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ORIGINAL ARTICLE **Experimental Models of Allergic Disease**

A prostacyclin agonist with thromboxane inhibitory activity for airway allergic inflammation in mice

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Summary

Background ONO-1301 is a novel drug that acts as a prostacyclin agonist with thromboxane A_2 (TxA_2) synthase inhibitory activity. We investigated the effect of ONO-1301 on development of airway allergic inflammation.

Methods Mice sensitized and challenged to ovalbumin (OVA) received ONO-1301, OKY-046 (TxA_2 synthase inhibitor), beraprost, a prostacyclin receptor (IP) agonist, ONO-1301 plus CAY10449 (selective IP antagonist) or vehicle during the challenge period. Twenty-four hours after the OVA challenge, airway hyperresponsiveness (AHR) to methacholine was assessed and bronchoalveolar lavage was performed. Lung specimens were excised for goblet cell staining and analysis of lung dendritic cells (DCs). Bone marrow-derived dendritic cells (BMDCs) were generated, in the presence or absence of drugs, for analysis of DC function.

Results Mice that received ONO-1301 showed significantly lower AHR, airway eosinophilia, T-helper type 2 cytokine levels, mucus production and lung DCs numbers than vehicle-treated mice. These effects of ONO-1301 were mostly reversed by CAY10449. BMDCs treated with ONO-1301 alone showed lower DC functions, such as expression of costimulatory factors or stimulation to spleen T cells.

Conclusions These data suggest that ONO-1301 may suppress AHR and airway allergic inflammation through modulation of DCs, mainly mediated through the IP receptor. This agent may be effective as an anti-inflammatory drug in the treatment of asthma.

Keywords dendritic cell, ONO-1301, prostacyclin, thromboxane synthase inhibitor
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Introduction

Arachidonic acid released from membrane phospholipids by phospholipase A2 is converted to various metabolites that play important roles in asthma [1]. Prostacyclin, a potent vasodilator and inhibitor of platelet aggregation, is also a major product of the cyclooxygenase pathway. There are several reports that describe the anti-inflammatory effect of prostacyclin using prostacyclin receptor (IP)-deficient mice [2, 3]. Idzko et al. [4] demonstrated that the prostacyclin analog, iloprost, inhibited characteristic T-helper type 2 (Th2)-mediated features such as eosinophil infiltration in the airways, Th2 cytokine elevation and airway hyperresponsiveness (AHR), by altering the function of lung dendritic cells (DCs).

Thromboxane A_2 (TxA_2), produced from arachidonic acid through the cyclooxygenase pathway, is considered to be an aggravating factor for bronchoconstriction in asthma [5]. In addition, the levels of TxA_2 in the plasma, bronchoalveolar lavage fluid (BALF) and urine from patients with bronchial asthma have been shown to be elevated [6]. In addition to affecting AHR, TxA_2 seems to play an important role in airway allergic inflammation associated with Th2 lymphocyte and eosinophil infiltration, because the TxA_2 synthase inhibitor, OKY-046, inhibited eosinophil accumulation and the production of Th2 cytokines in a mouse model [7].

Recently, we developed a new prostacyclin agonist, ONO-1301 (Fig. 1), which has long-lasting prostacyclin activity and an inhibitory effect on thromboxane synthase

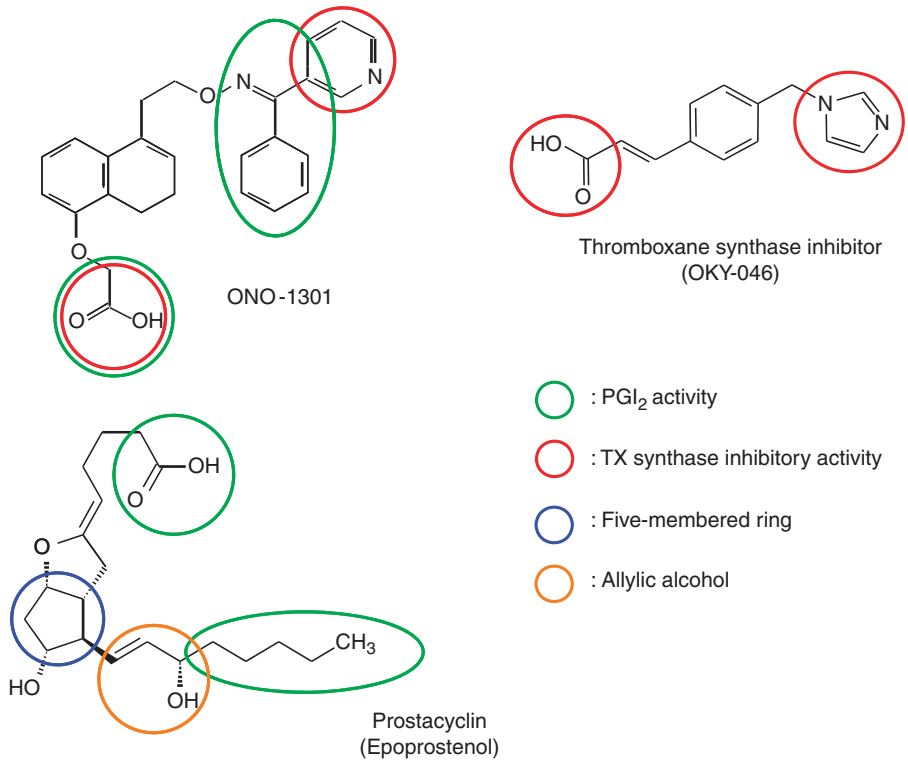


Fig. 1. Chemical structures of ONO-1301, epoprostenol (prostacyclin analog), and OKY-046 (thromboxane synthase inhibitor). Epoprostenol shares common characteristics with prostanoid structures, including a five-membered ring and an allylic alcohol (blue and yellow circles, respectively). In contrast, ONO-1301 has a carboxylic acid and a lipid-soluble functional group that activates the prostacyclin receptor (green circles), but does not have prostanoid structures, which allows long-lasting prostacyclin activity. Unlike epoprostenol, ONO-1301 has thromboxane synthase-inhibitory activity because of a 3-pyridine radical and carboxylic acid within its molecule (red circles), similar to OKY-046.

[8]. ONO-1301 does not contain prostanoid structures, such as a five-membered ring or allylic alcohol, which are digested by 15-hydroxyprostaglandin dehydrogenase (Fig. 1). Because ONO-1301 does not have prostanoid structures, it is not easily metabolized and its effects remain for a longer time compared with other prostacyclin analog, such as beraprost or iloprost. Previously, ONO-1301 has been shown to be effective in a rat pulmonary arterial hypertension (PAH) model [8, 9] and a murine bleomycin-induced pulmonary fibrosis model in mice [10].

In the present study, we investigated the effects of ONO-1301 on airway allergic inflammation in mice. In addition, we compared the effects of OKY-046, a thromboxane synthase inhibitor, IP agonist beraprost or IP antagonist CAY10449 to elucidate the underlying mechanisms responsible for the beneficial effects of this compound.

Materials and methods

Animals

Female BALB/c mice were purchased at 6–8 weeks of age from CLEA Japan Inc. (Tokyo, Japan). Animals were housed under specific pathogen-free conditions and maintained on an ovalbumin (OVA)-free diet. All animal experiments were conducted with the approval of the Niigata University ethics committee for animal experiments.

Ovalbumin-induced allergic airway inflammation

Mice were sensitized on days 0 and 14 by an intraperitoneal (i.p.) injection of 20 mg of OVA premixed with 2.25 mg of $Al(OH)_3$ in 100 μL of PBS. After sensitization, animals were exposed to aerosolized OVA (1% in saline) for 20 min on days 28, 29 and 30. ONO-1301 and OKY-046 (6.7 mg/kg/day), a specific TxA_2 synthase inhibitor, and beraprost (0.3 mg/kg/day), an IP agonist, were dissolved in 100 mL of saline or vehicle and administered subcutaneously twice a day for 4 consecutive days starting from day 27. CAY10449 (200 μg/day), a selective IP antagonist, dissolved in DMSO and diluted with saline, was subcutaneously administered once a day for 4 consecutive days from day 27. Twenty-four hours after the last OVA challenge, AHR was assessed and BAL fluid, serum and lungs were obtained for further analysis.

Airway responsiveness

Airway responsiveness to aerosolized methacholine was assessed by measuring changes in respiratory resistance

(R) and compliance (C) using the Flexivent system (SCIREQ, Montreal, QC, Canada). Anaesthetized (pentobarbital sodium 70–90 mg/kg, i.p.) and tracheostomized (18 G cannula) mice were mechanically ventilated (160 breaths/min, tidal volume of 10 mL/kg, positive end-expiratory pressure of 2–3 cmH_2O). Increasing concentrations (0–12.5 mg/mL) of methacholine aerosol were administered through an inline nebulizer for 10 s with a tidal volume of 30 mL/kg. After delivery of aerolized methacholine, the single-compartment model was used to assess R and C with a 2.5 Hz sinusoidal piston volume movement of 0.15 mL. This 2-s perturbation was applied consecutively every 10 s for 3 min. Peak responses during each 3-min period were determined. Baseline R and C values for each mouse were obtained by applying a 2-s perturbation three times after saline nebulization. Data are expressed as the percent change from baseline R and C values. Baseline values of R and C were not significantly different among the groups. The statistical analyses were performed on the percentage change.

Bronchoalveolar lavage fluid and lung histology

Immediately after measurement of AHR, the lungs were lavaged via the tracheal tube as described previously [11].

Lungs were fixed in 10% formalin and processed for paraffin embedding. Mucus-containing goblet cells were detected by staining paraffin sections (5 μm thick) with periodic acid-Schiff. Histological analyses were performed as described previously [12].

Cell preparations of lung cells

Lung cells were isolated as described previously [13] using collagenase digestion. Cells were resuspended in HBSS and MNCs were purified over 35% Percoll (Sigma-Aldrich, St. Louis, MO, USA). After gradient centrifugation, lung cells were washed three times with PBS.

Flow cytometry

The surface phenotype of lung cells or BMDCs was analysed with monoclonal antibodies by flow cytometry using the three-colour immunofluorescence test. To minimize non-specific binding, 5×10^5 cells were incubated with 0.25 μg Fc blocking solution (CD16/CD32; 2.4G2) for 10 min and subsequently treated with fluorochrome-labelled mAbs. The mAbs included fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, peridinin chlorophyll protein (PerCP) or allophycocyanin (APC)-conjugated anti-CD11b (M1/70), anti-CD11c (HL3), anti-CD80 (16-10A1), anti-CD86 (GL1) [all obtained from BD Pharmingen (San Diego, CA, USA)]. After washing, staining was analysed by flow cytometry on a FACS caliber using CellQuest software (BD Biosciences, San Jose, CA, USA).

Generation of bone marrow-derived dendritic cells

DCs were generated from bone marrow cells of naive BALB/c mice according to the procedure described previously [13]. Briefly, bone marrow cells were obtained from the femurs and iliac bones of mice and cultured in RPMI 1640 medium containing 10% heat-inactivated FCS, 50 μM 2-ME, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin (GIBCO, Carlsbad, CA, USA), 10 ng/mL recombinant mouse GM-CSF and 10 ng/mL recombinant mouse IL-4 (R&D Systems, Minneapolis, MN, USA) for 8 days.

On day 8, DCs were obtained with anti-mouse CD11c microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) from non-adherent cells, and were pulsed with OVA (200 μg/mL) with or without ONO-1301, OKY-046 or CAY10449 for 24 h and washed three times with PBS. As a control, DCs were cultured without OVA.

In vitro *co-culture of bone marrow-derived dendritic cells with spleen T cells*

Primed spleen T cells were isolated from mice treated with OVA sensitization and challenged using mouse T cell immunocolumns (Cedarlane, Hornby, ON, Canada). In 96-well culture plates, 2×10^5 purified T cells were mixed with ONO-1301-, OKY-046- or ONO-1301 plus CAY10449-treated BMDCs (6.7×10^3 cells) for 48 h.

Measurement of cytokines in supernatants from cell culture or bronchoalveolar lavage fluid

Supernatants from cell cultures, serum or BAL fluid were stored at −80 °C until used for cytokine measurements. The levels of cytokines and thromboxane B_2 (TxB_2) were determined using commercially available ELISA or EIA kits in accordance with the manufacturers' instructions. ELISA kits for detection of IL-4, IL-5 and IFN-γ were obtained from BD Pharmingen. The IL-13 ELISA kit was purchased from R&D Systems. The EIA kit for 11-dehydro TxB_2 was purchased from Cayman Chemical (Cayman Chemical Company, Ann Arbor, MI, USA).

Statistical analysis

Mann–Whitney *U*-tests were used to determine the levels of differences between all groups. The data were pooled from three independent experiments with four mice/group in each experiment ($n = 12$). Comparisons for all pairs were performed using the Kruskal–Wallis test. Significance was assumed at *P*-values of <0.05 for all tests. Values for all measurements are expressed as means±SEM.

Results

ONO-1301 suppresses airway hyperresponsiveness and airway eosinophilia in a dose-dependent manner

We initially examined the effects of ONO-1301 on AHR at different doses. As shown in Fig. 2a, in the high-dose ONO-1301 group (20 mg/kg/day), R and C were significantly attenuated compared with the vehicle group. To a lesser extent, in the middle-dose ONO-1301 group (2 mg/kg/day), R and C were also attenuated at the highest dose of methacholine (12.5 mg/mL) compared with the vehicle group, whereas in the low-dose ONO-1301 group (0.2 mg/kg/day), there were no differences in R and C compared with the vehicle group (Figs 2a and b). Baseline R and C were not significantly different among groups ($P = 0.505$ in R and $P = 0.555$ in C). In addition to AHR, the numbers of eosinophils and lymphocytes in BALF were also significantly decreased when the middle (2 mg/kg/day) and high doses (20 mg/kg/day) of ONO-1301 were administered, but this was not seen in the low-dose group (0.2 mg/kg/day) (Fig. 2c). With regard to drug toxicity, no obvious major side-effects such as death or massive haemorrhage in BALF were observed in mice treated with ONO-1301.

Levels of thromboxane B_2 in bronchoalveolar lavage fluid

To clarify the role of TxA_2 or prostacyclin in the development of lung allergic responses, mice that received OKY-046, beraprost or ONO-1301 were studied following an OVA challenge. We administered OKY-046 (6.7 mg/kg/day), beraprost (0.3 mg/kg/day) or ONO-1301 (20 mg/kg/day) to sensitized and challenged mice based on a previous report describing the activity of TxA_2 synthase inhibition and prostacyclin using ONO-1301 [14]. Levels of 11-dehydro TxB_2, a metabolite of TxA_2, in BAL fluid were significantly decreased in mice that received OKY-046 or ONO-1301 compared with mice that received vehicle or beraprost (Fig. 3).

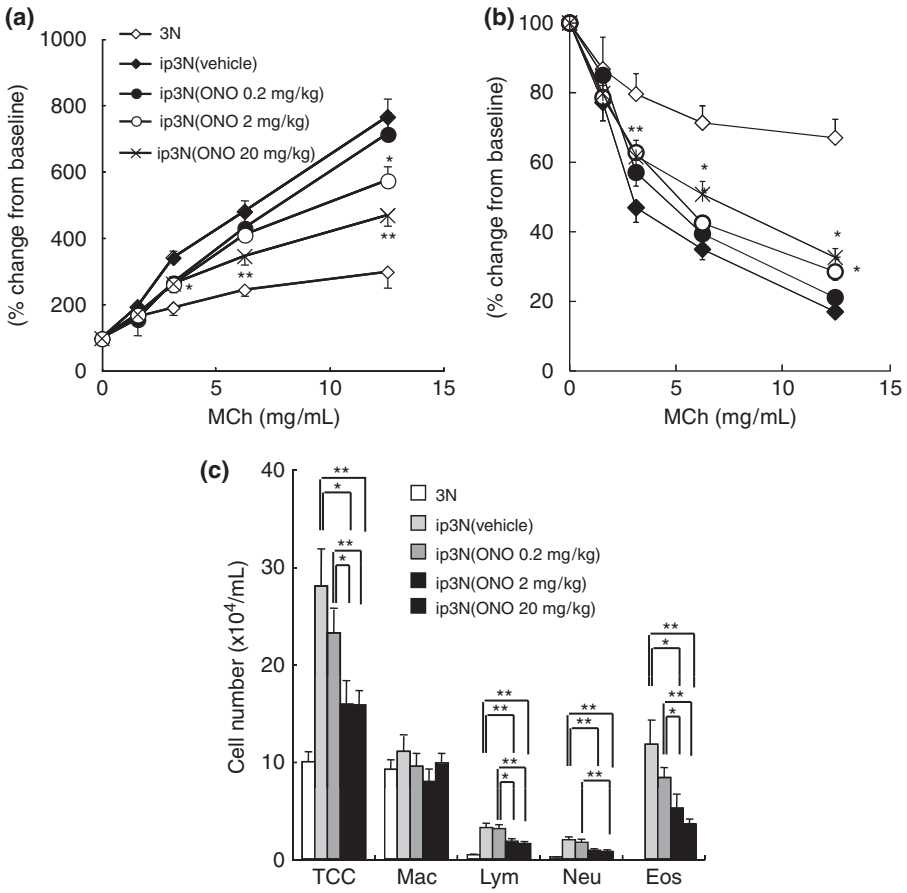


Fig. 2. Effect of ONO-1301 on lung allergic responses of DC at different doses. (a) Changes in respiratory resistance (R) and (b) changes in compliance (C) following ovalbumin (OVA) challenges. Increasing concentrations of nebulized MCh were administered through the tracheal cannula 24 h after the last OVA challenge in BALB/c mice. (c) bronchoalveolar lavage cellular composition in BALB/c mice sensitized and challenged with OVA. Data represent the means±SEM from three independent experiments consisting of four mice/group ($n = 12$). $^{*}P < 0.05$ or $^{**}P < 0.01$ compared with vehicle-treated mice following OVA sensitization and challenge or as indicated. 3N, non-sensitized mice exposed to three OVA challenges; ip3N, sensitized mice exposed to three OVA challenges; Mac, macrophages; Lym, lymphocytes; Neu, neutrophils; Eos, eosinophils.

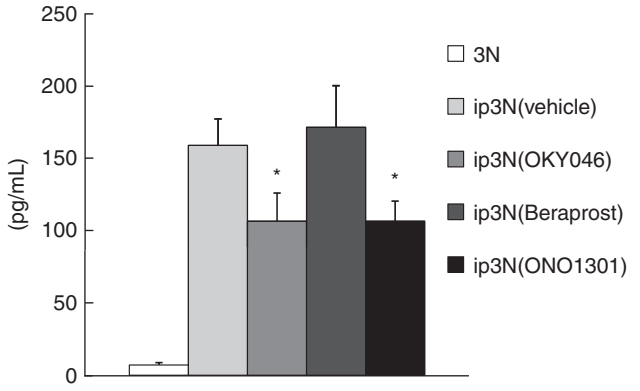


Fig. 3. Effect of ONO-1301 (20 mg/kg/day), OKY-046 (6.7 mg/kg/day) or beraprost (0.3 mg/kg/day) administration on 11-dehydro thromboxane B_2 (TxB_2) in BAL fluid. Data represent the means±SEM from three independent experiments consisting of 4 mice/group ($n = 12$). $^*P < 0.05$ compared with vehicle-treated mice following ovalbumin sensitization and challenge.

ONO-1301 but not OKY-046 suppresses airway hyperresponsiveness and airway inflammation, mainly through the IP receptor

Mice that received ONO-1301 showed suppressed AHR, airway lymphocytosis, neutrophilia and eosinophilia compared with mice that received vehicle or OKY-046 (Figs 4a–c). Mice that received beraprost also showed lower AHR and airway eosinophilia compared with mice that received vehicle, but to a lesser degree compared with ONO-1301-treated mice (Figs 4a–c). In contrast, combination treatment with ONO-1301 and CAY10449 had little effect on the suppression of AHR and airway inflammatory cell accumulation (Figs 4a–c). As a control, treatment with CAY10449 alone also had little effect on AHR and airway eosinophilia (Figs 4a–c). Baseline R and C were not significantly different among groups ($P = 0.359$ in R and $P = 0.418$ in C among groups). None of the drugs had effects on baseline AHR and cell composition in BALF of non-sensitized mice (data not shown). Levels of IL-5 and IL-13 in BAL fluid in ONO-1301-treated mice as well as IL-13 in beraprost-treated mice were significantly lower compared with vehicle-treated mice, whereas in mice treated with the combination of ONO-1301 and CAY10449, levels of IL-5 and IL-13 in BALF almost returned to the same levels as those seen in the vehicle groups. The levels of IL-4 or IFN-γ in BALF were not significantly different among the four groups (Fig. 4d). ONO-1301-treated mice also failed to develop goblet cell metaplasia and to increase mucus production compared with vehicle- or OKY-046-treated mice, and these effects were reversed by co-administration of CAY10449 (Fig. 4e).

ONO-1301 alters dendritic cell function

To determine the functional differences in allergic inflammation between ONO-1301 and OKY-046, DC function was investigated because previous reports indicated that prostacyclin had the potential to modify this function [4]. The percentages of lung $CD11c^+I\text{-}Ad^+$ DCs were lower in mice that received ONO-1301 compared with those in vehicle- or OKY-046-treated mice (Fig. 5a). The expression of CD80 or CD86 on lung DCs was also lower in mice that received ONO-1301 compared with those in vehicle- or OKY-046-treated mice (Fig. 5b). Also, the profiles of lung DCs from mice treated with ONO-1301 and CAY10449 were similar to vehicle-treated mice (Figs 5a and b). The ratio of the MHC-high fraction to MHC-low was significantly decreased in mice that received ONO-1301 compared with mice that received vehicle or OKY-046 (Fig. 5c). These data suggested that ONO-1301 had a potential to alter the amount of mature myeloid DCs. Next, an examination was performed to investigate whether ONO-1301 had the capacity to alter the expression of surface markers on BMDCs. BMDCs from naïve BALB/c mice were cultured in the presence or absence of ONO-1301/OKY-046 or with CAY10449 for 24 h. As shown in Fig. 6, for ONO-1301 alone, but not in combination with CAY10449-treated BMDCs, expression of CD80 or CD86 was significantly lower than vehicle- or OKY-046-treated BMDCs (Fig. 6).

In vitro *co-culture of bone marrow-derived dendritic cells with spleen T cells*

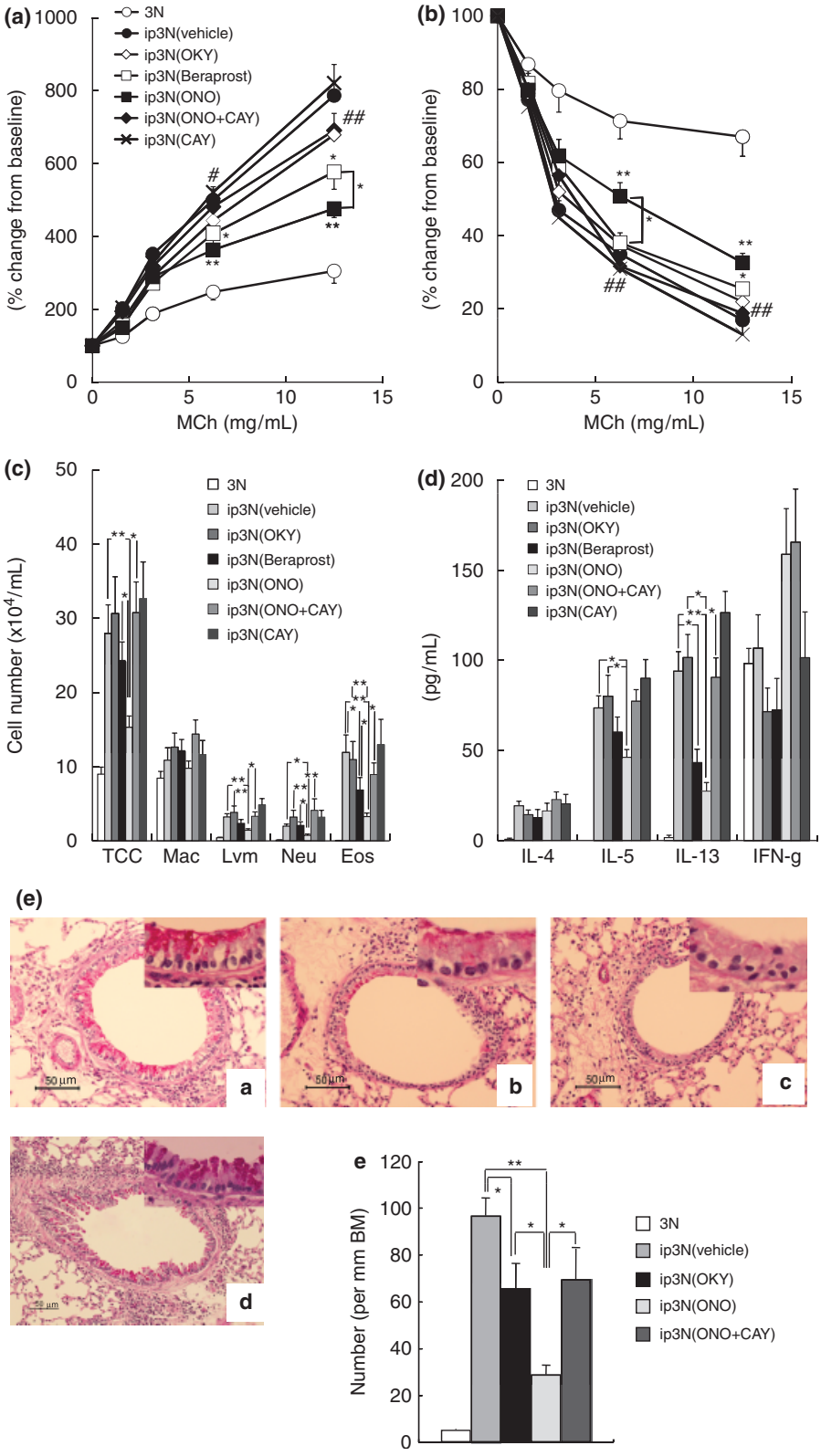
To clarify the effect of ONO-1301 on the T cell stimulatory activity of BMDCs, cytokines produced by OVA-primed T cells co-cultured with OVA-pulsed ONO-1301-treated BMDCs were examined. As shown in Fig. 7, T cells stimulated by ONO-1301-treated BMDCs released significantly lower levels of Th2 cytokines such as IL-4, IL-5 and IL-13. These effects were reversed when BMDCs were cultured and treated with a combination of ONO-1301 and CAY10449 (Fig. 7).

Discussion

In the present study, we demonstrated that (1) a novel prostacyclin agonist (ONO-1301) ameliorated the development of AHR, airway eosinophilia, Th2 type cytokine elevation in BALF and goblet cell metaplasia in a mouse model of airway allergic inflammation; (2) these effects of ONO-1301 were superior to those of OKY-046, a TxA_2 synthase inhibitor; (3) CAY10449, a selective IP antagonist, reversed the effects of ONO-1301 and (4) ONO-1301 suppressed the function of DCs.

Prostacyclin, which relaxes smooth muscle and inhibits platelet aggregation, is known to be a modulator of Th2-mediated inflammation. The deficiency of IP, a specific binding receptor in prostacyclin, enhanced allergic inflammation, sustained this inflammation through repeated challenges and resulted in airway remodelling

[2, 3]. Zhou et al. [15] reported that prostacyclin analogs had an important role in the inhibition of Th1 and Th2 cytokine production from CD4T cells. Another study showed that the prostacyclin-IP receptor system was important for regulating Th2-mediated airway inflammation through inhibition of the recruitment of Th2 cells in mice [16]. Our results showed that ONO-1301 suppressed Th2-type inflammation in the airway. However, we could



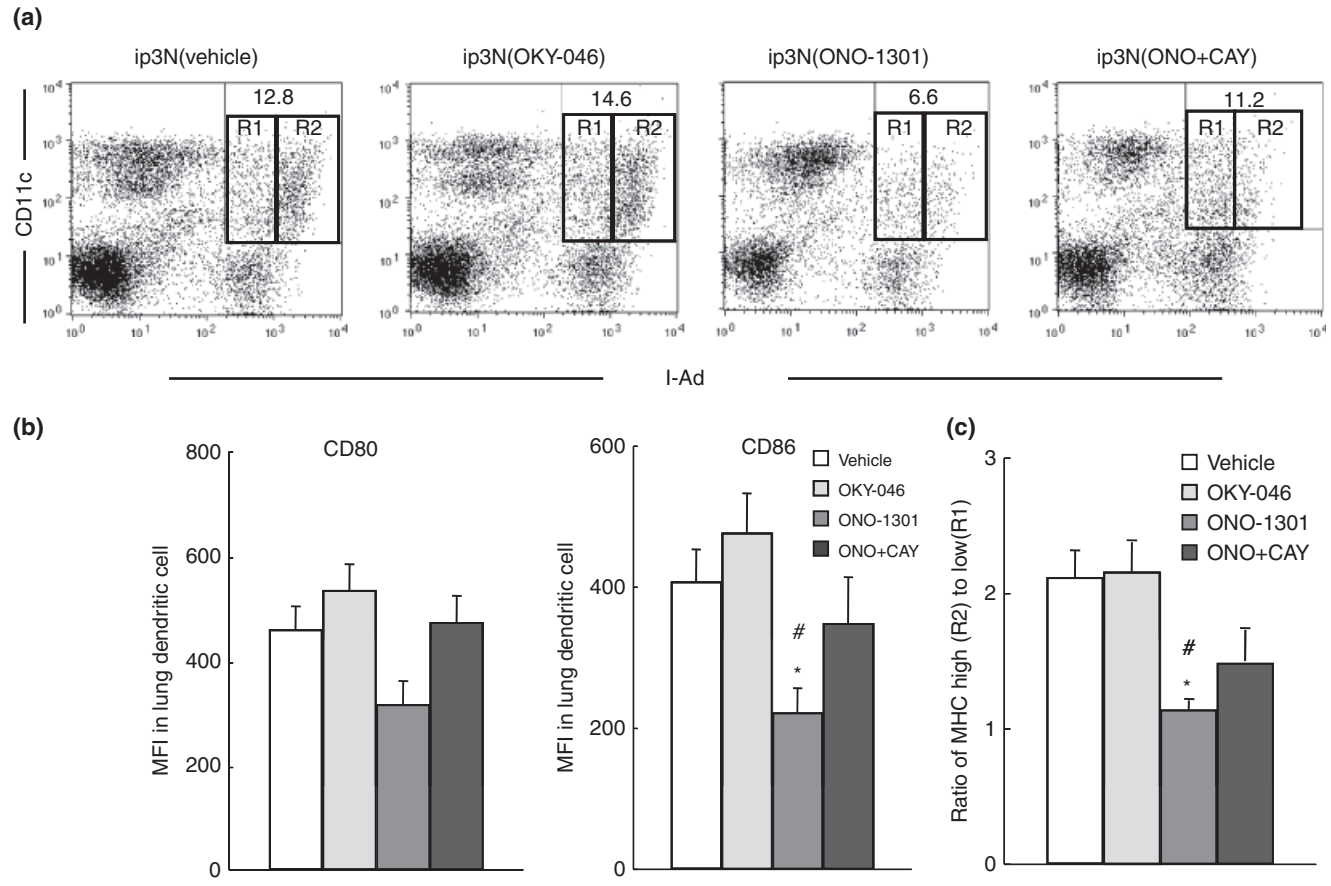


Fig. 5. Flow cytometric analysis of lung dendritic cells. (a) Anti-I-Ad (MHC class II for BALB/c mouse) and anti-CD11c staining of lung leucocytes from vehicle-treated (vehicle), OKY-046-treated (OKY-046: 5×10^{-5} M), ONO-1301-treated (ONO-1301: 5×10^{-5} M) or ONO-1301+CAY10449 (1×10^{-6} M)-treated mice that were sensitized following three ovalbumin challenges. Number is the percentage of the I-Ad$^+$CD11c$^+$ fraction. Data from a representative experiment are shown. (b) Mean fluorescence intensity (MFI) values of CD80 and CD86 in the I-Ad$^+$CD11c$^+$ fraction. (c) The ratio of the MHC-high fraction (R2) to the MHC-low fraction (R1) in I-Ad$^+$CD11c$^+$. Data represent the means±SEM from two independent experiments consisting of four mice/group ($n=8$). $^*P<0.05$ compared with vehicle-treated mice. $\#P<0.05$ compared with OKY-046-treated mice.

not directly prove that inhibition of cytokine production from lung-infiltrating CD4T cells or suppression of recruitment of CD4T cells were responsible for the beneficial effects of ONO-1301, as there was little change in flow cytometric analysis of lung-infiltrating T cells and cytokine production from lung CD4T cells following *in vitro* stimulation (data not shown). As an alternative mechanism, prostacyclin also has a role in modulating the function of DCs. Several reports have identified a critical role for DCs in allergic airway inflammation [13, 17]. Prostacyclin analogs inhibit the production of multiple proinflammatory cytokines and chemokines released by BMDCs, and antigen-specific activation of naïve CD4T cells. These modulatory effects appear to be mediated through c-AMP and NF-κB-signalling pathways, because prostacyclin analogs induced elevation of intracellular c-AMP production and a decrease in NF-κB activity [18]. Another report on the role of prostacyclin in the modulation of DC function described how iloprost, a prostacyclin analog, altered the function of lung DCs and resulted in

Fig. 4. Comparison of the effects of administration of OKY-046, ONO-1301 or ONO-1301 plus CAY10449 on lung allergic response. (a) Changes in respiratory resistance (R) and (b) changes in compliance (C) in recipients that received vehicle, OKY-046 (6.7 mg/kg/day), ONO-1301 (20 mg/kg/day) or ONO-1301 plus CAY10449 (200 μg/day). (c) Bronchoalveolar lavage (BAL) cellular composition. (d) Cytokine levels in BAL fluid. (e) Representative periodic acid-Schiff (PAS)-stained histological sections of lung tissues obtained 24 h after the last challenge in (a) vehicle-treated mice, (b) OKY-046-treated mice, (c) ONO-1301-treated mice, (d) ONO-1301 plus CAY10449-treated mice, (e) Quantitative analysis of PAS+cells. Data represent means±SEM from three independent experiments ($n=12$). $^*P<0.05$ or $^{**}P<0.01$ compared with vehicle-treated mice or as indicated. $\#P<0.05$ or $\#\#P<0.01$ compared with ONO-1301-treated mice. 3N, non-sensitized mice exposed to three ovalbumin (OVA) challenges; ip3N, sensitized mice exposed to three OVA challenges; Mac, macrophages; Lym, lymphocytes; Neu, neutrophils; Eos, eosinophils; BM, basement membrane.

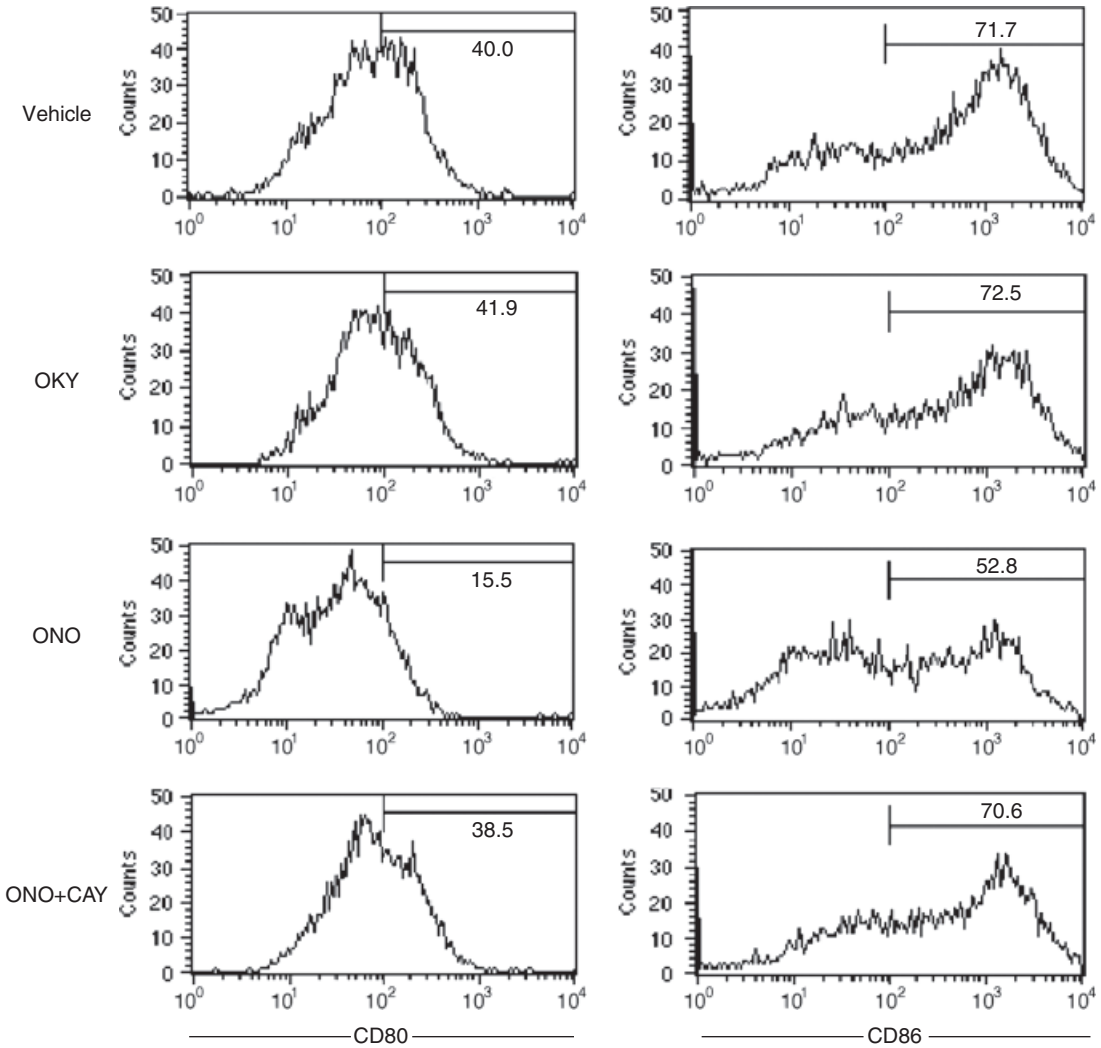


Fig. 6. The phenotypic profile of Bone marrow-derived dendritic cells in the presence of OKY-046 (5×10^{-5} M), ONO-1301 (5×10^{-5} M) or ONO-1301 (5×10^{-5} M) plus CAY10449 (1×10^{-6} M). Histogram profiles of CD80 and CD86 expression on cells of the CD11c-positive fraction. Results are representative for three independent experiments.

the suppression of AHR and airway eosinophilia, which are the important features of bronchial asthma [4]. In the current study, ONO-1301 showed significant effects in altering lung DCs and BMDCs in a murine model of airway allergic inflammation. In particular, the *in vitro* function of ONO-1301-treated BMDCs was clearly suppressed, compared with vehicle or treatment with OKY-046. With regard to the mechanisms underlying our results, we did not measure the levels of intracellular c-AMP, but we speculate that c-AMP is involved in this mechanism, at least in part, based on previous reports, which indicate that ONO-1301 up-regulated plasma and intracellular c-AMP levels [8, 10].

In the present study, we focused on alteration of DC function by ONO-1301 mainly through IP signalling because CAY10449, a selective IP antagonist, largely reversed the effects of ONO-1301. Recently, prostacyclin and its mimetics have been shown to be capable of activating a nuclear hormone receptor, peroxisome proliferator-activated receptor δ (PPAR δ) [19]. Some of these studies reported the importance of PPAR δ in the signalling pathway of prostacyclin [20, 21]. Although the role of PPAR δ in DCs remains unclear, PPAR δ signalling may have mediated some of the findings observed in this study.

ONO-1301 also possesses potent inhibitory activity against TxA_2 synthase. However, in the group treated with OKY-046, a TxA_2 synthase inhibitor, AHR, airway eosinophilia and Th2 cytokine elevation in BALF were not decreased compared with the vehicle group, whereas the levels of TxB_2 in BALF were significantly decreased. These results might be attributable to the low overall dose of OKY-046, because the protocols in previous reports used higher and more frequent dosing of OKY-046 [7]. Based on these results, further investigations to determine the optimal dosage of ONO-1301 need to be carried out, although the

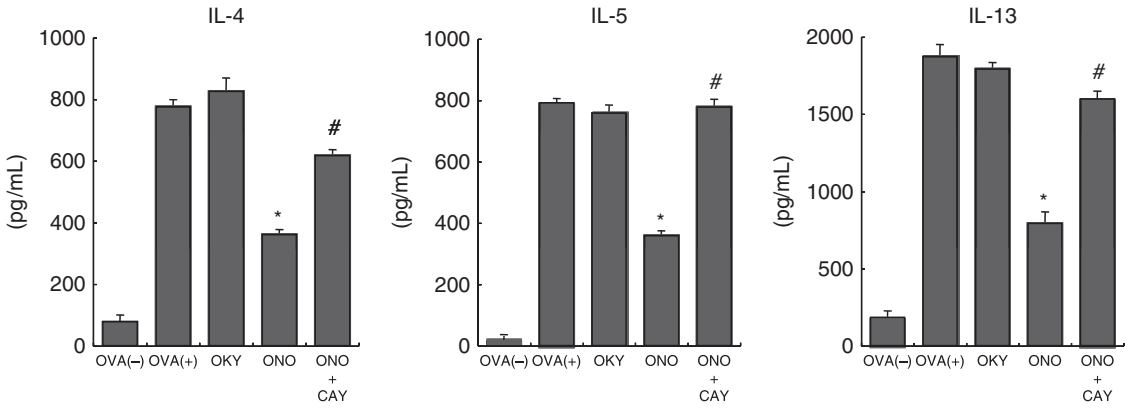


Fig. 7. Cytokine levels in the culture supernatants from ovalbumin (OVA)-primed spleen T cells co-cultured with vehicle, OKY-046-, ONO-1301- or ONO1301 plus CAY10449-treated bone marrow-derived dendritic cells (BMDCs). Spleen T cells were cultured with BMDCs-treated with the respective reagents and OVA (10 μg/mL) for 48 h. The ratio of BMDCs to spleen T cells was 1:33. Data represent the means±SEM from three independent experiments carried out in triplicate. OVA(−), vehicle-treated BMDCs without OVA; OVA(+), vehicle-treated BMDCs with OVA; OKY, OKY-046- (5×10^{-5} M) treated BMDCs with OVA; ONO, ONO-1301- (5×10^{-5} M) treated BMDCs with OVA; ONO+CAY, ONO-1301 (5×10^{-5} M) plus CAY10449- (1×10^{-6} M) treated BMDCs with OVA. $^{*}P<0.05$ or $\#P<0.05$ compared with vehicle-treated or ONO-1301-treated BMDCs, respectively.

current study utilized one-third of the dose of OKY-046 compared with ONO-1301, based on *in vitro* data [14]. Our data, however, should not be interpreted to exclude a role for inhibition of TxA_2 synthase in the suppressive effect of ONO-1301 on AHR, airway eosinophilia, Th2 cytokine up-regulation or goblet cell metaplasia. ONO-1301 suppressed AHR and allergic airway inflammation more effectively compared with beraprost. These data suggested that the additional TxA_2 inhibition present in ONO-1301 might synergistically enhance the beneficial effects in allergic airway inflammation. As an alternative explanation, Itoh et al. [22] reported that selective TxA_2 synthase inhibitors reduce the production of TxA_2 from PGH_2, and accelerate the production of prostacyclin and PGE_2. From this perspective, our data suggest that the TxA_2 synthase-inhibitory activity of ONO-1301 may have amplified the effects of prostacyclin through up-regulation of this production.

Prostacyclin has been used for many years in the treatment of PAH, but its efficacy has not met the initial expectations. One reason is thought to be the development of tolerance to prostacyclin during long-term use [23, 24]. The mechanism of this tolerance appears to be an imbalance between thromboxane and prostacyclin, which have opposing effects on platelet aggregation and pulmonary vascular smooth muscle. Further, earlier studies demonstrated impaired prostacyclin synthesis and increased thromboxane production in patients with PAH, suggesting that an imbalance in the release of thromboxane and prostacyclin plays an important role in the development of PAH [25]. From this knowledge, ONO-1301, a new type of prostacyclin agonist having a potent inhibitory effect on TxA_2 synthase, is thought to be a more effective agent for the treatment of PAH. In bronchial asthma, TxA_2 has been regarded as one of the important chemical mediators because it is a potent inducer of bronchoconstriction [26]. Although a thromboxane-receptor antagonist and a thromboxane synthase inhibitor have been commercially available as anti-asthma drugs in Japan, the clinical effects have not met expectations. This suggests that the pathogenesis of asthma is not associated with just one or two factors, but multiple mechanisms that lead to the establishment and persistence of the disease. ONO-1301 appears to affect not only TxA_2 synthase or the prostacyclin receptor, but also other prostanoids, growth factors or residual cell function, and these multiple actions are expected to influence the clinical treatment of asthma in the near future.

In the present study, we investigated the role of ONO-1301 in airway allergic inflammation in the mouse. ONO-1301 proved effective in decreasing AHR, eosinophil infiltration in the airways, Th2 cytokine levels in BALF and mucus production in bronchial epithelium compared with vehicle, at least in part through modulation of lung DC function. ONO-1301-treated BMDCs showed a lower expression of costimulatory molecules and suppressed Th2 cytokine production from primed spleen T cells compared with vehicle or OKY-046. These effects of ONO-1301 were reversed when co-administered with CAY10449, a selective IP antagonist. These data suggest that ONO-1301 may suppress AHR and airway allergic inflammation through inhibition of DC function, mainly through the IP-signalling pathway. This agent may be effective as an anti-inflammatory drug in the treatment of asthma.

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