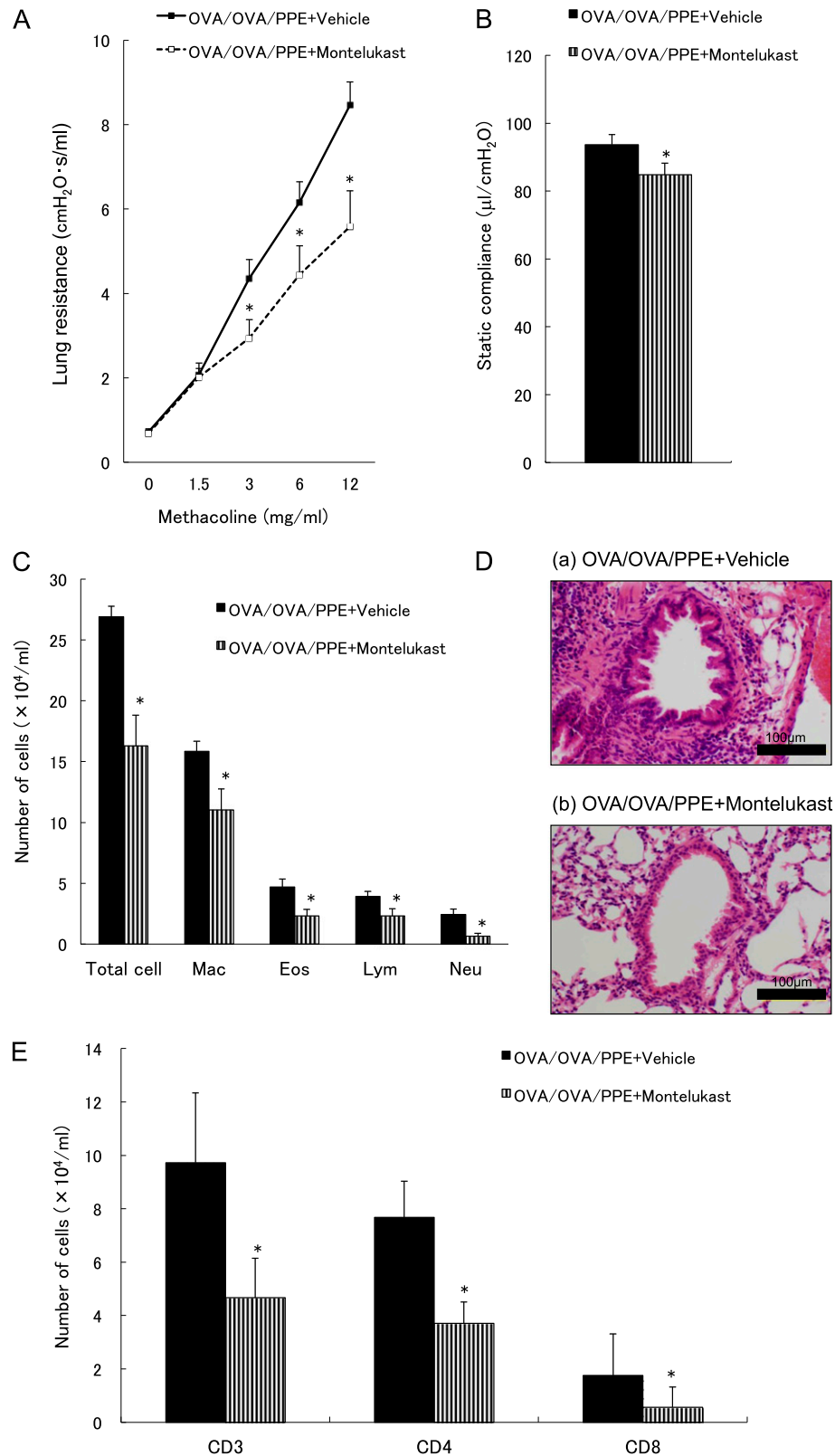


Figure 5. Effect of montelukast on the combined model. AHR (A), static compliance (B), inflammatory cells in the BAL fluid (C), lung histology (D) ([a] OVA/OVA/PPE + Vehicle; [b] OVA/OVA/PPE + Montelukast), and CD3⁺, CD4⁺, and CD8⁺ T cells in the BAL fluid (E). The results for each group are expressed as the mean (± SEM; *n* = 8–12 in each group). *Significant difference (*P* < 0.05) between the OVA/OVA/PPE + Vehicle and OVA/OVA/PPE + Montelukast mice. Eos, eosinophil; Lym, lymphocyte; Mac, macrophage; Neu, neutrophil.

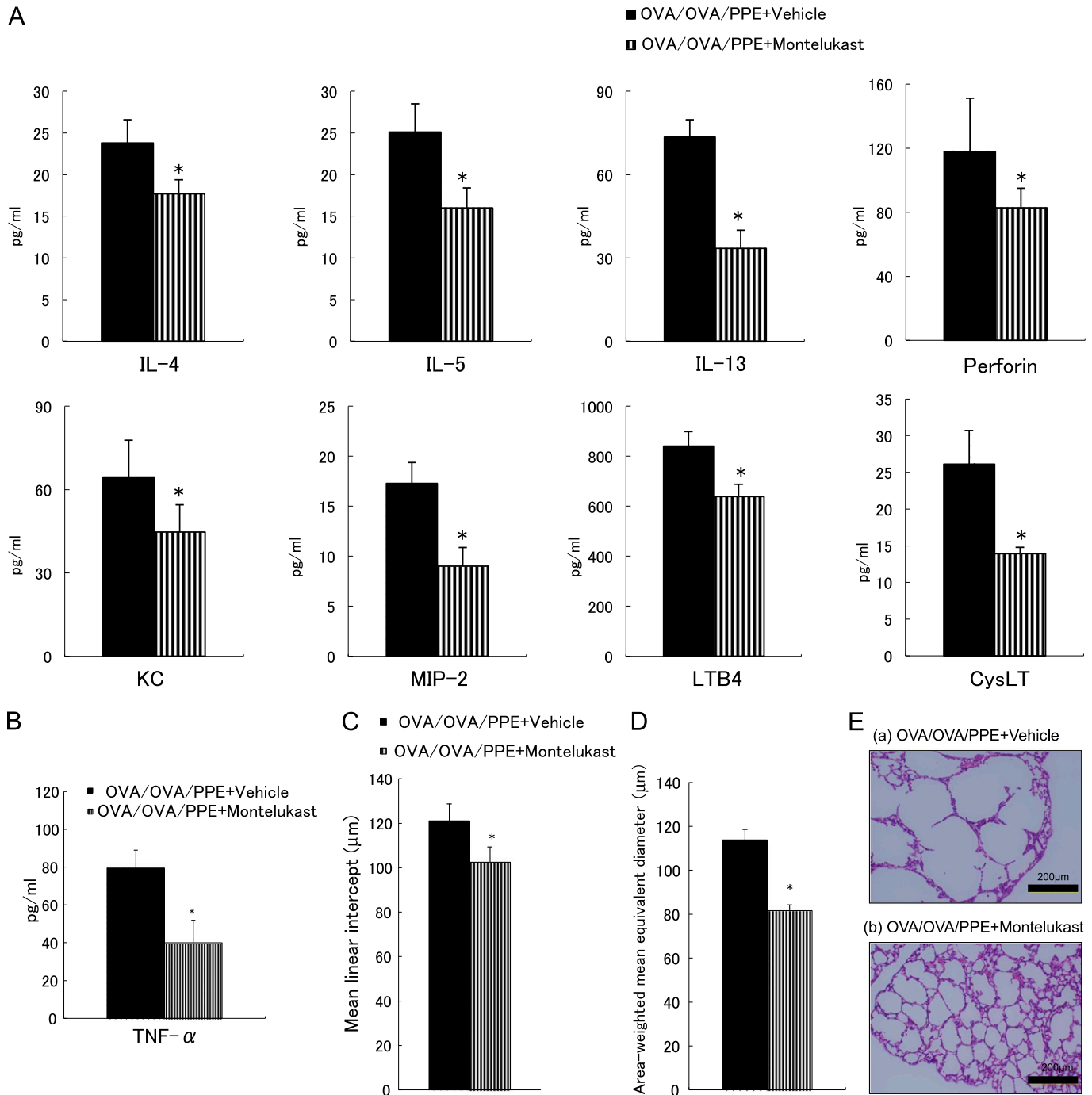


Figure 6. Treatment with montelukast prevents the increase in cytokine, chemokine, LT, and perforin levels in the BAL fluid (A), TNF- α in the lung (B), Lm values (C), D2 (D), and lung histology (E) in the combined model. The results for each group are expressed as the mean ($\pm$ SEM; $n = 8-12$ in each group). *Significant difference ($P < 0.05$) between the OVA/OVA/PPE + Vehicle and OVA/OVA/PPE + Montelukast mice. KC, keratinocyte chemoattractant; MIP, macrophage inflammatory protein.

eosinophils in bronchial biopsies, the pathology of emphysema is associated with an increase in the proportion of mast cells, neutrophils, CD8⁺ T cells, and macrophages in the lung parenchyma (41, 42). These inflammatory cells and

bronchial epithelial cells are the main source of LTs in the lungs (7, 43, 44). Furthermore, in active and passive smokers, an increased number of mast cells, neutrophils, eosinophils, lymphocytes, and macrophages is evident, and activation of

these cells has been shown in the bronchiole and pulmonary parenchyma (45, 46). Anderson and colleagues (14) demonstrated that preincubation of neutrophils with montelukast resulted in a dose-related inhibition of the generation

of reactive oxygen species and of LTB₄ by chemoattractant-activated neutrophils, in addition to a release of elastase by a mechanism involving the inhibition of cyclic nucleotide phosphodiesterases and the cAMP-mediated attenuation of Ca²⁺ influx. These data suggest that one of the mechanisms underlying the action of montelukast may involve neutrophil-directed, cAMP-dependent anti-inflammatory effects, which, in turn, lead to a reduction in neutrophilic airway responses in the PPE-treated model. Additional studies are needed to explore the cAMP-dependent anti-inflammatory effects of montelukast on additional types of inflammatory cells.

Perforin levels in the BAL fluid from OVA-sensitized and OVA-challenged asthma mice, PPE-treated emphysema mice, and OVA-sensitized and OVA-challenged asthma combined with emphysema mice were significantly increased, and treatment with montelukast significantly decreased the levels of perforin in the BAL fluid in the PPE-treated mice in the current study. Perforin, a roughly 70-kD protein, is stored as a monomer in the secretory granules of CD8⁺ T cells. Perforin acts in parallel with granzyme B, which is also stored in the secretory granules of CD8⁺ T cells, to induce apoptosis in target cells. The

percentage of T cells expressing intracellular perforin that were isolated from the BAL fluid of former and current smokers with COPD was reported to be significantly higher than that in BAL fluid from individuals who had never smoked (47). Recently, Shiratsuchi and colleagues (48) showed that the level of perforin in the fluid of the epithelial lining in both the central and peripheral airways, which is a biomarker of CD8⁺ T cell activation, was significantly higher in patients with COPD than in healthy control subjects. Moreover, the perforin level in the peripheral airways was significantly higher than that in the central airways, and was correlated with % FEV₁, FEV₁/forced vital capacity, and the carbon monoxide diffusion capacity of the lung in patients with COPD (48). Chrysafakis and colleagues (49) also reported that the perforin expression in CD8⁺ T cells present in the induced sputum was significantly higher in smokers with COPD than in those without COPD and in healthy nonsmokers. These data suggest that montelukast affects perforin secretion in CD8⁺ T cells, which, in turn, may lead to a reduction in PPE-induced lung damage. Taken together, our findings suggest that montelukast may be a therapeutic agent, as it treats the underlying mechanisms of the disease by

reducing the amount of inflammatory cells (e.g., CD8⁺ T cells) and their derivative inflammatory mediators (e.g., perforin). Mouse models are not predictive of human asthma or smoking-related COPD, but rather offer potential insights into the mechanism of action of montelukast and other basic biological questions. Our results indicate that montelukast may have a broader spectrum of anti-inflammatory activity than is currently thought.

In summary, we have demonstrated that treatment with montelukast attenuates the AHR and eosinophilic and neutrophilic airway inflammation, not only in a PPE-induced emphysema model, but also in an OVA sensitization and challenge model with PPE instillation. In addition, montelukast treatment suppresses the development of PPE-induced lung damage in both the PPE-treated model and the combined model. These data suggest that CysLT₁R antagonists may be effective in the management of emphysema, asthma, and bronchial asthma in combination with emphysema. Future studies are needed to confirm these observations and their long-term efficacy in a human population with emphysema and asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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