

Lipid A Analogue, ONO-4007, Inhibits IgE Response and Antigen-Induced Eosinophilic Recruitment into Airways in BALB/c Mice

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Key Words

ONO-4007 · Lipid A analogue · IgE · IL-4 · IL-5 ·
Eosinophil · Th2 · Dendritic cell

Abstract

Background: Since antigen-specific IgE and eosinophils are major inducing factors of allergic inflammation of the airways, both factors are therapeutic targets of asthma. We investigated the effects of ONO-4007, a nontoxic lipid A analogue, on antigen-specific antibody response and the recruitment of eosinophils into airways in murine systems. **Methods:** BALB/c mice were injected ONO-4007 intraperitoneally during sensitization with ovalbumin (OVA) and aluminium hydroxide to determine its effects on the antigen-specific antibody response. ONO-4007 was also injected intravenously during either systemic sensitization and inhalation with OVA, or sensitization or inhalation alone to determine its effects on antigen-induced airway inflammation. In vitro effects of ONO-4007 on the functional differentiation of naive CD4⁺ T cells were investigated by culturing naive CD4⁺ T cells derived from DO11.10 mice and OVA-pulsed dendritic

cells (CDCs) with ONO-4007. **Results:** ONO-4007 inhibited antigen-specific IgE and IgG₁, but not IgG_{2a} responses. ONO-4007 decreased the recruitment of eosinophils and the levels of IL-5 in bronchoalveolar lavage fluid, not only when it was injected during systemic sensitization and inhalation with OVA, but also during inhalation alone. ONO-4007 inhibited the differentiation of IL-4- and IL-13-producing CD4⁺ T cells in vitro, which was partly mediated by DCs. **Conclusions:** ONO-4007 inhibited antigen-specific IgE and IgG₁ responses and antigen-induced eosinophil recruitment into the airways in BALB/c mice. These effects were mediated, at least partly, by the modulation of DCs, although there may also be other mechanisms.

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Introduction

Th2 type cytokines, such as IL-4, IL-5 and IL-13, which are produced by allergen-specific CD4⁺ T cells, play a central role in the development of allergic diseases [1, 2]. IL-4 and IL-13 promote the production of IgE and

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IL-5 is involved in the growth, differentiation and activation of eosinophils [3–5]. Since these allergen-specific Th2 cells differentiate from naive CD4⁺ T cells under the influences of many environmental factors in allergic patients, a trial to skew the cytokine production profile of allergen-specific CD4⁺ T cells in allergic patients from Th2 to other subsets may lead to new strategies for the treatment of allergic diseases. In fact, some studies were reported that inhibited antigen-induced IgE production by skewing the cytokine production profile from Th2 to Th1, or reduced airway hyperresponsiveness by expressing an immunomodulatory cytokine in antigen-specific CD4⁺ T cells [6–9].

Bacterial lipopolysaccharides or endotoxins evoke a variety of toxic, pharmacological and immunomodulatory effects, such as Th1-inducing activities via the induction of IL-12 [10, 11]. Most of these biological effects of bacterial lipopolysaccharides are mediated by the bis-(phospho)lipid A or diphosphoryl lipid A moiety of the molecule [12, 13]. However, lipid A as well as lipopolysaccharides are extremely toxic in most species and could not be used as therapeutic agents in vivo. Sodium 2-deoxy-2-[3*S*-(9-phenylnonanoyloxy)tetradecanoyl]amino-3-*O*-(9-phenylnonanoyl)-*D*-glucopyranose 4-sulfate (ONO-4007) was chemically synthesized as a lipid A analogue, which had potent immunopharmacological activities, but with reduced toxicity (less than 1/1,000 that of natural *Escherichia coli* lipopolysaccharide) [14]. ONO-4007 has been shown to exhibit antitumor activity on various experimental models [15]. In the course of our study, to modulate the functional differentiation of naive CD4⁺ T cells, we tried a synthetic lipid A analogue, ONO-4007. Here, we describe the effects of ONO-4007 on the antigen-specific antibody responses and eosinophil recruitment into the airways by antigen inhalation in vivo, and the functional differentiation of naive CD4⁺ T cells in vitro in murine systems.

Materials and Methods

Reagents

ONO-4007 was a gift from Ono Pharmaceutical Co., Ltd. (Osaka, Japan). Recombinant human IL-2 was a gift from Shionogi Pharmaceutical Co. (Osaka, Japan). Murine IL-4 and GM-CSF were obtained from R&D Systems, Inc. (Minneapolis, Minn., USA) and Kirin Brewery Co., Ltd. (Gunma, Japan), respectively.

Mice

Eight-week-old BALB/c mice were purchased from SLC (Shizuoka, Japan). DO11.10 mice, which are transgenic for T cell receptor (TCR) recognizing ovalbumin peptide 323–339 (pOVA^{323–339}) and

backcrossed to BALB/c, were provided by Dr. S. Sakaguchi (Kyoto University, Japan) [16]. All these mice were maintained in our animal facility and cared for in accordance with the institutional guidelines for animal welfare.

Immunization and Antibody Measurement

BALB/c mice were sensitized with 10 µg OVA (Seikagaku Co., Tokyo, Japan) that was precipitated on 2 mg aluminium hydroxide (alum), resuspended in 200 µl saline and injected intraperitoneally on day 0. The booster dose was given 2 weeks after the priming dose; serum was collected 14 days later. ONO-4007 was dissolved in 50% ethanol at a concentration of 50 mg/ml and diluted with 5% glucose. ONO-4007 (10 mg/kg) was injected intraperitoneally on days –1 to 3, and 13–17.

To measure OVA-specific IgE by ELISA, 96-well plates were coated with anti-IgE, 2 µg/ml (R35–72; BD PharMingen, San Diego, Calif., USA). Bound serum antibody was detected by OVA that had been biotinylated, followed by streptavidin-horseradish peroxidase conjugate (SAv-HRP; BD PharMingen). For the detection of OVA-specific IgG₁ and IgG_{2a}, 96-well plates were coated with OVA (50 µg/ml), and bound serum antibodies were detected by biotinylated anti-mouse IgG₁ (A85–1; BD PharMingen) and IgG_{2a} (R19–15; BD PharMingen), followed by SAv-HRP. To measure serum total IgE, 96-well plates were coated with anti-mouse IgE (R35–72; BD PharMingen). Bound IgE was detected by biotinylated anti-mouse IgE (R35–92; BD PharMingen), followed by SAv-HRP. The antibody titers were calculated by comparison with internal standards. Anti-OVA IgE and IgG serum standards were obtained by pooling sera from mice sensitized intraperitoneally with OVA and alum. The standard curve was constructed by a linear regression analysis of the absorbances versus serial dilutions of the positive reference sera. Results are expressed as arbitrary units relative to an internal isotype standard run in each assay.

Determination of Cell Numbers and IL-5 Levels in Bronchoalveolar Lavage Fluid

Cell numbers and IL-5 levels in bronchoalveolar lavage fluid (BALF) were determined as previously described [17]. Briefly, BALB/c mice were sensitized on days 0 and 12 by intraperitoneal injection of 50 µg OVA emulsified in 1 mg alum. The mice were challenged with aerosolized OVA (10 mg/ml in 0.9% NaCl solution) for 30 min on days 22, 26 and 30. Control animals were exposed to saline in a similar manner. The aerosol was generated using an ultrasonic nebulizer NE-U12 (Omron Co., Tokyo, Japan). Eight hours after the final inhalation of antigen, the mice were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg). Then, the tracheae were cannulated, and BAL was performed by flushing the lungs and airways 3 times with 1 ml saline containing 0.1% bovine serum albumin. Then, the first BALF was collected and centrifuged, and the supernatants were recovered for the measurement of cytokines. The cell pellets were resuspended with the second and third BALF and centrifuged. The pellets were resuspended in 1 ml saline and the number of nucleated cells in total BALF was counted using a hemacytometer, and a differential count was determined on slide preparations stained by the May-Giemsa method by counting at least 500 cells. IL-5 levels were determined using ELISA (Endogen, Inc., Woburn, Mass., USA). ONO-4007 was injected intravenously 1 h before sensitization, inhalation, or both with the indicated doses. Prednisolone (5 mg/kg) was administered orally from day 21 for 10 days.

Preparation of OVA-Specific Naive CD4⁺ T Cells

Spleen cells from DO11.10 (OVA TCR Tg) mice were isolated by Histopaque-1083 (Sigma Diagnostics, Inc., St. Louis, Mo., USA) density gradient centrifugation, then CD4⁺ T cells were positively selected from splenocytes using Dynabeads Mouse CD4 and DETACHA-BEAD mouse CD4 (DynaL AS, Oslo, Norway) according to the manufacturer's protocol. Naive CD4⁺ T cells were further positively selected using anti-CD62L MicroBeads and MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol.

Preparation of Dendritic Cells

Dendritic cells (DCs) were prepared from BALB/c splenocytes as previously described [18]. In brief, spleens were treated with collagenase D (Boehringer, Mannheim, Germany) for 20 min at 37°C, and homogenized. Red blood cells were lysed with NH₄Cl-Tris/HCl (pH 7.2). Then, cells were suspended in a dense BSA solution ($p = 1.080$), overlaid with 2 ml of PBS, and centrifuged in a swing bucket rotor at 9,500 *g* for 20 min at 4°C. DCs and monocytes at the interface were collected, washed and allowed to adhere to plastic dishes for 2 h at 37°C. After washing nonadherent cells by vigorous pipetting, cells were incubated in the presence or absence of 10 µg/ml of ONO-4007 for an additional 18 h to allow DCs to detach from the plastic dishes. Nonadherent cells containing DCs were recovered and incubated with MACS CD11c MicroBeads (Miltenyi Biotec) for 15 min at 4°C. CD11c⁺ DCs were positively selected using MACS magnet.

In vitro Stimulation of OVA-Specific CD4⁺ T Cells from DO11.10 Mice with OVA Peptide

Purified CD4⁺CD62L⁺ T cells (4×10^5) were stimulated in X-Vivo 15 (BioWhittaker, Walkersville, Md., USA), containing 0.5% inactivated mice serum and 2-mercaptoethanol (50 µM) with pOVA^{323–339} (1 µM) (Takara Shuzo Co., Shiga, Japan), and DCs (2×10^4) in the presence or absence of ONO-4007 (10 µg/ml) for 4 days in 96-well U-bottom plates. Stimulated CD4⁺ T cells were washed and expanded in IL-2-containing medium (50 IU/ml) for an additional 3 days. These CD4⁺ T cells were rested with irradiated BALB/c splenocytes for 5 days and then stimulated with pOVA^{323–339} and T-depleted splenocytes of BALB/c mice for 24 h. The levels of IL-4, INF-γ and IL-2 in supernatants were determined using the OptEIA™ mouse cytokine ELISA system set (BD PharMingen). The levels of IL-13 were determined using DuoSet for mIL-13 (R&D Systems, Inc.).

Flow Cytometric Two-Color Analysis of DCs

Purified DCs were stained with PE-labeled antimouse CD80 (16–10A1), CD86 (GL1), CD54 (3E2) (BD PharMingen) and FITC-labeled antimouse I-A/I-E^d (2G9) for 30 min at 4°C. Stained cells were analyzed using FACSCalibur (Becton Dickinson, San Diego, Calif., USA).

In vitro Stimulation of DCs with Anti-CD40 mAb

Purified DCs (5×10^5 /well) were stimulated with 10 µg/ml of anti-CD40 mAb (HM40–3; BD PharMingen) in the presence of GM-CSF (20 ng/ml) and IL-4 (10 ng/ml) with or without ONO-4007 (10 µg/ml) for 18 h in flat-bottom, 96-well plates. The level of IL-12 in each supernatant was determined using DuoSet for mIL-12 (R&D Systems, Inc.).

Results

Effects of ONO-4007 on Humoral Immune Response of BALB/c Mice Sensitized with OVA and Alum

At first, we investigated the *in vivo* effects of ONO-4007 on humoral immune response as well as its toxic effects. BALB/c mice were sensitized and boosted intraperitoneally with OVA and alum as described in Materials and Methods. Two weeks after boosting, the mice were sacrificed and serum was collected for measurement of each OVA-specific immunoglobulin with ELISA. ONO-4007 (10 mg/kg weight) was injected intraperitoneally on days –1 to 3 and 13–17 (fig. 1). Preliminary experiments revealed that this protocol of sensitization with OVA and alum accompanied by treatment with ONO-4007 (10 mg/kg weight) did not affect the activity or body weight of each treated mouse (data not shown). As shown in figure 1, ONO-4007 suppressed the total IgE, OVA-specific IgE and IgG₁ response significantly, but not IgG_{2a}. The treatment with ONO-4007 less than 10 mg/kg (0.1 and 1 mg/kg) did not affect antibody response (data not shown). Since IgE and IgG₁ are dependent on Th2-type cytokines [19], these findings suggested that ONO-4007 selectively inhibited the Th2-type immune response without affecting Th1 response.

Effects of ONO-4007 on the Antigen-Induced Recruitment of Eosinophils and IL-5 Production in BALF

Eosinophil recruitment by antigen inhalation into the airways is a key step for the development of bronchial asthma as well as immediate-type hypersensitivity evoked by IgE and mast cells [2]. Therefore, we also examined the effects of ONO-4007 on the antigen-induced recruitment of eosinophils into BALF from BALB/c mice sensitized with OVA and alum. The mice were sensitized intraperitoneally twice with OVA and alum on days 0 and 12, and challenged with aerosolized OVA on days 22, 26, and 30. ONO-4007 (0.1, 1, 10 mg/kg) was injected intravenously 5 times on the same days of sensitization and challenge (fig. 2). To compare the effects of ONO-4007 on allergic inflammation to those of prednisolone, another group of mice was administered prednisolone (5 mg/kg) orally from day 21 for 10 days. OVA challenge by inhalation in the sensitized mice induced recruitment of eosinophils into BALF. Treatment with ONO-4007, however, inhibited this recruitment depending on the dose of ONO-4007 (fig. 2). The inhibition with 10 mg/kg ONO-4007 (5 times) was almost similar to that with 5 mg/kg/day prednisolone for 10 days. To examine the mechanism of inhi-

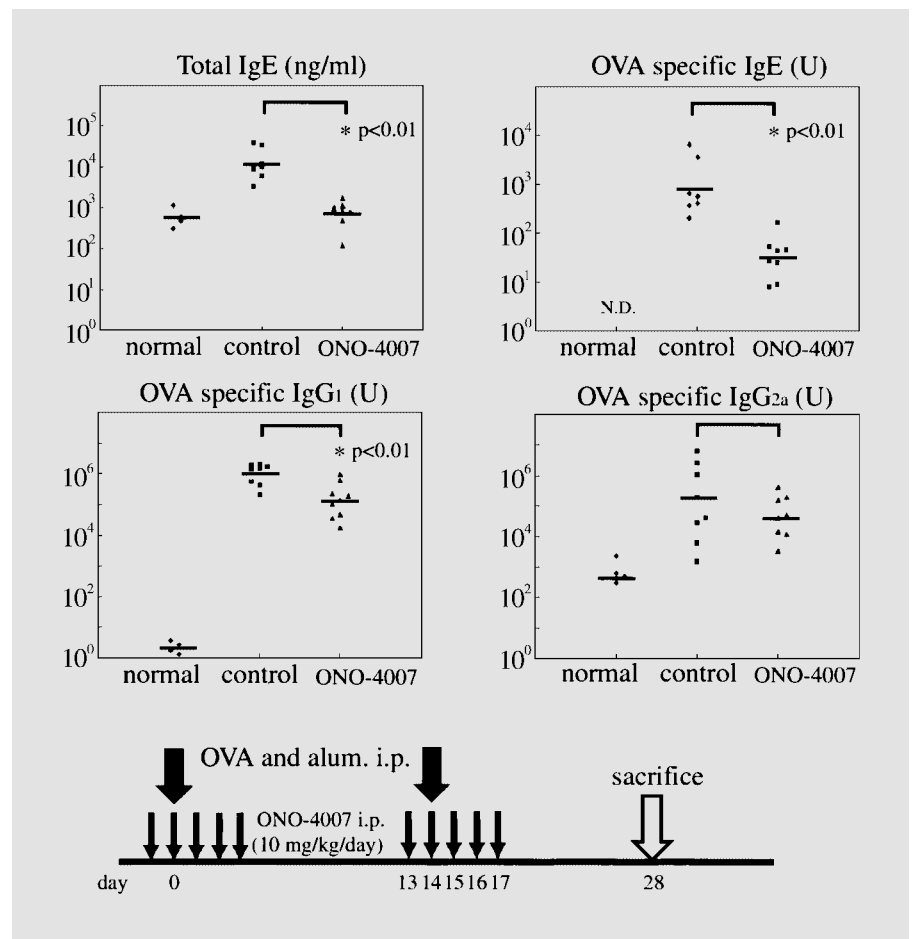


Fig. 1. Effects of ONO-4007 on total IgE and OVA-specific IgE, IgG₁ and IgG_{2a} antibody levels. The mice were sensitized intraperitoneally twice with OVA and alum with (n = 8) or without treatment (n = 8) with ONO-4007, as shown at the bottom of the figure. The number of the unsensitized mice was 4. * p < 0.01 by Student's t test.

bition of eosinophil recruitment by ONO-4007, the IL-5 concentration in BALF recovered from each mouse was measured. Intravenous injection with ONO-4007 decreased the concentration of IL-5 in BALF as well as the recruitment of eosinophils dose dependently (fig. 2). These findings demonstrated that ONO-4007 not only inhibited the antigen-specific IgE response, but also antigen-induced eosinophil recruitment and the production of IL-5 in BALF.

Effects of ONO-4007 on the Antigen-Induced Recruitment of Eosinophils after Systemic Sensitization

Although we have demonstrated that intravenous injection with ONO-4007 at sensitization and inhalation inhibited antigen-induced recruitment of eosinophils into BALF, these findings may have been due to the decreased levels of antigen-specific IgE (fig. 1), which plays a key role at the first stage of antigen-induced airway inflamma-

tion. Therefore, we investigated the effects of ONO-4007 on airway eosinophil recruitment at antigen challenge after sensitizing the mice systemically. The BALB/c mice were sensitized twice and challenged 3 times as shown in figure 2. The mice in group 1 were injected intravenously with 10 mg/kg ONO-4007 at sensitization alone. The mice in group 2 were injected at antigen challenge alone. The mice in group 3 were injected at both sensitization and antigen challenge (fig. 3). Then, the eosinophils and the concentration of IL-5 in BALF 8 h after antigen challenge were measured. As shown in figure 3, any type of treatment with ONO-4007 almost completely inhibited eosinophil recruitment and IL-5 production in BALF from antigen-challenged mice. These findings demonstrated that ONO-4007 is effective on the inhibition of eosinophil recruitment into BALF even after systemic sensitization and subsequent elevation of antigen-specific IgE.

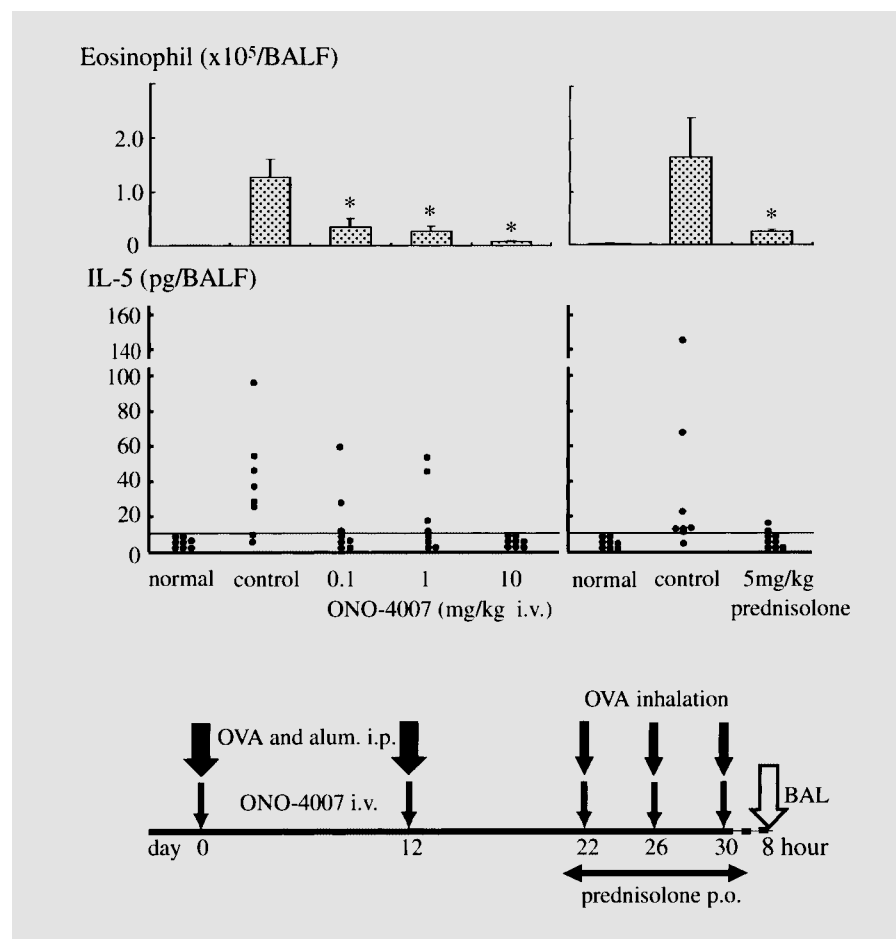


Fig. 2. ONO-4007 suppresses antigen-induced recruitment of eosinophils and IL-5 production in BALF. The mice were sensitized and challenged as shown in the figure. Three groups of mice (n = 8 for each group) were treated with each dose of ONO-4007. Another group of mice (n = 8) was sensitized, challenged and treated with prednisolone to compare the effects of ONO-4007 to that of prednisolone. * p < 0.01 by the Dunnett 2-sided test.

In vitro Effect of ONO-4007 at Primary Stimulation on the Differentiation of Naive CD4⁺ T Cells

To elucidate the cellular mechanism of suppression of IgE response and antigen-induced eosinophil recruitment into the airway by ONO-4007, we investigated the in vitro effects of ONO-4007 on the differentiation of naive CD4⁺ T cells. OVA-specific naive CD4⁺ T cells purified from DO11.10 mice were stimulated with OVA peptides and DCs in the presence or absence of ONO-4007. Then, the cells were washed and expanded in IL-2-containing medium. These T cells were stimulated with OVA peptides and T-cell-depleted splenocytes from the BALB/c mice for 24 h, and the supernatants were recovered for the measurement of cytokines. OVA-specific CD4⁺ T cells stimulated in the presence of ONO-4007 produced significantly less IL-4 and IL-13, and a considerable amount of IFN- γ without affecting IL-2 production upon secondary stimulation compared with those primed in the absence of ONO-4007 (fig. 4). Furthermore, to clarify whether these

effects of ONO-4007 were mediated by DCs, we also prepared ONO-4007-pretreated and untreated DCs as described in Materials and Methods and used these DCs to stimulate OVA-specific CD4⁺ T cells with OVA peptides. OVA-specific CD4⁺ T cells stimulated with ONO-4007-pretreated DCs and OVA peptides produced significantly less IL-4 and IL-13 upon secondary stimulation compared with those primed with control DCs independent of the presence of exogenous ONO-4007 at primary stimulation (fig. 4). These findings demonstrated that ONO-4007 inhibited the differentiation of IL-4- and IL-13-producing CD4⁺ T cells from naive CD4⁺ T cells, at least partly, by modulating DCs in vitro.

ONO-4007 Did Not Affect Surface Expression of Costimulatory and Class II Molecules on DCs

Since the expression of both class II molecules and costimulatory molecules on antigen-presenting cells has been suggested to regulate Th1 vs. Th2 polarization, the

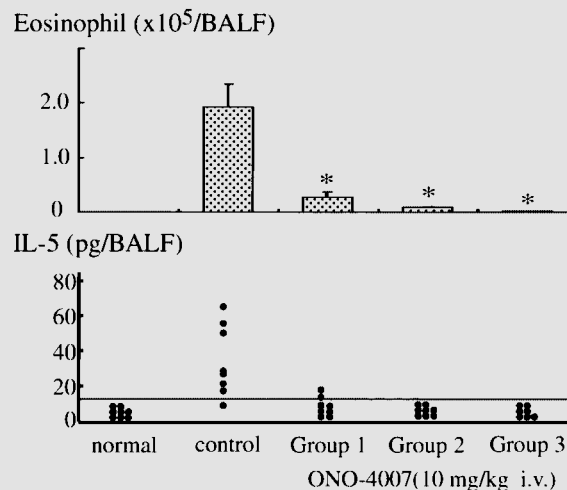


Fig. 3. ONO-4007 at inhalation suppresses antigen-induced recruitment of eosinophils and IL-5 production in BALF after systemic sensitization. The mice were sensitized and challenged with OVA as shown in the figure. Three groups of mice were treated with ONO-4007: group 1 ($n = 8$) at sensitization only, group 2 ($n = 8$) at inhalation only, group 3 ($n = 8$) both at sensitization and inhalation. * $p < 0.01$ by Student's t test.

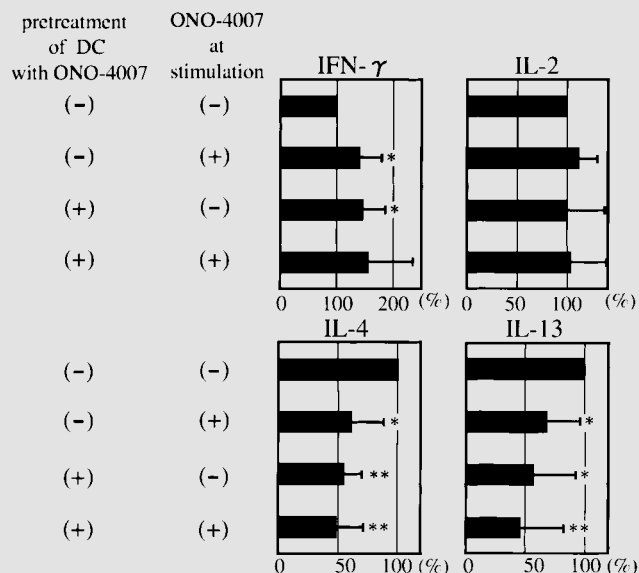
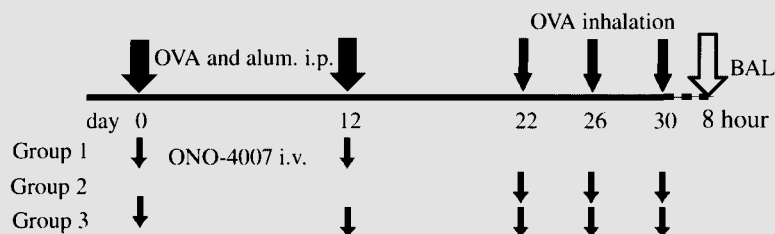


Fig. 4. ONO-4007 at primary stimulation inhibits IL-4 and IL-13 production of OVA-specific CD4^+ T cells. Purified OVA-specific CD4^+ T cells were stimulated with antigen and either DCs or ONO-4007 pretreated DCs. The amount of each cytokine was expressed as a percentage of that produced by T cells primed in the absence of ONO-4007. * $p < 0.05$, ** $p < 0.01$ by Student's t test ($n = 4$).

surface expression of these molecules was examined. As shown in figure 5, there was no significant change in the expression of I-A^d, CD80, CD86 and CD54 between the ONO-4007-treated and untreated DCs.

In vitro Effects of ONO-4007 on IL-12 Production by DCs

To clarify the inhibitory effects of ONO-4007 on the differentiation of IL-4- and IL-13-producing CD4⁺ T cells, we also examined IL-12 production by DCs stimulated with anti-CD40 mAb in the presence or absence of ONO-4007. ONO-4007 did not significantly increase IL-12 production by DCs stimulated with anti-CD40 mAb, indicating that IL-12 from DCs treated with ONO-4007 does not play a primary role in modulating the differentiation of naive CD4⁺ T cells (table 1).

Discussion

Lipid A is a major component of biological effects of lipopolysaccharide which constitutes the cell wall of gram-negative bacteria. When peripheral immature DCs and macrophages capture gram-negative bacteria, lipopolysaccharide plays an important role in inducing maturation of these antigen-presenting cells and production of IL-12, a key cytokine for the differentiation to Th1, at antigen presentation to naive CD4⁺ T cells [20, 21]. Despite these powerful biological effects, it is very difficult to apply lipopolysaccharide or lipid A to an immunomodulatory agent because of their strong toxicity.

ONO-4007, a synthetic lipid A analogue, has been reported to exert antitumor activities on various experimental tumors with less toxicity than bacterial lipopolysaccharides [14, 15]. Furthermore, the low toxicity of this analogue in humans was confirmed by a phase I study on patients with cancer [22].

In the present study, we investigated the effects of ONO-4007 on antigen-specific IgE production and antigen-induced eosinophil recruitment into the airways in mice. The injection of ONO-4007 inhibited antigen-specific IgE and IgG₁ but not IgG_{2a} production in BALB/c mice sensitized with OVA and alum (fig. 1). The production of IgE and IgG₁ by B cells depends on the IL-4 and IL-13 from Th2 cells, and that of IgG_{2a} depends on IFN- γ from Th1 cells. Although lipopolysaccharide has been reported to induce various effects, such as Th1-inducing, Th2-inducing and tolerance-inducing effects in macrophages and DCs, mainly depending on duration [23, 24], the present findings suggest that ONO-4007 selectively

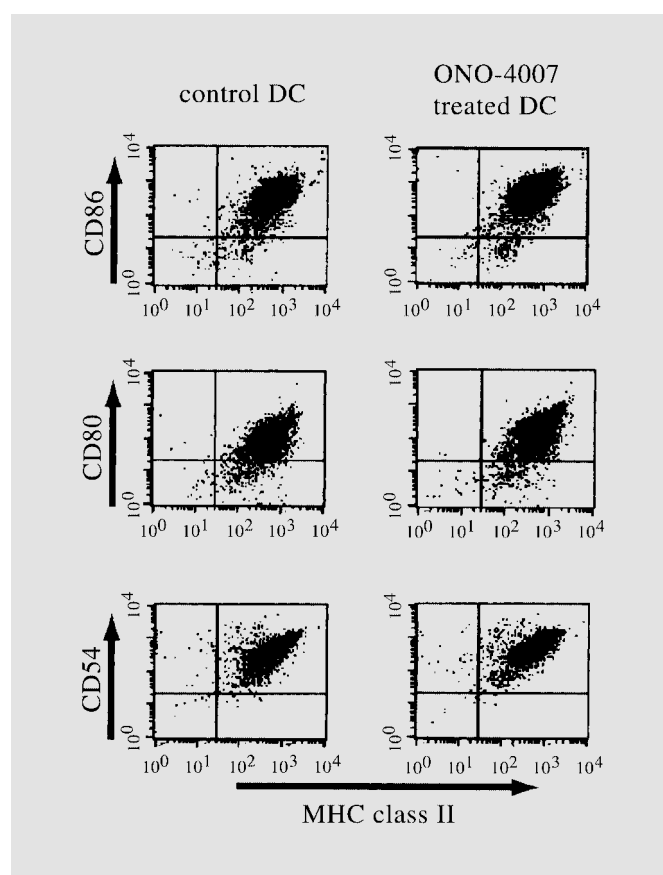


Fig. 5. Surface expression of CD54, CD80, CD86 and MHC class II on control DCs and ONO-treated DCs.

Table 1. IL-12 production by DCs stimulated with anti-CD40 mAb¹

Treatment	IL-12 production, pg/ml
–	<3
Anti-CD40 mAb	4.8 ± 0.7
Anti-CD40 mAb + ONO-4007	6.7 ± 2.1
ONO-4007	<3

Purified DCs were stimulated with anti-CD40 mAb (10 μ g/ml) with or without ONO-4007 (10 μ g/ml) in the presence of GM-CSF (20 ng/ml) and IL-4 (10 ng/ml). ONO-4007 did not significantly increase IL-12 production by DCs stimulated with anti-CD40 mAb ($p > 0.05$ by the unpaired Student's t test).

¹ Mean $\pm$ SD of 4 independent experiments.

inhibited the differentiation of IL-4- and IL-13-producing CD4⁺ T cells in vivo.

Several studies document the importance of eosinophils in the airways for the development of bronchial asthma [25–27]. Injection of ONO-4007 at both sensitization and inhalation also completely inhibited antigen-induced eosinophil recruitment into the airways with a concomitant decrease in IL-5 concentration in BALF, as observed following treatment with prednisolone (fig. 2). Furthermore, the injection of ONO-4007 only at inhalation of antigen after sensitization with OVA and alum, which preferentially induce the Th2 response, inhibited this antigen-induced eosinophil recruitment (fig. 3). These findings could not be explained only by inhibiting effects of ONO-4007 on Th2 differentiation. ONO-4007 may affect the production of cytokines and chemokines by DCs, mast cells and Th2 cells which have fully differentiated and existed in the airways. Further studies are necessary to determine the mechanism of the inhibition of antigen-induced eosinophil recruitment into the airways by ONO-4007.

To elucidate the mechanism of the suppression of the IgE response using ONO-4007, naive CD4⁺ T cells from DO11.10 mice were stimulated with antigen peptides and DCs in the presence or absence of ONO-4007. Without affecting the proliferation of CD4⁺ T cells (data not shown), ONO-4007 inhibited the acquisition of IL-4- and IL-13-producing ability by CD4⁺ T cells. Furthermore, these effects of ONO-4007 on cytokine production were mediated, at least partly, by ONO-4007-pretreated DCs (fig. 4). Recently, murine Toll-like receptor 4 was reported to be responsible for immune responses to lipopolysaccharide, suggesting that those receptors on murine DCs may be involved in regulating the differentiation of naive CD4⁺ T cells by ONO-4007 [28, 29].

The differentiation of naive CD4⁺ T cells was regulated by many factors, such as the strength of signals via

their TCRs and the quantity and quality of costimulation as well as cytokines [30, 31]. Although ONO-4007 did not affect the expression of class II molecules, CD86, CD80 or CD54 on DCs (fig. 5), new costimulatory molecules, such as ICOS and ICOS-ligand which were recently demonstrated to regulate the differentiation of naive CD4⁺ T cells and IgE production, may be involved in the effects of ONO-4007 [32, 33]. Maldonado-Lopez et al. [34] reported two different subsets of DCs, IL-12-producing CD8 α ⁺ DCs and nonproducing CD8 α [−] DCs, which induce the differentiation of Th1 and Th2, respectively. We used a mixture of both subsets in the present experiments. However, pretreatment of ONO-4007 did not significantly affect either the recovery of DCs or the ratio of CD8 α ⁺ to CD8 α [−] DCs (data not shown). Furthermore, ONO-4007 did not significantly increase IL-12 production by DCs stimulated with anti-CD40 mAb (table 1). These results may rule out the primary role of IL-12 in modulating the differentiation of naive CD4⁺ T cells by ONO-4007. Although the precise mechanism remains to be clarified, our findings suggest that ONO-4007 inhibited the acquisition of IL-4- and IL-13-producing ability by naive CD4⁺ T cells, at least partly, by modulating DCs, and subsequently suppressing the IgE response.

We speculated that it may be possible to use the modulating effects of ONO-4007 on IgE production and antigen-induced recruitment of eosinophils into the airways as a therapeutic reagent for allergic diseases. In particular, since most patients with asthma have already been sensitized with allergens at the time of drug treatment, it was very interesting that treatment with ONO-4007 during inhalation, even after systemic sensitization, inhibited the recruitment of eosinophils into the airways. Further studies are necessary to elucidate the mechanism by which ONO-4007 suppresses the IgE response and allergic inflammation.

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