A Novel Prostacyclin Agonist Protects against Airway Hyperresponsiveness and Remodeling in Mice

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Airway remodeling in bronchial asthma results from chronic, persistent airway inflammation. The effects of the reversal of airway remodeling by drug interventions remain to be elucidated. We investigated the effects of ONO-1301, a novel prostacyclin agonist with thromboxane inhibitory activity, on the prevention and reversibility of airway remodeling in an experimental chronic asthma model. Mice sensitized and challenged to ovalbumin (OVA) three times a week for 5 consecutive weeks were administered ONO-1301 or vehicle twice a day from the fourth week of OVA challenges. Twenty-four hours after the final OVA challenge, airway hyperresponsiveness (AHR) was assessed, and bronchoalveolar lavage was performed. Lung specimens were excised for staining to detect goblet-cell metaplasia, airway smooth muscle, and submucosal fibrosis. Mice administered ONO-1301 showed limited increases in AHR compared with mice administered the vehicle. The histological findings of airway remodeling were improved in ONO-1301–treated mice compared with vehicle-treated mice. Presumably, these therapeutic effects of ONO-1301 are attributable to the up-regulation of production of hepatocyte growth factor (HGF) in lung tissue, because the neutralization of HGF by antibodies prevented the effects of ONO-1301 on AHR and airway remodeling. Mice administered ONO-1301 showed similar levels of AHR and airway remodeling as mice administered montelukast, a cysteinyl–leukotriene–1 receptor antagonist, and lower levels were observed in mice administered dexamethasone. These data suggest that ONO-1301 exerts the effect of reversing airway remodeling, at least in part through an elevation of HGF in the lungs, and may be effective as an anti-remodeling drug in the treatment of asthma.

Keywords: bronchial asthma; hepatocyte growth factor; montelukast; ONO-1301; prostacyclin

CLINICAL RELEVANCE

Airway remodeling in bronchial asthma results from chronic, persistent airway inflammation and the effects of reversing airway remodeling by drug interventions remain to be elucidated. ONO-1301 has the effect of reversing airway remodeling, at least in part through the elevation of hepatocyte growth factor in the lungs, and may be effective as an anti-remodeling drug in the treatment of asthma.

Bronchial asthma is characterized by recurrent episodes of airway obstruction, airway hyperresponsiveness (AHR) to environmental stimuli, chronic airway inflammation, and structural changes in the airway walls. Those structural changes, referred to as airway remodeling (1), include goblet-cell hyperplasia (2), subepithelial collagen deposition, and smooth muscle hypertrophy (1, 3). Airway remodeling is thought to be the result of persistent airway allergic inflammation, and appears to be irreversible, at least in response to conventional asthma reagents such as inhaled corticosteroids (ICS) (4). In murine models of experimental asthma focusing on the chronic phase, few reagents exert distinct effects on airway remodeling except for a cysteinyl–leukotriene receptor antagonist, montelukast (5, 6).

Prostacyclin is an arachidonic acid–derived mediator that acts on a specific receptor (prostacyclin receptor: IP), and it is known to be a regulator of tissue homeostasis and modulator of inflammatory reactions. Nagao and colleagues demonstrated in a chronic asthma model that IP deficiency augments airway inflammation and airway remodeling, suggesting that prostacyclin may play an important regulatory role in asthma (7). Prostacyclin was reported to induce hepatocyte growth factor (HGF) expression in cultured cells (8), and prostacyclin analogues can attenuate tissue remodeling by inhibiting the release of fibronectin (9).

ONO-1301 is a synthetic prostacyclin agonist without the typical prostanoid structure of a five-member ring and allylic alcohol, making it less easily metabolized. This enhances stability with long-lasting prostacyclin activity when administered *in vivo*. ONO-1301 also has thromboxane-synthase inhibitory activity because of the presence of a 3-pyridine radical. We previously demonstrated that ONO-1301 prevents acute airway inflammation in murine models of allergic asthma through the inhibition of lung dendritic cell functions (10). In the present study, we investigated whether this compound exerted an effect on the alteration of AHR and airway remodeling in a model of chronic asthma. We also compared its effects with those of beraprost (an IP agonist), OKY-046 (a specific thromboxane A_2 synthase inhibitor), montelukast (a cysteinyl–leukotriene–1 receptor antagonist), and dexamethasone. The mechanism of action of ONO-1301 was identified through the modulation of HGF in the development of changes seen with airway remodeling.

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MATERIALS AND METHODS

Animals

Female BALB/c mice, 8 weeks of age and free of murine-specific pathogens, were purchased from CLEA Japan, Inc. (Tokyo, Japan). Animals were housed under specific pathogen-free conditions and maintained on an ovalbumin (OVA)–free diet. All experiments were performed under a protocol for animal experiments approved by the Ethics Committee of Niigata University.

OVA-Induced Chronic Asthma Model and Treatment Protocol

OVA-sensitized mice were challenged with aerosolized OVA for 20 minutes, three times a week for 5 consecutive weeks, for a total of 15 OVA challenges. Twenty-four hours after the final OVA challenge, AHR was assessed, and bronchoalveolar lavage (BAL) fluid, serum, and lungs were obtained for further analyses (Figure 1A). ONO-1301 dissolved in a NaOH/saline solution was administered subcutaneously during the final 2 weeks of the aerosolized OVA challenge protocol.

To examine the importance of HGF on AHR and airway inflammation, some animals receiving ONO-1301 were also administered anti-rat HGF raised in rabbits (Figure 1B). Montelukast was administered subcutaneously, and dexamethasone was administered intraperitoneally (Figure 1C). The details of procedures are provided in the online supplement.

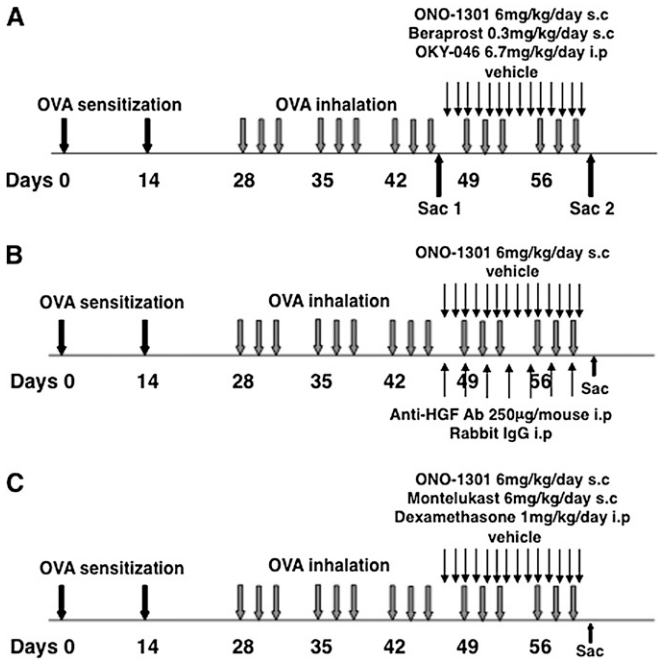

Figure 1. Experimental protocols. (*A*) Mice were sensitized after two intraperitoneal injections of ovalbumin (OVA)/alum, and were then exposed to 3 days of aerosolized OVA challenges/week for 5 consecutive weeks (for a total of 15 OVA challenges). Mice were killed 24 hours after the final challenge (Sac 2). Animals were administered ONO-1301, beraprost, or vehicle every day for the last 2 weeks before they were killed. Some animals were killed 24 hours after receiving nine OVA challenges, and before receiving any treatment (Sac 1). (*B*) To evaluate the importance of hepatocyte growth factor (HGF) in airway hyperresponsiveness (AHR) and airway fibrosis, mice were administered intraperitoneal injections of anti-rat HGF IgG (240 μg/mouse) or rabbit IgG control every other day, concomitant with ONO-1301 treatment. (*C*) To compare the effects of ONO-1301 with those of other treatments, some mice received montelukast or dexamethasone in the final 2 weeks of OVA challenge. Ab, antibody; i.p., intraperitoneal; s.c., subcutaneous.

Determination of AHR

AHR was assessed by measuring changes in respiratory resistance, using the Flexivent system (SCIREQ, Montreal, PQ, Canada) in response to increasing doses of inhaled methacholine (MCh), as previously reported (10).

BAL Fluid and Measurement of Cytokine Concentrations in BAL Fluid

Immediately after the measurement of AHR, BAL was performed via a tracheal tube, as previously described (11).

Supernatants from the BAL fluid were used for the measurement of cytokines and growth factors. The details of procedures are provided in the online supplement.

Histology and Immunohistochemistry Staining

Left lungs were fixed in 10% formalin and immersed in paraffin. After they were deparaffinized, samples were stained with periodic acid–Schiff (PAS) and Masson trichrome for histological analysis. Right lungs were fixed at 4°C in periodate-lysine–paraformaldehyde and embedded in OCT compound, frozen in dry ice–acetone, and cut on a cryostat for the detection of α-smooth muscle actin (α-SMA) or HGF expression. The details of procedures are provided in the online supplement.

Western Blot Analysis for HGF

Tissue lysates of right lungs were subjected to 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes, and immunostained with the anti-HGF. The details of procedures are provided in the online supplement.

RNA Isolation and Quantitative Real-Time PCR

Total RNA was isolated from the homogenized lung tissue, and cDNA was generated by reverse transcription. The primers of murine HGF mRNA (forward primer, 5′-agaaatgcagtcagcaccatcaag-3′; reverse primer, 5′-gatggcacatccacgaccag-3′) were used in a real-time PCR. The results determined the relative expression of HGF to glyceraldehyde 3–phosphate dehydrogenase mRNA in each treatment group. The details of procedures are provided in the online supplement.

Statistical Analysis

Mann-Whitney U tests were used to determine levels of difference between all groups. Comparisons for all pairs were performed using the Kruskal-Wallis test. Significance was assumed at $P < 0.05$ for all tests. Values for all measurements are expressed as means ± SEM.

RESULTS

Effect of ONO-1301 on AHR, Airway Inflammation, and Airway Remodeling

Sensitized mice were challenged nine times with OVA and developed AHR to MCh in a dose-dependent manner. AHR was sustained at similar levels in mice sensitized and challenged 15 times with OVA. Mice administered ONO-1301 showed limited increases in AHR throughout the MCh dose–response curve, compared with mice administered vehicle or mice not receiving vehicle (Figure 2A). Mice administered beraprost also showed lower AHR at the higher dose of MCh (12.5 mg/ml) compared with mice administered vehicle, but to a lesser degree compared with those administered ONO-1301, whereas AHR was little affected in mice administered OKY-046.

The number of inflammatory cells in BAL fluid was determined 24 hours after the final allergen challenge. The number of inflammatory cells and eosinophils in mice sensitized with OVA and challenged nine times was much higher than in mice challenged 15 times. Although the administration of ONO-1301

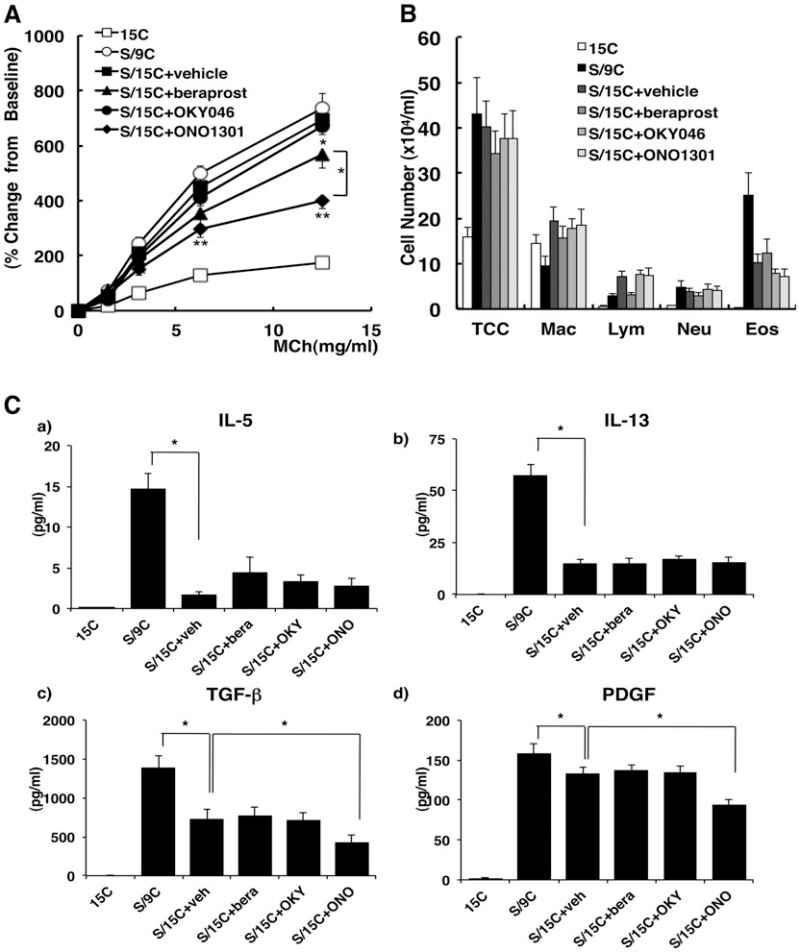

Figure 2. Comparison of the effects of the study drugs on the development of AHR and inflammation. We studied AHR and airway inflammation in nonsensitized mice with 15 OVA challenges (15C), in OVA-sensitized mice with nine OVA challenges (S/9C), and in OVA-sensitized mice with 15 OVA challenges (S/15C) + vehicle, beraprost, OKY-046, or ONO-1301 (S/15C + vehicle, S/15C + beraraprost, S/15C + OKY046, and S/15C + ONO1301, respectively). (*A*) Changes in airway resistance with increased concentrations of nebulized methacholine (MCh). (*B*) Changes in cell composition in bronchoalveolar lavage (BAL) fluid. (*C*) Changes in concentrations of cytokines IL-5, IL-13, transforming growth factor–β (TGF-β), and platelet-derived growth factor (PDGF) in BAL fluid. (*a*) IL-5. (*b*) IL-13. (*c*) TGF-β. (*d*) PDGF. TCC, total cell count; Mac, macrophages; Lym, lymphocytes; Neu, neutrophils; Eos, eosinophils. $^*P < 0.05$ or $^{**}P < 0.01$, compared with the S/15C + vehicle group, or as indicated. Data represent mean ± SEM for $n = 12$ per group.

tended to attenuate the increase in eosinophil counts, statistical significance was not achieved in the total or differential cell counts between vehicle-treated, ONO-1301–treated, beraprost-treated, or OKY-046–treated groups (Figure 2B). The concentrations of IL-5 and IL-13 in BAL fluid were not significantly different among the four groups (vehicle, beraprost, OKY-046, or ONO-1301). However, the concentrations of transforming growth–β (TGF-β) and platelet-derived growth factor (PDGF) in BAL fluid from the ONO-1301–treated group were lower when compared with the other three groups. No statistically significant differences were evident in BAL fluid TGF-β and PDGF concentrations between beraprost-treated and vehicle-treated mice.

The presence of goblet-cell metaplasia, one characteristic of chronic asthma, can be easily identified by the PAS staining of mucus glycoproteins. Mice sensitized and challenged nine times with OVA developed goblet-cell metaplasia, which was sustained to a lesser degree in mice sensitized and challenged 15 times with OVA. The ONO-1301–treated group showed decreased numbers of airway PAS^+ cells compared with the vehicle-treated group (Figures 3A and 3D). Similar results were found when comparing the beraprost-administered group with the vehicle-treated group, but to a lesser extent than in the ONO-1301 group. In addition to goblet-cell metaplasia, there was a similar level of thickness of smooth muscle stained with anti–α-SMA antibody and bronchial submucosal collagen deposition stained with Masson trichrome in mice sensitized and challenged nine times with OVA and in mice challenged 15 times with OVA. ONO-1301 dramatically reduced airway smooth muscle hypertrophy and collagen deposition in the bronchial submucosal area, compared with the vehicle group (Figures 3B–3D). Beraprost also reduced airway smooth muscle hypertrophy and collagen deposition, but to a significantly lesser extent than did ONO-1301 (Figures 3B–3D).

ONO-1301 Alters the Expression of HGF in Lungs of Repetitively Challenged Mice

Based on previous findings that ONO-1301 is potent inducer of HGF in cultured fibroblasts (12, 13), we hypothesized that differences in the levels of HGF expression in the lung were at the root of the attenuation of AHR and airway remodeling in ONO-1301–treated mice. We first evaluated the expression of HGF mRNA by real-time PCR in lung homogenates and the concentrations of HGF by ELISA in BAL fluid. We found an up-regulation of both HGF mRNA concentrations and concentrations of HGF in the lung in the mice administered ONO-1301, compared with those concentrations in animals administered vehicle or beraprost (Figures 4A and 4B). Immunohistochemical analysis of lung samples stained with anti-HGF showed that ONO-1301 treatment was associated with higher concentrations of HGF compared with mice that received vehicle or beraprost (Figure 4C). These results were confirmed by Western blot analysis (Figure 4D). To further elucidate the role of HGF in ONO-1301–administered mice, anti-HGF was administered to mice in the same term of ONO-1301 administration, to neutralize the activity of HGF. The administration of anti-HGF restored

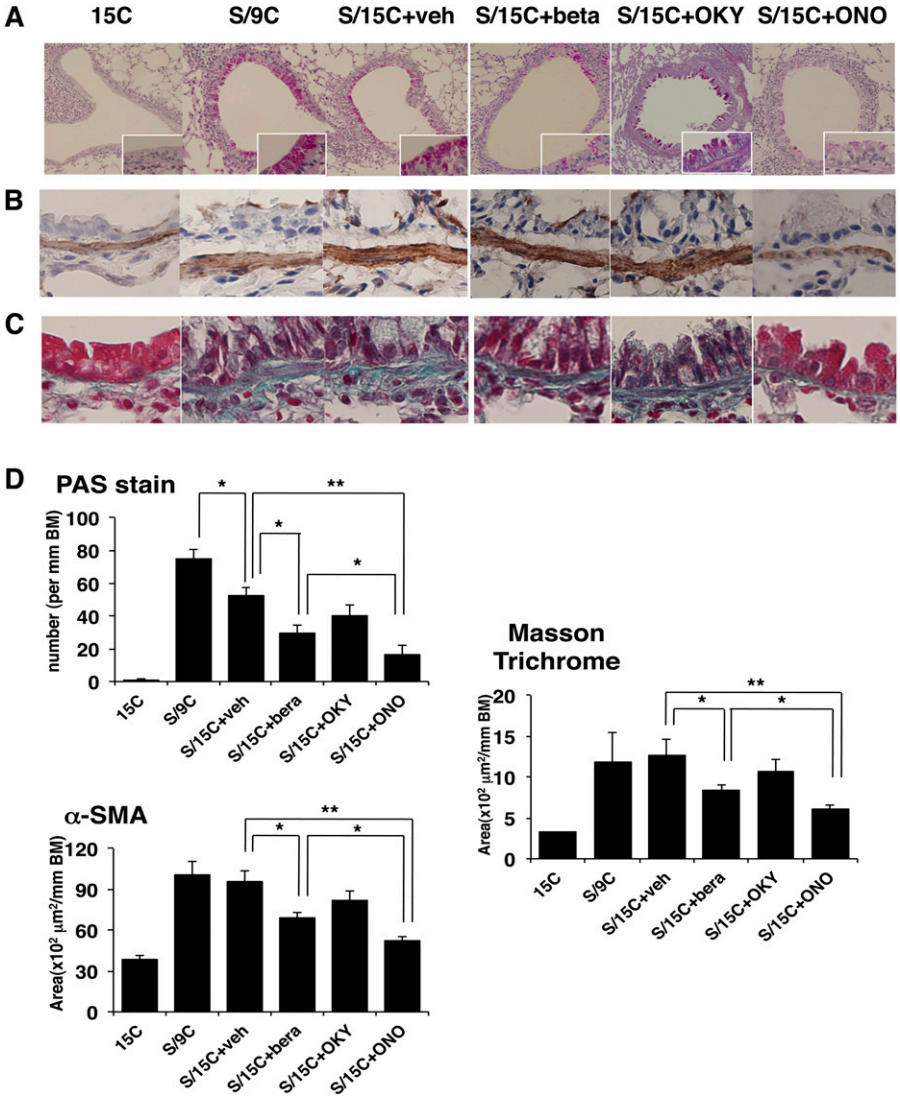

Figure 3. Comparison of the effects of the study drugs on features of airway remodeling. Representative periodic acid–Schiff (PAS) staining (*A*), immunohistochemical staining of the airway smooth muscle cells for α–smooth muscle actin (α-SMA) (*B*), and Masson trichrome staining (*C*) of lung sections (original magnification, ×100; *insets*, ×400 in PAS staining; ×400 in α-SMA and Masson trichrome staining) were obtained 24 hours after the final challenge from nonsensitized animals with 15 OVA challenges (15C), animals sensitized and challenged nine times with OVA (S/9C), 15 OVA challenges + vehicle (S/15C + veh), 15 OVA challenges + beraprost (S/15C + bera), 15 OVA challenges + OKY-046 (S/15C + OKY), and 15 OVA challenges + ONO-1301 (S/15C + ONO). (*D*) Quantitative analysis of PAS-positive cells, α-SMA layer, and peribronchiolar collagen deposition in bronchial tissue. Data represent means ± SD from three independent experiments ($n = 9$). *$P < 0.05$ or **$P < 0.01$ between the groups indicated. BM, basement membrane.

the development of AHR to vehicle-treated levels, and increased the total number of inflammatory cells and eosinophils in BAL fluid (Figures 5A and 5B). In addition, goblet-cell metaplasia, smooth muscle hypertrophy, and submucosal collagen deposition, which were reduced in the lungs of mice administered ONO-1301, developed in mice that received anti-HGF together with ONO-1301 (Figures 5C and 5D). These data suggest that HGF plays a pivotal role in the amelioration of AHR and airway remodeling in ONO-1301–treated mice in this model of chronic asthma.

Comparison of ONO-1301 Treatment with Other Therapies

To date, the reversal of the effects of allergen-induced airway remodeling in a chronic asthma model has only been observed with montelukast (6). To determine the ameliorative effects on AHR and airway remodeling with ONO-1301, we compared the effects of ONO-1301, montelukast, and dexamethasone. All three groups showed lower levels of AHR at the higher dose of MCh (12.5 mg/ml), compared with the vehicle group. Mice administered dexamethasone showed higher levels of AHR compared with the ONO-1301 group. Even though AHR tended to be lower in the ONO-1301 group compared with the montelukast group, no statistically significant difference was evident (Figures 6A and 6B). Similarly, the features of airway remodeling showed amelioration to a striking degree in the ONO-1301–treated and montelukast-treated groups, and to a lesser degree in the dexamethasone-treated group (Figures 6C and 6D).

DISCUSSION

In the present study, we show that a novel prostacyclin agonist, ONO-1301, was able to interfere with the development of increased levels of AHR and airway remodeling after repetitive allergen challenges in an experimental model of chronic asthma. ONO-1301 also proved to be as effective as another antiasthma compound, montelukast, which was previously shown to be effective in a similar model (5, 6). We also demonstrated that in this model, the beneficial effects of ONO-1301 were, at least in part, attributable to the increased expression of HGF.

Prostacyclin is known to be associated with the suppression of airway remodeling in experimental asthma models, and is known to be a modulator of Th2-mediated inflammation. A deficiency of IP, a specific prostacyclin-binding receptor, was shown to enhance and sustain allergic inflammation through repeated challenges for 3 weeks, and to result in airway remodeling (7, 14).

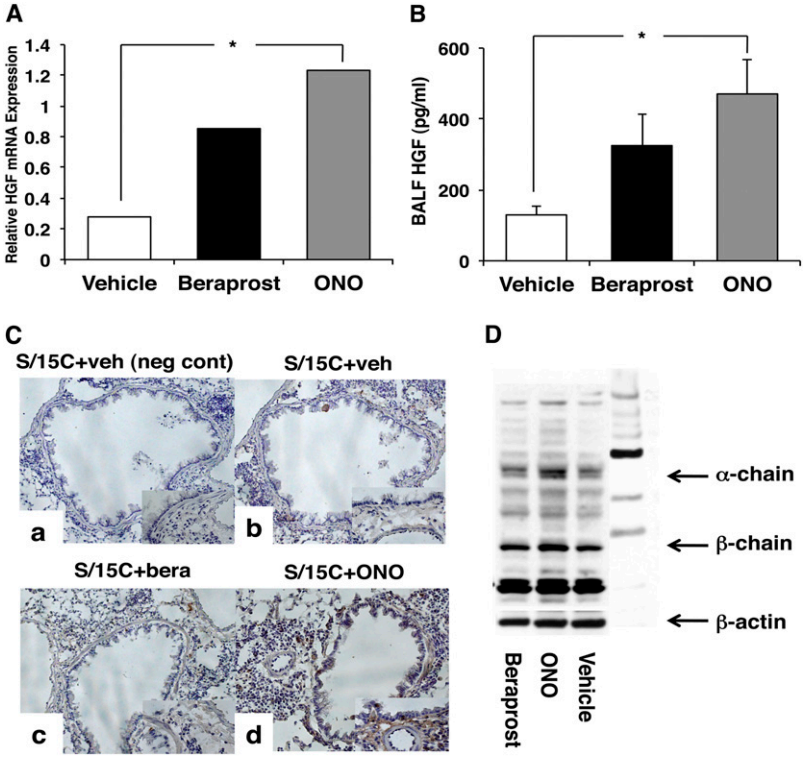

Figure 4. Expression of HGF in lung tissue. (*A*) HGF mRNA expression in the lung. Total RNA was prepared from the lung, and real-time PCR analysis for HGF mRNA was performed. Vehicle refers to sensitized animals with 15 OVA challenges (S/15C) + vehicle. Beraprost refers to S/15C + beraprost. ONO refers to S/15C + ONO-1301. (*B*) Concentrations of HGF in BAL fluid. (*C*) Example of immunohistochemical staining of HGF in lung tissue. (*a*) S/15C + Veh (neg cont), negative control for HGF in a sample of OVA-sensitized/15 OVA challenges (S/15C) + vehicle. (*b*) S/15C + veh, S/15C + vehicle. (*c*) S/15C + bera, S/15C + beraprost. (*d*) S/15C + ONO, S/15C + ONO-1301. (*D*) Western Blot analysis of HGF in the lung. After the protein was harvested from lung tissue, lysates were transferred to membranes and immunostained with anti-HGF antibody. See Materials and Methods for details. *$P < 0.05$, between the groups indicated. BALF, bronchoalveolar lavage fluid.

Several mechanisms altering Th2-mediated inflammation have been reported. Zhou and colleagues described the inhibition of Th1 and Th2 cytokine production from CD4 T cells (15). Activation of the prostacyclin–IP receptor system was important in regulating Th2-mediated airway inflammation through the inhibition of Th2 cell recruitment in mice (16). Prostacyclin can also modulate the function of dendritic cells (17, 18). However, the mechanism underlying the inhibition of airway remodeling induced by prostacyclin administration remains unclear. In this study, we focused on the role of HGF and its elevated concentrations in the lungs after ONO-1301 administration.

HGF, a pleiotropic factor, is known to regulate diverse biological responses, including cell proliferation, survival, migration, and differentiation in different organs. Evidence is increasing that HGF plays an essential role in parenchymal repair and protection. HGF has been shown to antagonize, *in vitro*, the profibrotic action of TGF-β, such as the expression of α-SMA, collagen type 1, and fibronectin in rat alveolar epithelial cells (19). Recent studies suggest that both endogenous and exogenous HGF are protective against the onset and progression of different models of chronic disease (e.g., renal, cardiac, and liver) (20–22). Yaekashiwa and colleagues reported that exogenous HGF prevented the progression of lung fibrosis induced by bleomycin (23), and Ito and colleagues reported that the administration of exogenous HGF significantly decreased tissue fibrosis, remodeling, and dysfunction by suppressing the production of the growth factors TGF-β, PDGF, and nerve growth factor (NGF) in a chronic asthma model (24). In the present study, we found that ONO-1301 attenuated the development of AHR and reversed the histological findings of airway remodeling in association with an increase in HGF production in the lungs. These antifibrotic effects of ONO-1301 were diminished by the administration of anti-HGF, suggesting that the effects of the compound were mediated, at least in part, through HGF induction.

The mechanisms whereby ONO-1301 induced the production of HGF have yet to be elucidated. Morishita and colleagues reported that prostacyclin stimulated local vascular HGF production from *in vitro* cultured vascular smooth muscle cells (VSMCs) and endothelial cells (25). Nakamura and colleagues reported that the production of HGF by ONO-1301 was augmented in a cyclic adenosine monophosphate (cAMP)–dependent manner, and that the administration of a cAMP inhibitor, Rp-8-Br-cAMP (Rp–cAMP), inhibited the effect of ONO-1301 (12). We determined the concentrations of cAMP in serum, and showed that the administration of ONO-1301 did indeed elevate cAMP concentrations, perhaps accounting for the increased production of HGF in this system (data not shown).

Interestingly, the administration of anti-HGF did not fully inhibit the benefits of ONO-1301. Prostacyclin itself exhibits suppressive effects on the growth of smooth muscle cells by suppressing the activation of extracellular regulated kinase through cAMP, as well as by the induction of endogenous HGF (26). Based on these data, ONO-1301 may also exhibit effects through an alternative therapeutic pathway other than the induction of endogenous HGF.

ONO-1301 also exhibits potent thromboxane A_2 (TxA_2) synthase inhibitory activity. However, in the group administered OKY-046, a TxA_2 synthase inhibitor, AHR and airway remodeling were unchanged, compared with the vehicle group. In a previous report, OKY-046 was not found to be a major contributor to the control of AHR and airway allergic inflammation in a model of acute asthma, although the concentrations of TxB_2 in BAL fluid were significantly decreased (10). Because ONO-1301 suppressed AHR and airway remodeling features more effectively than beraprost, this suggested that the additional TxA_2 inhibition seen with ONO-1301 might synergistically enhance the beneficial effects on allergic airway inflammation. Alternatively, Itoh and colleagues reported that selective TxA_2 synthase inhibitors reduced the production of TxA_2 from prostaglandin H2 (PGH_2), and accelerated the production of prostacyclin and prostaglandin E2 (PGE_2) (27). Taken together, the present findings suggest that the TxA_2 synthase inhibitory activity

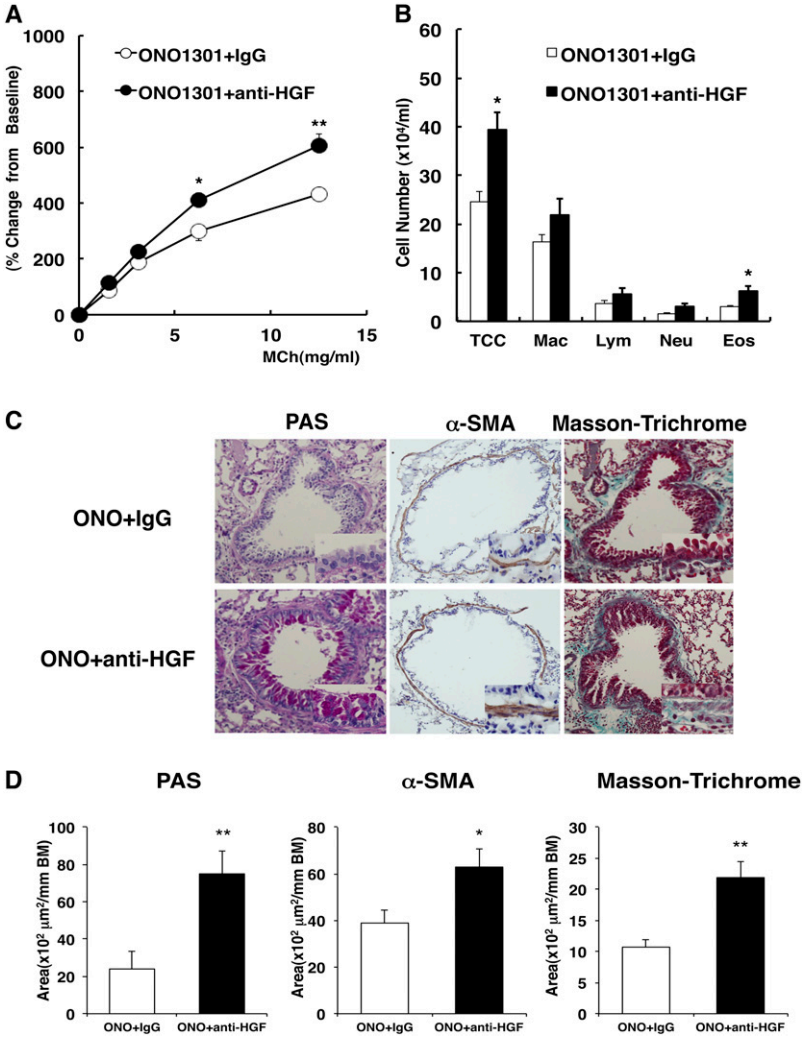

Figure 5. Effects of administration of anti-HGF together with ONO-1301 on AHR and airway fibrosis. (*A*) Changes in airway resistance in response to increased concentrations of nebulized MCh. ONO-1301 + IgG, sensitized animals with 15 OVA challenges + ONO-1301 and coadministration of control rabbit IgG; ONO-1301 + anti-HGF, sensitized animals with 15 OVA challenges + ONO-1301 and coadministration of anti-HGF antibody. (*B*) Changes in cell composition in BAL fluid. TCC, total cell count; Mac, macrophages; Lym, lymphocytes; Neu, neutrophils; Eos, eosinophils. $^{*}P < 0.05$ or $^{**}P < 0.01$, compared with ONO-1301 + IgG group. Data represent means ± SEM for $n = 6$ per group. (*C*) Representative periodic acid–Schiff (PAS) staining, immunohistochemical staining of airway smooth muscle cells for α–smooth muscle actin (α-SMA), and Masson trichrome staining of lung sections (original magnification, ×100; *insets*, ×400 in PAS staining; ×400 in α-SMA and Masson trichrome staining) were obtained 24 hours after the final challenge from animals sensitized with 15 OVA challenges + ONO-1301 and the coadministration of control rabbit IgG (ONO + IgG), or the coadministration of anti-HGF antibody (ONO + anti-HGF). (*D*) Quantitative analysis of PAS-positive cells, α-SMA layer, and peribronchiolar collagen deposition in bronchial tissue. Data represent means ± SD from three independent experiments ($n = 6$). $^{*}P < 0.05$ or $^{**}P < 0.01$, compared with the ONO + IgG group. BM, basement membrane.

of ONO-1301 may have amplified the effects of prostacyclin through the up-regulation of production.

In the present study, we compared the effects of ONO-1301 on AHR and airway remodeling to those of dexamethasone and the cysteinyl–leukotriene (CysLT)–1 receptor antagonist, montelukast. Montelukast is the only drug with demonstrable efficacy on airway remodeling in an established airway fibrosis model (5, 6). Our data suggest that ONO-1301, similar to montelukast, has the potential to reverse AHR and features of remodeling such as goblet-cell metaplasia, submucosal collagen deposition, and smooth muscle hypertrophy. CysLTs are well established to play an important role in bronchoconstriction and airway eosinophilia after allergen challenge, in nocturnal asthma, and in exercise-induced bronchospasms (28–31). Several studies indicated that CysLTs also play a role in the pathophysiology of remodeling (32). *In vitro* studies have shown that leukotriene D4 (LTD4) augments epidermal growth factor–induced human airway smooth muscle proliferation, and that leukotriene C4 (LTC4) up-regulates the expression and synthesis of collagenase in human lung fibroblasts (33, 34). Despite these reports, the precise mechanism of montelukast in the modification of airway remodeling remains to be determined. Our preliminary data show that AHR to methacholine, the composition of inflammatory cells in BALF, and the extent of PAS staining in the bronchus were not changed significantly by the administration of anti-HGF antibody in montelukast-treated mice, at least in our protocol (data not shown). Building on these data, details about the roles of cysteinyl leukotrienes, prostacyclin, and HGF for the establishment of airway remodeling remain to be elucidated in the near future.

In our data, dexamethasone was less effective at suppressing AHR to methacholine and the features of airway remodeling compared with ONO-1301 or montelukast (Figures 6A–6D). Henderson and colleagues demonstrated that montelukast, but not dexamethasone, reversed the established increase in airway smooth muscle mass and subepithelial collagen deposition (6). Christie and colleagues reported that the treatment of OVA-sensitized/challenged mice with dexamethasone reduced the airway expression of laminin and laminin-1 receptor, but not AHR to methacholine, through noninvasive plethysmography (35). In a rat model of chronic asthma, the increased fibronectin deposition that is one of strong features of airway remodeling persisted after the final allergen inhalation, and was not reversed by fluticasone treatment (36). Thus, the effect of reversing established airway remodeling by steroid is known to be difficult in animal or human models (4). The clinical relevance of remodeling and the therapeutic modalities controlling these features of asthma remain to be defined.

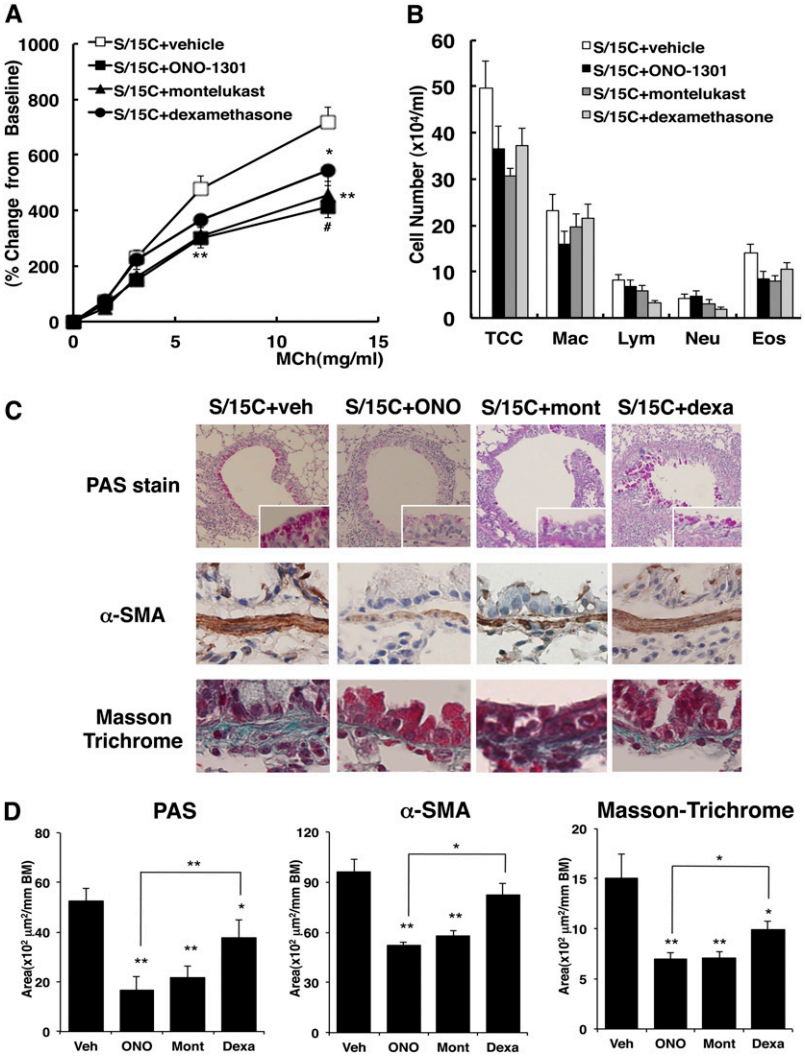

Figure 6. Comparison of ONO-1301 with other drugs on AHR and airway fibrosis. (*A*) Changes in airway resistance to increased concentrations of nebulized MCh. S/15C + vehicle, sensitized animals with 15 OVA challenges + vehicle; S/15C + ONO-1301, sensitized animals with 15 OVA challenges + ONO-1301; S/15C + montelukast, sensitized animals with 15 OVA challenges + montelukast; S/15C + dexamethasone, sensitized animals with 15 OVA challenges + dexamethasone. (*B*) Changes in cell composition in BAL fluid. TCC, total cell count; Mac, macrophages; Lym, lymphocytes; Neu, neutrophils; Eos, eosinophils. Data represent means ± SEM for $n = 8$ per group. (*C*) Representative periodic acid–Schiff (PAS) staining, immunohistochemical staining of airway smooth muscle cells for α smooth muscle actin (α-SMA), and Masson trichrome staining of lung sections (original magnification, ×100; *insets*, ×400 in PAS staining; ×400 in α-SMA and Masson trichrome staining) were obtained from animals 24 hours after the final challenge. S/15C + veh, animals sensitized with 15 OVA challenges + vehicle; S/15C + ONO, animals sensitized with 15 OVA challenges + ONO-1301; S/15C + mont, animals sensitized with 15 OVA challenges + montelukast; S/15C + dexa, animals sensitized with 15 OVA challenges + dexamethasone. (*D*) Quantitative analysis of PAS-positive cells, α-SMA layer, and peribronchiolar collagen deposition in bronchial tissue. Data represent means ± SD from three independent experiments ($n = 6$). $^{*}P < 0.05$ or $^{**}P < 0.01$, compared with the vehicle group or as indicated. $^{\#}P < 0.05$, compared with the dexamethasone group. BM, basement membrane; Dexa, dexamethasone; Mont, montelukast; Veh, vehicle.

In the present model, AHR to methacholine was still sustained, although signs of airway inflammation, such as airway eosinophilia or Th2 cytokine elevation in BAL fluid, diminished. Previous reports stated that prolonging allergen exposure in sensitized animals was associated with the disappearance of inflammatory changes (37–39). In the present study, the administration of ONO-1301, initiated after nine challenges with OVA inhalation, did not appear to be effective at ameliorating airway allergic inflammation, because no differences were evident in the BAL fluid between the vehicle group and the ONO-1301 group (Figures 2B and 2C). However, in our previous report about an acute model of airway allergic inflammation, the administration of ONO-1301 in the OVA challenge phase suppressed AHR to methacholine and airway eosinophilia and Th2 cytokine production (10). In our preliminary data, the administration of ONO-1301 in conjunction with OVA challenges for 7 consecutive days also suppressed AHR to methacholine and airway allergic inflammation (data not shown). Based on these data, ONO-1301 may play some roles in modulating airway inflammation, even in the current protocol.

In conclusion, ONO-1301, a prostacyclin agonist with TxA_2 synthase inhibitory activity, suppressed AHR to inhaled MCh and reduced features of airway remodeling such as goblet-cell metaplasia, airway smooth muscle hypertrophy, and submucosal collagen deposition. The administration of anti-HGF reduced the benefits of ONO-1301 on AHR and airway remodeling. ONO-1301 demonstrated comparable effects to those of montelukast, and greater efficacy than dexamethasone. The data suggest that ONO-1301 has the important potential to suppress the development of airway remodeling, at least in part, through an elevation of HGF in the lungs.

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