



A carbohydrate fraction, AIP1 from *Artemisia iwayomogi* suppresses pulmonary eosinophilia and Th2-type cytokine production in an ovalbumin-induced allergic asthma. Down-regulation of TNF- α expression in the lung

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TNF

Abstract

Asthma is a chronic inflammatory disease of the airways characterized by reversible airway obstruction, airway hyperreactivity, and remodeling of the airways. The incidence of asthma is on the rise despite ongoing intensive asthma research. *Artemisia iwayomogi*, a member of the Compositae, is a perennial herb easily found around Korea and has been used as a traditional anti-inflammatory medicine in liver diseases. We investigated suppressive effects of AIP1, a water-soluble carbohydrate fraction from *A. iwayomogi* on ovalbumin-induced allergic asthma in BALB/c mice and studied the possible mechanisms of its anti-allergic action. AIP1 significantly reduced pulmonary eosinophilia and Th2 cytokine expression in the lungs as well as serum IgE levels. Flow cytometric analysis of lung-infiltrating cells showed that the surface levels of CD11c and MHC II in CD11c+MHC II+ cells, potent dendritic cells, decreased in animals treated with AIP1. Expression of TNF- α , one of several proinflammatory cytokines released into the airway during episodes of asthma, was down-regulated by AIP1 injection, suggesting that reduced expression of TNF- α could account for the suppression of pulmonary eosinophilia and Th2-type cytokine production by AIP1. © 2007 Elsevier B.V. All rights reserved.

Abbreviations: AIP1, a carbohydrate fraction of *Artemisia iwayomogi*; OVA, ovalbumin; DC, dendritic cell.

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1. Introduction

Asthma is a chronic inflammatory disease of the airways characterized by reversible airway obstruction, airway hyperreactivity (AHR), and remodeling of the airways. The incidence of asthma is on the rise despite ongoing intensive asthma research. One of the characteristics of asthma is the infiltration of the airways with various cell types including eosinophils, mast cells, and T lymphocytes [1,2]. Several mediators secreted from these cells amplify the inflammatory cascade, increasing AHR and stimulating mucus secretion, which contribute to airway obstruction and airway remodeling [1,3,4]. Interleukin (IL)-4 and IL-5, two cytokines secreted from T helper (Th) 2 lymphocytes, have been shown to play a pivotal role in allergic airway inflammation. In IL-4 deficient (IL-4 $-/-$) mice immunized to aerosolized ovalbumin (OVA), there was markedly reduced eosinophilia and inflammation compared with wild type mice [5,6]. In addition, IL-5 and immunoglobulin (Ig)E production in wild type mice was inhibited when anti-IL-4 antibody was administered during the immunization. IL-5 induces eosinophil differentiation, activates them and acts as an eosinophil chemotactic factor [7,8]. In contrast, interferon (IFN)- γ and IL-12, Th1-type cytokines, inhibit IgE secretion and eosinophil infiltration [9–11].

A number of studies have been reported that tumor necrosis factor (TNF)- α plays an important role in the pathogenesis of asthma. TNF is released into the airway during episodes of asthma, and increased levels of TNF mRNA and protein are detected in bronchoalveolar lavage fluid (BALF) from asthma patients with symptoms compared with asymptomatic asthmatics [12–14]. The levels of TNF- α in the BALF were also increased in mice sensitized and challenged with ovalbumin [15]. TNF- α aggravates airway inflammation and bronchial hyperresponsiveness in animals and human beings as well as recruitment of inflammatory cells into the airways [16–18].

Dendritic cells (DC) are the most potent antigen-presenting cells (APC) and are particularly important in initiating primary T cell-mediated immune responses [19]. DC exist in different maturation stages, which are characterized by the expression of key accessory molecules and the functional capacity of the cells. Immature DC express relatively low levels of major histocompatibility complex (MHC) class II and costimulatory molecules such as B7-2 (CD86) and have a high capacity of antigen uptake but poor T cell stimulating activity. Upon encounter of antigen, inflammatory stimuli or bacterial products, they migrate to the T cell areas of lymphoid tissues up-regulating MHC class II and B7-2 molecules and become mature to activate T cells. DC have multiple subsets based on expression patterns of cell surface molecules and potential functions: myeloid, lymphoid, and plasmacytoid DC, and Langerhans cells [20,21]. Pulmonary DC play a pivotal role in sampling and presenting antigen in the lung, activating CD4 $^{+}$ T cells and preferentially inducing Th2-biased adaptive immune responses such as allergic asthma. CD8-myeloid DC induced Th2 responses to inhaled ovalbumin antigen in mice whereas CD8 $^{+}$ lymphoid DC reduced Th2 cytokines leading to weak eosinophilic inflammation [22]. In a similar study, depletion of plasmacytoid DC during inhalation of normally inert antigen led to IgE sensitization, eosinophilia, and Th2 cytokine production,

indicating that plasmacytoid DC provide intrinsic protection against inflammatory responses to harmless antigen [23].

Corticosteroids have become the most effective therapy currently available for persistent asthma [24]. However, a small proportion of asthma patients fail to respond to these drugs [25]. Patients with a chronic disease such as asthma often use complementary or alternative therapies: acupuncture, aromatherapy, breathing techniques, and herbal medicine. There are many reports on the effectiveness of herbal medicines on asthma, although a large proportion of those studies were not adequately designed trials [26].

Artemisia iwayomogi, a member of the Compositae, is a perennial herb easily found around Korea and the extract of *A. iwayomogi* has been used for treatment of various liver diseases including hepatitis [27,28]. *A. iwayomogi* has been shown to have various biological functions. Methanol extracts of *A. iwayomogi* inhibited nitric oxide production of lipopolysaccharide-activated macrophages [29] and two sesquiterpenes from *A. iwayomogi*, 3-O-methyl-isosecotanapartholide and iso-secotanapartholide, were shown to inhibit expression of inducible nitric oxide synthetase (iNOS) [30]. In another study methanol extracts of *A. iwayomogi* displayed scavenging activity of peroxynitrite (ONOO^{-}), a potent cytotoxic oxidant, formed by the reaction between nitric oxide (NO) and superoxide radical (O_2^{-}) [31]. NO synthesized by the action of iNOS and its derivatives such as ONOO^{-} play a detrimental role in inflammation and asthma [32]. AIP1, a water-soluble carbohydrate fraction from *A. iwayomogi*, showed anti-tumor and immuno-modulating activities [33] and suppressed spontaneous or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced apoptotic death of mouse thymocytes probably by down-regulating Fas gene expression [34,35].

In the present study we examined therapeutic effects of AIP1 on OVA-induced allergic asthma in mice and investigated mechanisms underlying the anti-allergic function of AIP1.

2. Materials and methods

2.1. Mice

BALB/c mice, 6–12 weeks of age, were purchased from the Korean Institute for Chemistry (Taejeon, Korea). The animals were housed 5 mice per cage in a laminar air flow room maintained at $22 \pm 2^{\circ}\text{C}$ with relative humidity of $55 \pm 5\%$. Mice were cared and treated in accordance with the guidelines established by the Public Health Service Policy on the Human Care and Use of Laboratory Animals.

2.2. Chemicals and reagents

Lipopolysaccharide (LPS) was purchased from Sigma-Aldrich (USA). Murine GM-CSF (Granulocyte-Monocyte Colony Stimulating Factor) and IL-4 were from Pierce (USA) and R & D (USA), respectively.

2.3. Preparation of *A. iwayomogi* extract and purification of AIP1 fraction

A. iwayomogi was purchased from Daewon herbal medicine dealer (Changwon, Korea). A voucher specimen (CNUBio00101) was deposited at the Herbarium of the Department of Biology, Changwon National University. A crude extract was prepared by percolating dried leaves of *A. iwayomogi* with distilled water at 100°C for 2 h.

Table 1 Primers used in RT-PCR

Name	Nucleotide sequence	Annealing temperature (°C)	Cycles ^a	Size ^b (bp)
mIL4-fw	5'-acaggagaaggacgcat-3'	60	35	95
mIL4-rv	5'-gaagccctacagacgagctca-3'			
mIL12p40-fw	5'-ggaagcacggcagcagaata-3'	60	35	180
mIL12p40-rv	5'-aacttgaggagaagtaggaatgg-3'			
mIL5-fw	5'-agcacagtgggtgaaagagacctt-3'	60	35	117
mIL5-rv	5'-tccaatgcatagctggtgattt-3'			
mTNF α -fw	5'-catcttctcaaaattcgagtacaa-3'	60	35	175
mTNF α -rv	5'-tgaggtagacaaggtacaaccc-3'			
mIFN γ -fw	5'-tcaagtggcatagatgtggaaga-3'	60	35	92
mIFN γ -rv	5'-tggtctgcaggatttctatg-3'			
β -actin-F	5'-gcaccacaccttctacaatgag-3'	60	30	150
β -actin-R	5'-aatagcacagcctggatagcaac-3'			

^a Indicates the number of cycles of amplification.^b Indicates the expected size of PCR products.

The extract was filtered with Whatman No.1 paper and lyophilized. The yield of dried extract from starting crude materials was about 10%. The dried extract was dissolved in phosphate buffered saline (PBS), autoclaved and stored at 4 °C. AIP1 fraction was isolated from *A. iwayomogi* as described by Koo et al. [33] and Ji et al. [35]. Briefly, a water-soluble crude extract was fractionated using sephadex G-50 size exclusion chromatography with distilled water. The fractions containing carbohydrates or similar compounds were determined by the modified phenol/sulfuric acid method of total sugar determination [36], producing a minor peak around 50 kDa and a major peak smaller than 2 kDa. The fractions smaller than 2 kDa were pooled and used as AIP1 fraction [33]. The potential presence of endotoxin in AIP1 was tested by the Limulus amoebocyte lysate assay (Sigma, USA). No detectable endotoxin was present in AIP1.

2.4. Antigen sensitization, challenge and administration of AIP1

Sensitization and challenge with OVA was performed according to the method of Kung et al. [37] with minor modifications. On day 0, mice were sensitized with an intraperitoneal (i.p.) injection of 15 μ g of OVA and 2 mg of alum dissolved in 0.2 ml phosphate buffered saline (PBS). The animals received a booster injection of this alum-OVA mixture 5 days later. Twelve days after the first sensitization, the mice were exposed for 1 h to aerosolized OVA (0.5% dissolved in 0.9% saline) which was produced by an ultrasonic nebulizer at a flow rate of 6 l/min. At the 13th day, mice were challenged again with OVA as performed on day 12. Mice were sacrificed at day 14. AIP1 (0.5–50 mg/kg body weight) in 0.1 ml was i.p. injected six times at days 0, 5, 12 and 13.

2.5. Bronchoalveolar lavage fluid (BALF) preparation

The tracheas of mice were cannulated and lavaged with two 0.8 ml aliquots of cold PBS. Number of cells in the BALF was counted and used for cytospin and staining.

2.6. Measurement of total IgE in serum

Serum samples from each experimental group were analyzed for total IgE. Complementary capture and detection antibody pairs for mouse IgE antibody were purchased from Pharmingen (USA), and the IgE enzyme-linked immunosorbent assay (ELISA) was performed according to the manufacturer's directions. Duplicate serum samples were diluted 1:100. IgE levels in each sample were measured from optical density readings at 405 nm, and IgE concentrations were

calculated from a standard curve generated using recombinant IgE (5–2000 pg/ml).

2.7. Isolation of dendritic cells from lungs

Animals were euthanized and lungs were collected. Lungs were mechanically disrupted before a 40-min incubation in 5 ml of RPMI 1640 with collagenase D (1 mg/ml), DNase I (0.02 mg/ml) (Sigma-Aldrich, USA), 0.2% BSA and 10 mM HEPES at 37 °C. The tissue fragments were passed through a stainless-steel sieve and the resulting cell suspension was washed by centrifugation in RPMI 1640. After lysing the RBCs with 0.165 M NH₄Cl, cells were stained with antibodies and flow-cytometrically analyzed.

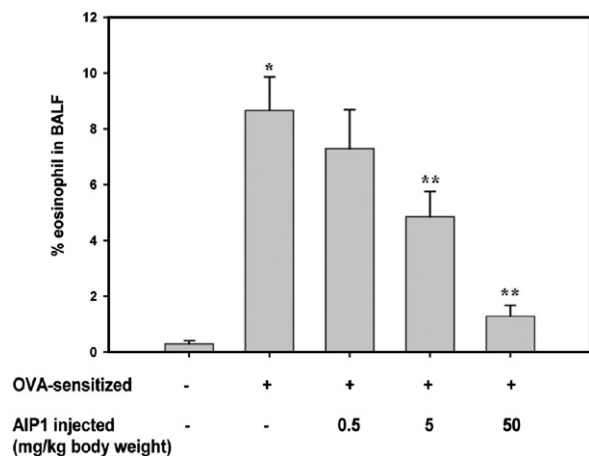


Figure 1 AIP1 effects on eosinophil infiltration into the lungs after 24 h OVA challenge. Five female mice were sensitized with alum-OVA mixture on days 0 and 5, and challenged on days 12 and 13. On day 14, mice were sacrificed for collection of BALF. For AIP1 administration, AIP1 was i.p. injected as described in the Materials and methods. Cells were fixed in methanol, and stained with 'Hemacolor for microscopy' and identified under the microscope according to the criteria described in the protocol (Merck, USA). Data are presented as mean $\pm$ SD (5 mice per group) and are representative of three separate experiments. Statistical significance was analyzed using the Student's *t*-test. * p <0.05 compared with nonsensitized. ** p <0.05 compared with OVA-sensitized but not injected with AIP1.

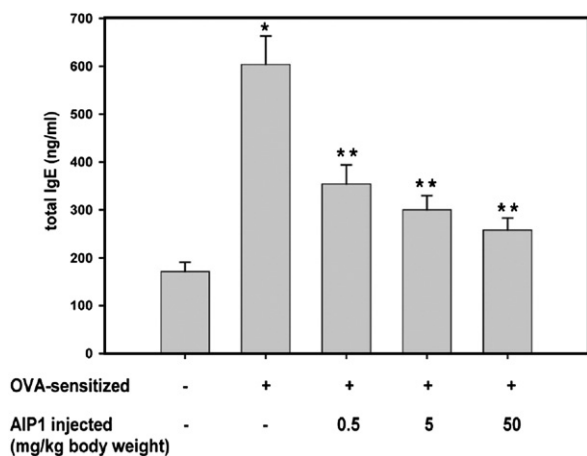


Figure 2 AIP1 reduced total serum IgE levels in OVA-sensitized mice. Five female mice were sensitized with alum-OVA mixture on days 0 and 5, and challenged on days 12 and 13. On day 14, mice were sacrificed for lung DC. For AIP1 administration, AIP1 was i.p. injected as described in the Materials and methods. Sera were prepared and assayed for total IgE by ELISA. The data are the means $\pm$ SD of three independent experiments performed in triplicate. Statistical significance was analyzed using the Student's *t*-test. * $p < 0.05$ compared with nonsensitized. ** $p < 0.05$ compared with OVA-sensitized but not injected with AIP1.

2.8. RNA preparation, reverse transcription-polymerase chain reaction (RT-PCR), and Real-Time PCR

Total cellular RNA was extracted from cells using the RNAzol method (TEL-TEST, INC., USA). For PCR analysis, RNA was used after contaminating DNA was completely removed by DNase I treatment. RT-PCR analysis was performed using pairs of oligonucleotide primers. The PCR products were confirmed to correspond to their original sequence by DNA sequencing. PCR products were comparatively quantitated by GS-700

densitometer (BIO-RAD, USA). Gene specific primers, number of cycles of amplification, annealing temperature, and expected size of PCR product are listed in Table 1. Real-Time PCR was performed to confirm the RT-PCR results. Power SYBR green PCR Master Mix and Real-Time PCR system (7300, Applied Biosystems, USA) were used.

2.9. Flow cytometry

Cells were kept on ice or at 4 °C at all time. Immunofluorescence staining was carried out in 96-well, round-bottomed cell culture plates. The cells were centrifuged and resuspended in 10 μ l of primary antibody diluted with PBS at a previously determined optimal concentration. The following antibodies were used: CD11c, clone HL3 (Pharmingen, USA); MHC II, clone M5/114.15.2 (BioLegend, USA). After 15 min of incubation, the cells were washed with washing buffer (PBS/0.5% BSA/0.09% sodium azide) three times and fixed with 0.9% buffered formalin. For biotin-conjugated primary antibodies, streptavidin-APC conjugate (Pharmingen, USA) was secondarily used. Cells were analyzed within 1 week on a FACSCalibur flow cytometer (Becton Dickinson, USA).

2.10. Cytospin preparations and Giemsa staining

Cells (4×10^4 cells) were centrifuged in 100 μ l PBS with 3% BSA onto a microscope slide using a CellSpin (Hanil Science, Korea). Cells were fixed in methanol, and stained with 'Hemacolor for microscopy' and identified under the microscope according to the criteria described in the protocol (Merck, USA).

2.11. Measurement of cytokine in the lung

Whole lungs were collected from mice 24 h after aerosolized OVA challenge. Frozen lung tissue was chopped after thawing and was suspended in 1 ml of 50 mM HEPES buffer containing 1 mM EDTA and 50 mM phenylmethylsulphonyl fluoride (PMSF). Samples were homogenized using Dounce tissue grinder (Wheaton Science, USA) and centrifuged (at 15,000 g, for 15 min at 4 °C). The levels of cytokines were measured using enzyme-linked immunosorbent assay (ELISA) kit purchased from R & D Systems (USA).

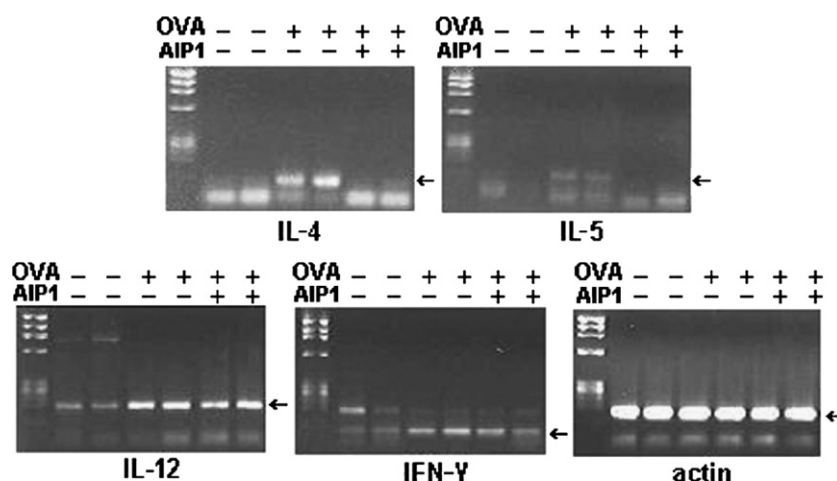


Figure 3 Effects of AIP1 on the expression of Th1 and Th2 cytokines in the lung. Mice were sensitized with alum-OVA mixture on days 0 and 5, and challenged on days 12 and 13. On day 14, mice were sacrificed for the lung. For AIP1 administration, AIP1 was i.p. injected (5 mg/kg body weight) as described in the Materials and methods. RNA was prepared from the lungs and examined for gene expression by RT-PCR. Three female mice for each group were chosen for RT-PCR analysis and the data represent two samples for each group. Actin was used as an internal control. Molecular weight markers are shown to the left in each gel photograph. The arrows indicate the expected PCR product for each gene.

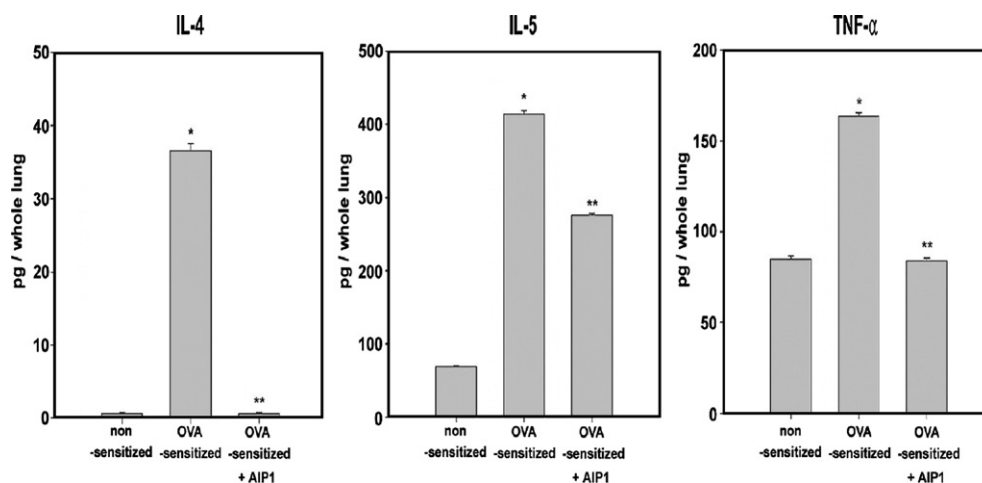


Figure 4 Cytokine generation in lung tissue. Five female mice were sensitized with alum-OVA mixture on days 0 and 5, and challenged on days 12 and 13. On day 14, mice were sacrificed and whole lung was collected for cytokine. For AIP1 administration, AIP1 was i.p. injected (5 mg/kg body weight) as described in the Materials and methods. Lung extracts were prepared and assayed for IL-4, IL-5, and TNF- α by ELISA. The data are the means $\pm$ SD of three independent experiments performed in triplicate. Statistical significance was analyzed using the Student's *t*-test. * $p < 0.05$ compared with nonsensitized. ** $p < 0.05$ compared with OVA-sensitized but not injected with AIP1.

2.12. Statistical analysis

Data were analyzed with the paired Student's *t* test. The level of significance was $p < 0.05$.

3. Results

3.1. Reduced pulmonary eosinophilia in BALB/c mice treated with AIP1

BALB/c mice were sensitized with alum-precipitated OVA and challenged with aerosolized OVA. 24 h after the last challenge of OVA, mice were sacrificed. BALF cells were centrifuged onto a microscope slide, stained with Hemacolor, and observed under a

microscope. Percentages of eosinophils present in the total leukocytes are shown in Fig. 1. Percentages of eosinophils in OVA-sensitized mice significantly increased compared to unsensitized counterparts. When AIP1 was i.p. administered at concentrations ranging from 0.5 to 50 mg/kg body weight, percentages of eosinophils dose-dependently reduced.

3.2. Reduced serum IgE concentration in BALB/c mice treated with AIP1

Next, we measured levels of serum IgE in BALB/c mice which were sensitized with alum-precipitated OVA and challenged with aerosolized OVA. The serum levels of IgE antibodies were determined 24 h after the last challenge of OVA. Unsensitized

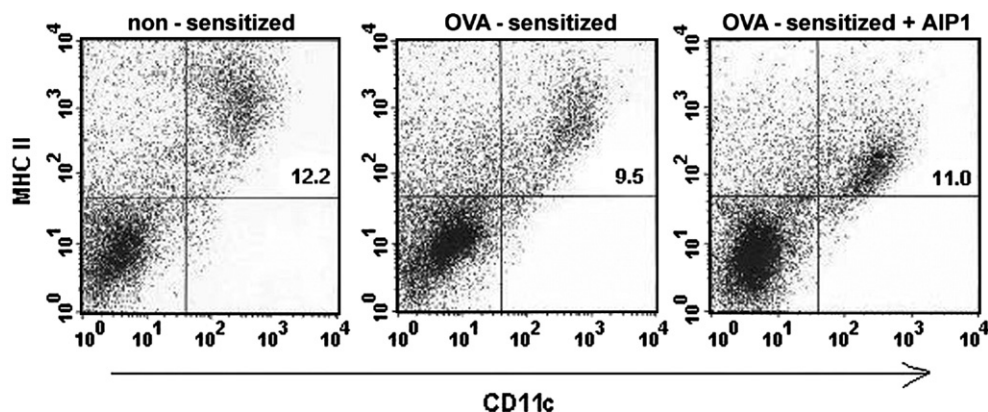


Figure 5 Effects of AIP1 on cell surface expression of CD11c and MHC II on lung DC. Female mice were sensitized with alum-OVA mixture on days 0 and 5, and challenged on days 12 and 13. On day 14, mice were sacrificed for lung DC. For EAC administration, EAC was i.p. injected (5 mg/kg body weight) as described in the Materials and methods. Lung cells were harvested, stained with stained with CD11c and MHC II, and analyzed on a FACSCalibur flow cytometer using the Cellquest program. The data shown here are representative of three separate experiments ($n = 3-5$ mice per group). Numbers on the plots represent percentages of gated cells within the indicated quadrants.

BALB/c mice produced lower levels of IgE (about 180 ng/ml of serum) whereas the OVA group produced significantly increased levels of IgE (about 600 ng/ml of serum) (Fig. 2). Levels of serum IgE dose-dependently decreased when AIP1 was i.p. administered at concentrations ranging from 0.5 to 50 mg/kg body weight. From this point, all the experiments were carried out at the AIP1 concentration of 5 mg/kg body weight.

3.3. Expression of type 2 cytokines, IL4 and IL-5, were suppressed in mice injected with AIP1

Next, we determined the levels of IL-4, IL-5, IL-12 (p40), and IFN- γ in lungs of BALB/c mice. 24 h after the last challenge of OVA, lungs from the unsensitized, OVA-sensitized, or OVA-sensitized and AIP1-injected groups were harvested for RT-PCR analysis. Three mice from each group were randomly selected and their lungs were assayed for cytokine expression (Fig. 3). All three mice of the OVA-sensitized group expressed significantly increased levels of IL-4 and IL-5 over the unsensitized group (more than 3–5 fold increase for both cytokines based on Real-Time PCR). When AIP1 was injected to OVA-sensitized mice, the levels of IL-4 and IL-5 mRNAs in three mice fell back to those of the unsensitized group. To confirm the mRNA levels of cytokines expressed in the lung, the amount of Th2 cytokines IL-4 and IL-5 was directly measured in the lung. The levels of IL-4 and IL-5 proteins which increased upon OVA-sensitization significantly reduced by AIP1 administration (Fig. 4). The levels of IFN- γ and IL-12 were increased in the OVA-sensitized group compared to the unsensitized group. However, AIP1 injection was not able to revert the increased levels of IFN- γ and IL-12 observed in the OVA-sensitized group to those of the unsensitized group.

3.4. Reduced surface expression of MHC II molecules on CD11c+ lung cells in mice injected with AIP1

Next, we examined whether AIP1-suppression of eosinophilia, IgE secretion, and Th2 cytokine production in OVA-sensitized mice might be associated with function and differentiation of DC. To study these possibilities, we determined the levels of MHC II in CD11c+ populations in lungs of BALB/c mice. CD11c is generally used as a molecular marker for mouse DC. 24 h after the last challenge of OVA, lungs from the members of each group were harvested and treated with collagenase. The resulting cells were examined for the expression of CD11c and MHC II. Although there is little alterations in percentages of CD11c+ MHC II+ cells among the three groups (Fig. 5), MHC II-related mean fluorescence intensity (MFI) of the AIP1-injected group significantly decreased compared to the unsensitized or OVA-sensitized group (Fig. 5, Table 2).

Table 2 Flow cytometric analysis of molecules characterizing pulmonary dendritic cells

	Nonsensitized	OVA-sensitized	OVA-sensitized+AIP1
CD11c	303 $\pm$ 45	434 $\pm$ 96	275 $\pm$ 21
MHC II	1946 $\pm$ 131	1207 $\pm$ 324	348 $\pm$ 85

Results are expressed as mean fluorescence intensity (MFI) and are the means $\pm$ SD of three independent experiments.

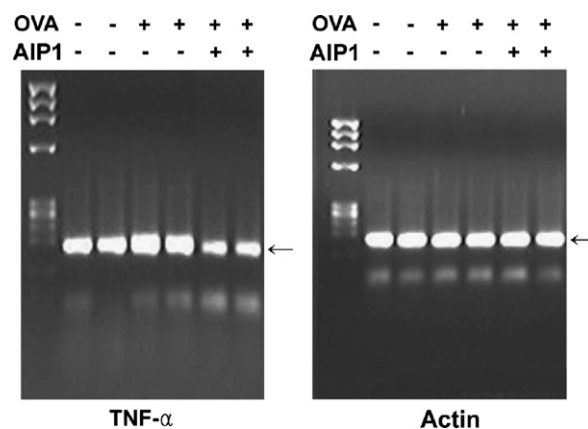


Figure 6 TNF- α expression in the lung is reduced upon administration of AIP1. Female mice were sensitized with alum-OVA mixture on days 0 and 5, and challenged on days 12 and 13. On day 14, mice were sacrificed for lung DC. For AIP1 administration, AIP1 was i.p. injected (5 mg/kg body weight) as described in the Materials and methods. RNA was prepared from the lungs and examined for gene expression by RT-PCR. Three mice for each group were chosen for RT-PCR analysis and the data represent two samples for each group. Actin was used as an internal control. Molecular weight markers are shown to the left in each gel photograph. The arrows indicate the expected PCR product for each gene.

3.5. Expression of TNF- α in the lungs was reduced in mice treated with AIP1

As mentioned in the Introduction section in this study, TNF- α plays an important role in the pathogenesis of asthma. Increased levels of TNF mRNA and protein are detected in BALF from asthma patients with symptoms compared with asymptomatic asthmatics [12–14]. The levels of TNF- α in the bronchoalveolar lavage fluid (BALF) were also increased in mice sensitized and challenged with ovalbumin [15]. Thus, we measured mRNA levels of TNF- α in the lung from the unsensitized, OVA-sensitized, or OVA-sensitized and AIP1-injected groups. All three mice of the OVA-sensitized group expressed significantly increased levels of TNF- α over the unsensitized group (more than 2–4 fold increase based on Real-Time PCR). When AIP1 was injected to OVA-sensitized mice, the levels of TNF- α mRNAs in three mice drastically decreased (Fig. 6). To confirm the mRNA levels of TNF- α expressed in the lung, the amount of TNF- α was directly measured in the lung. The level of TNF- α protein which increased upon OVA-sensitization significantly was reduced by AIP1 treatment (Fig. 4).

4. Discussion

There have been a few studies that certain traditional herbal medicines have therapeutic effects in allergic asthma [38]. In this study, we have demonstrated that AIP1, a water-soluble carbohydrate fraction from *A. iwayomogi*, significantly reduced pulmonary eosinophilia and Th2 cytokine expression in the lungs of BALB/c mice sensitized and challenged by OVA. Flow cytometric analysis of lung-infiltrating cells showed that surface levels of MHC II in CD11c+ MHC II+ cells decreased upon treatment of AIP1.

Expression of proinflammatory cytokine TNF- α was down-regulated by AIP1 injection, suggesting that reduced expression of TNF- α could account for the suppression of pulmonary eosinophilia and Th2-type cytokine production by AIP1.

Elevated serum IgE levels are associated with bronchial asthma, a disease characterized by eosinophilic inflammation of the airways. Activation of antigen-specific T helper 2 cells in the lung with the subsequent release of IL-4 and IL-5 is believed to play an important role in the pathogenesis of this disease. That the reduced expression of IL-4 and IL-5 in the lungs from mice treated with AIP1 is associated with a decrease in pulmonary eosinophilia and serum IgE levels also supports the central role of IL-4 and IL-5 in allergic asthma.

Immature DC express relatively low levels of major histocompatibility complex (MHC) class II compared to mature DC. In the present study, CD11c⁺ populations isolated from the lung of mice sensitized with OVA and injected with AIP1 expressed significantly reduced levels of MHC II compared to OVA-sensitized or unsensitized counterparts, suggesting that AIP1 could interfere with functional differentiation of pulmonary immature DC to mature DC. Different subsets of lung DC are differentially distributed [39]. CD11c⁺ myeloid DC are predominant and plasmacytoid DC positive for Gr-1, B220, and 120G8 are recently identified [40]. Most CD11c⁺ myeloid DC in the lung is immature assessed by the low expression of the costimulatory molecules CD40, CD80, and CD86 and by the high expression of several receptors for inflammatory cytokines and endocytic receptors [39]. Our results that AIP1 reduced expression of MHC class II molecules on pulmonary DC could provide a piece of evidence for the modulatory role of AIP1 on functional differentiation of pulmonary DC in vivo. In other study related to AIP1, we were able to show that AIP1 not only reduced expression of MHC II molecules on DC derived from bone marrow (BM-DC) cultured in the presence of granulocyte monocyte-colony stimulating factor (GM-CSF) and IL-4, but also diminished the allogeneic T cell stimulating ability of BM-DC (in preparation for submission), suggesting that AIP1 could inhibit functional differentiation of pulmonary DC in vivo, thereby resulting in the reduced expression of MHC II on them.

In this study, we have shown that expression of TNF- α was inhibited by AIP1 administration. TNF- α is first produced as a cell membrane-bound 26 kDa molecule that is cleaved from the cell surface by a TNF- α converting enzyme to generate a secreted 17 kDa form of the TNF molecule. Secreted forms of TNF- α interact as homotrimers with two structurally related TNF receptors (TNF-Rs), namely TNF-R1 and TNF-R2 [41]. TNF- α is among the most potent inflammatory cytokines produced by myeloid cells such as mast cells and macrophages and thus has a variety of immunomodulatory functions. TNF- α released at sites of allergic inflammation contributes to leukocyte recruitment by increasing the expression of endothelial cell adhesion molecules, including intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and E-selectin [42,43]. In studies done in an ovalbumin-induced asthma model in mice, eosinophilic inflammation was inhibited in TNF-R p55 deficient mice [44,45], resulting in significant inhibition of the late phase airway hyperresponsiveness. TNF- α produced by mast cells contributes to the pathogenesis of mast cell-dependent and IgE-dependent, ovalbumin-induced allergic inflamma-

tion in mice, probably by enhancing Th2 cell proliferation and activation such as the increased production of IL-4 [46]. TNF- α blocks or enhances the expression of MHC class II molecules depending on cell type and cellular differentiation state [47,48]. In immature cells such as HL-60 or THP-1, TNF- α enhances IFN- γ -induced class II expression. However, TNF- α down-regulates the IFN- γ -induced class II expression in differentiated cells such as human skin fibroblasts or activated macrophages. Such a reduced expression of class II molecules could decrease the capacity of antigen-presenting cells to stimulate T cells [49]. Dendritic cells (DC), the most potent antigen-presenting cells, can be generated from bone marrow (BM) precursor cells in the presence of GM-CSF and IL-4. TNF- α plays an important role in the development of immature DC (CD11c⁺, MHC class II^{low}, CD40^{low}, CD86^{low}) to mature DC (CD11c⁺, MHC class II^{high}, CD40^{high}, CD86^{high}) [50].

Water soluble extracts of *A. iwayomogi* was previously shown to have anti-allergic effects in a mast cell-mediated allergy model [51]. *A. iwayomogi* extract inhibited compound 48/80-induced systemic reactions and plasma histamine release. The AIP1 fraction used in this study is a mixture of water-soluble reducing sugars with side chains of UV-absorbing ability. The average molecular weight of the mixture is less than 1000 Da based on the high performance size exclusion chromatography (unpublished observations, Youn HJ). The AIP1 fraction has modulatory effects on the expression of several genes in murine thymocytes [34,35]. We are currently trying to identify the active component of AIP1 and to reveal the mechanisms underlying the functions of AIP1.

In conclusion, the results obtained in this study suggest that a new antiallergic therapy using AIP1, a carbohydrate fraction of *A. iwayomogi* may be promising. Future studies will elucidate the active components of AIP1 and the mechanisms underlying the functions of AIP1.

Acknowledgment

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