Biochemical and Biophysical Research Communications 390 (2009) 103–108

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Demethylallosamidin, a chitinase inhibitor, suppresses airway inflammation and hyperresponsiveness

Takafumi Matsumoto [a], Hiromasa Inoue [a,*], Yosuke Sato [b], Yoshihiro Kita [c], Takako Nakano [a], Naotaka Noda [a], Miyuki Eguchi-Tsuda [a], Atsushi Moriwaki [a], Keiko Kan-o [a], Koichiro Matsumoto [a], Takao Shimizu [c], Hiromichi Nagasawa [b], Shohei Sakuda [b,*], Yoichi Nakanishi [a]

[a] Research Institute for Diseases of the Chest, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[b] Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan
[c] Department of Biochemistry and Molecular Biology, Faculty of Medicine, The University of Tokyo, Tokyo, Japan

ARTICLE INFO

Article history:
Received 6 September 2009
Available online 24 September 2009

Keywords:
Asthma
Chitinase inhibitor
IL-13
Inflammation
Lipid mediator

ABSTRACT

Acidic mammalian chitinase is upregulated in response to allergen exposure in the lung. We investigated the effects of chitinase inhibitors, allosamidin (Allo) and demethylallosamidin (Dma), on asthmatic responses. Mice were subjected to IL-13 instillation into the airways or to ovalbumin sensitization plus exposure with or without treatment of Allo or Dma. Airway hyperresponsiveness (AHR) and inflammation were evaluated. Allo and Dma attenuated airway eosinophilia and the upregulation of eotaxin after IL-13 instillation, while Dma, but not Allo, suppressed AHR in IL-13-induced asthma. Allo or Dma suppressed the elevated chitinase activity in BAL fluids after IL-13 to similar levels. The bronchoprotective PGE_2 levels in BAL fluids were elevated after IL-13 instillation. Allo, but not Dma, suppressed the overproduction of PGE_2 and the expression of COX-2 and PGE synthase-1 induced by IL-13. In ovalbumin-induced asthma, Dma suppressed AHR more strongly than Allo. These findings suggest that Dma attenuates asthmatic responses induced by IL-13 without affecting PGE_2 synthesis. Dma may have potential as therapeutic agents for asthma.

Introduction

Bronchial asthma is a chronic inflammatory disease characterized by eosinophilic infiltration, airway hyperresponsiveness (AHR) to non-specific stimuli, and remodeling of the airways [1,2]. T-helper-2 (Th2) cytokines, including interleukin (IL)-4, IL-5, and IL-13, are essential for generating these abnormalities. The *in vivo* blockade of IL-13 markedly inhibits allergen-induced AHR, eosinophilia, and mucus overproduction [3,4]. Furthermore, local administration of recombinant IL-13 to non-immunized mice induces the asthma phenotype [4,5]. Among Th2 cytokines, IL-13 is now considered particularly critical.

Chitinases are enzymes that cleave chitin, an abundant polysaccharide present in fungal walls, the cuticles of helminths, and the exoskeletons of arthropods. Although chitin is not present in mammals, two chitinases, chitotriosidase and acidic mammalian chitinase (AMCase), are present in human and mouse [6]. The physiological roles of the chitinases are not clear enough. AMCase is upregulated in response to allergen exposure or IL-13-induced inflammation in the lung [7,8]. Inhibition of AMCase with anti-AMCase sera led to lower eosinophil counts as well as to the reduction of AHR without affecting Th2 cytokine expression in a mouse model of asthma [8]. Recent studies demonstrated that AMCase stimulates chemokine production from pulmonary epithelial cells and regulates epithelial cell apoptosis [9,10]. Although it has been reported that transgenic mice overexpressing AMCase showed no signs of allergic inflammation [11], it is possible that AMCase may act as a proinflammatory mediator in IL-13 effector responses.

Allosamidin (Allo), a metabolite of *Streptomyces*, inhibits all family 18 chitinases [12]. Allo has been used to investigate the physiological role of chitinases involved in a variety of organisms. It has been reported that Allo can suppress allergen-induced airway eosinophilia [8], whereas its effects on AHR after allergen challenge or on IL-13-induced asthmatic responses are not known. Compared to Allo, demethylallosamidin (Dma), an allosamidin congener, showed much stronger inhibitory activity toward yeast chitinases [13] and human chitotriosidase, which has 52% amino acid sequence similarity to AMCase [14]. These facts prompted us to investigate the effects of Allo and Dma on asthmatic responses as well as their inhibitory activity toward AMCase.

* Corresponding authors. Fax: +81 92 642 5389 (H. Inoue), +81 3 5841 8022 (S. Sakuda).
E-mail addresses: inoue@kokyu.med.kyushu-u.ac.jp (H. Inoue), asakuda@mail.ecc.u-tokyo.ac.jp (S. Sakuda).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.09.075

In the present study, we demonstrate much stronger activities of Dma than of Allo on attenuating asthmatic responses, especially AHR, in both IL-13-induced and allergen challenge mouse models and the AMCase inhibitory activity of Allo and Dma.

Materials and methods

Additional details regarding the methods are provided in Supplementary material.

Animals. Male A/J mice and male Balb/C mice of 8–10 weeks of age were used. All procedures and protocols were approved by the Kyushu University Animal Care and Use Committee.

Allosamidin and demethylallosamidin. Allo and Dma were prepared according to the method described previously [15].

IL-13 treatment in vivo. A/J mice received an intratracheal instillation of 0.5 μg recombinant mouse IL-13 solution or PBS on Days 1, 3, and 5. Allo (10 mg/kg), Dma (1 mg/kg), or vehicle acetic acid was administered intraperitoneally 24 h before the first instillation of IL-13 and 1 h before each instillation of IL-13 on 5 consecutive days (Day 0–5). Measurements of the airway responsiveness and bronchoalveolar lavage (BAL) were performed 24 h after the last instillation. A selective cyclooxygenase-2 (COX-2) inhibitor, NS-398 (1 mg/kg) [16], or vehicle was administered intraperitoneally 24 h before the first instillation of IL-13 and 2 h before each instillation of IL-13 on 5 consecutive days.

OVA sensitization and challenge. Balb/C mice were sensitized with intraperitoneal injections of 20 μg OVA (Grade V) plus 2.25 mg aluminum hydroxide on days 1 and 14. On days 26–28, mice received an aerosol challenge containing either saline or 1% OVA for 20 min per day. On day 30, 36 h after the last aerosol challenge, mice were ventilated to measure AHR [17].

Measurement of airway responsiveness. Mice were anesthetized, and their tracheas were cannulated via tracheostomy. The animals were ventilated to measure AHR to acetylcholine aerosol as described previously [5].

BAL and cell counting. Mice were given a lethal dose of pentobarbital, and lungs were gently lavaged one time with 1.0 ml saline via the tracheal cannula. Total cell counts and differential counts were performed [5].

Measurement of eotaxin and lipid mediators. Mouse eotaxin and PGE_2 in the supernatant of BAL fluids were measured using ELISA kits. The concentration of lipoxin A_4 was measured using commercially available Abs and according to the procedures supplied by the manufacturer (Neogen, Lexington, KY). The concentration of LTB_4, LTC_4, PGF_{2a}, PGD_2, 6-ketoPGF$_{1a}$, or 11-HETE was measured by a multiplex quantitation method using column-switching reversed-phase liquid chromatography–tandem mass spectrometry [18].

Expression of recombinant mouse AMCase. Primers flanking the coding region of mouse AMCase and incorporating EcoRI and XhoI sites were synthesized as the following: sense, 5′-ATCAGAATTCGCCGCCACCATGGCCAAGCTAC-3′; and anti-sense, 5′-TTTCTCTCGAGATGGCATTAGGTTCATGGC-3′. Using these primers and the lung cDNA from mice as the template, a cDNA fragment was obtained by PCR and cloned into the pcDNA 3.1 vector with the CMV immediate-early enhancer/promoter for expression in mammalian cells. AMCase was isolated after transfection to COS-7 cells. The expression of AMCase was confirmed by the activity staining analysis and the presence of two values of optimum pH characteristic of AMCase [6].

Chitinase assay. A chitinase assay was performed using 4-methylumbelliferyl-*N*,*N*′,*N*″-triacetyl chitotrioside as a substrate [19]. The liberated 4-methylumbelliferone was measured with a fluorescence spectrometer. The value of the fluorescence strength was used for the calibration of the relative chitinase activity.

Chitinase activity staining. After SDS–polyacrylamide gel electrophoresis, the gel was transferred to a polyacrylamide gel containing glycolchitin. The chitinase lytic bands on the gel were detected using a UV transilluminator.

Cell culture and reagents. A mouse tracheal epithelial cell line, TGMBE-02–3 cells, was cultured in a D-MEM/F-12 medium supplemented with 10% FCS, 1% ITES (2 μg/ml insulin, 2 μg/ml transferrin, 122 ng/ml ethanolamine, and 9.14 ng/ml sodium selenite), and 10 ng/ml mouse EGF.

Quantitative real-time PCR for COX-2, PGE synthase-1,15-PGDH. PGE_2 pathway-related mRNA expression in mouse lung was determined by means of reverse transcription followed by real-time quantitative PCR. The primers and probes of PGE_2-related pathway (COX-2, Mm00478374_m1; microsomal PGE synthase-1, Mm00452105_m1; 15-hydroxyprostaglandin dehydrogenase, 15-PGDH, Mm00515122_m1) and mouse β-actin (4352341E) were purchased from Applied Biosystems (Foster City, CA).

Data analysis. Values are expressed as means ± SEM. Differences between groups were analyzed by analysis of variance, and the significance of differences between values was assessed by Bonferroni correction. Values of $p < 0.05$ were considered significant.

Results

Effect of chitinase inhibitors on IL-13-induced asthma

To examine the effects of chitinase inhibitors on IL-13-induced asthmatic responses and allergen-induced responses, the dose of Allo or Dma that abolished eosinophilia induced by IL-13 was determined. Allo or Dma decreased the eosinophil counts in BAL fluid dose-dependently, and 10 mg/kg Allo or 1.0 mg/kg Dma almost completely inhibited IL-13-induced eosinophilia (Fig. 1A). Treatment with Allo or Dma did not change the number of macrophages, neutrophils, or lymphocytes in BAL fluid after IL-13 instillation (data not shown). We used 10 mg/kg Allo or 1.0 mg/kg Dma for subsequent studies.

Intratracheal administrations of IL-13 increased the concentration of eotaxin in BAL fluid significantly more than those of the control. Treatment with Allo or Dma attenuated eotaxin production in BAL fluids after IL-13 instillation in addition to inhibiting eosinophilia (Fig. 1B).

IL-13 also induced AHR to inhaled acetylcholine in A/J mice. Surprisingly, Dma, but not Allo, suppressed IL-13-induced AHR (Fig. 1C). There were no significant differences in the baseline values of airway pressure among the control, IL-13 instillation alone, IL-13 plus Allo, and IL-13 plus Dma.

Chitinase activity in BAL fluids

BAL fluids were prepared from vehicle-treated, IL-13-treated, IL-13 plus Allo-treated, and IL-13 plus Dma-treated mice, and the chitinase activity in the BAL samples was measured. IL-13 instillation increased the chitinase activity in the BAL fluids. The inhibitory effect on the enhanced chitinase activity in BAL fluids after IL-13 instillation was similar in the treatments with Allo and with Dma (Fig. 2A). This indicated that the decreased levels of chitinase activity in BAL fluids did not coincide with the inhibitory activity on AHR of the two compounds mentioned above.

Proteins with chitinase activity in the BAL fluids were also analyzed by activity staining (Fig. 2B). It is known that Allo or Dma is released from the Allo- or Dma-chitinase complex during activity staining. Therefore, the density of a band on the gel detected by staining shows the amount of chitinase protein in the BAL fluids. IL-13 instillation clearly enhanced the expression of a chitinase that had the same molecular mass (50 kDa) as AMCase. A band at

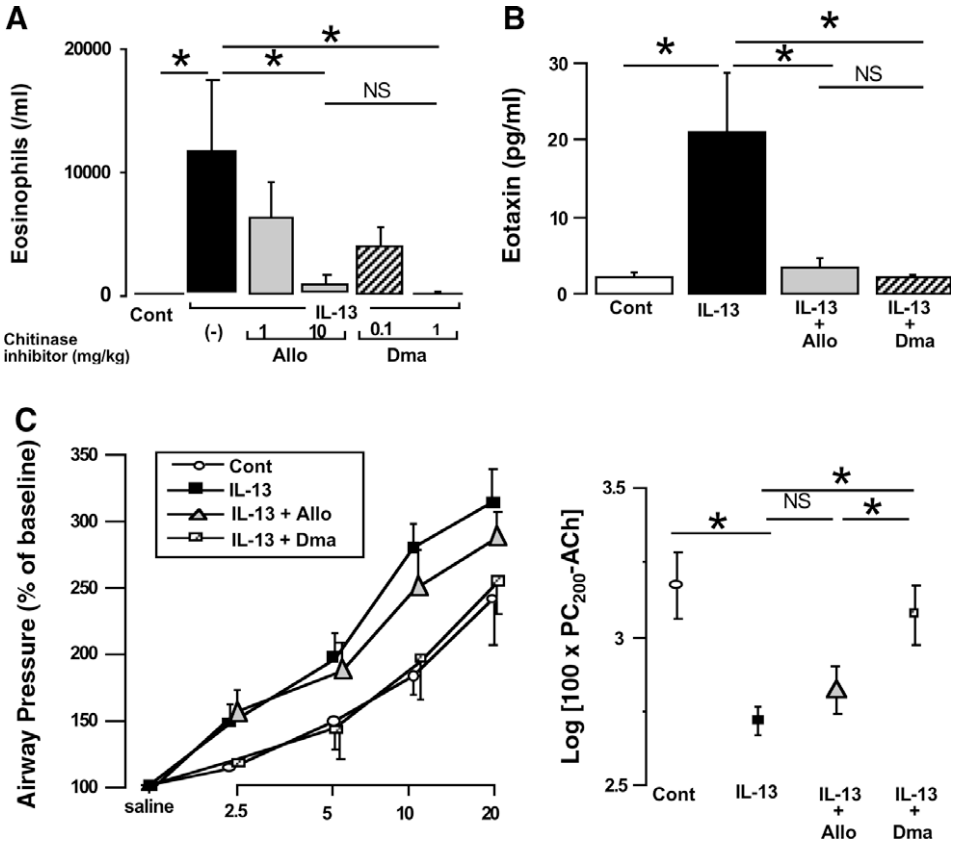

Fig. 1. Effect of chitinase inhibitors on eosinophilia, eotaxin expression, and airway responsiveness following IL-13 treatment. (A) Effect of treatment with Allo or with Dma on eosinophil counts in BAL fluid after intratracheal instillation of IL-13 *in vivo*. The values are means ± SEM. $n = 5–6$. $^{*}p < 0.05$. (B) Eotaxin levels in BAL fluids obtained from vehicle-treated (Cont), IL-13 instillation alone (IL-13), IL-13 plus 10 mg/kg Allo (IL-13 + Allo), or IL-13 plus 1 mg/kg Dma (IL-13 + Dma) mice. The values are means ± SEM. $n = 5–6$. $^{*}p < 0.05$. (C) Airway responsiveness was determined by the acetylcholine-dependent change in airway pressure (*left panel*), and the provocative concentration 200 (PC_{200}-ACh), at which airway pressure was 200% of its baseline value, is expressed as log (PC_{200}-ACh × 100, *right panel*). $n = 8–10$. $^{*}p < 0.05$. NS, not significant.

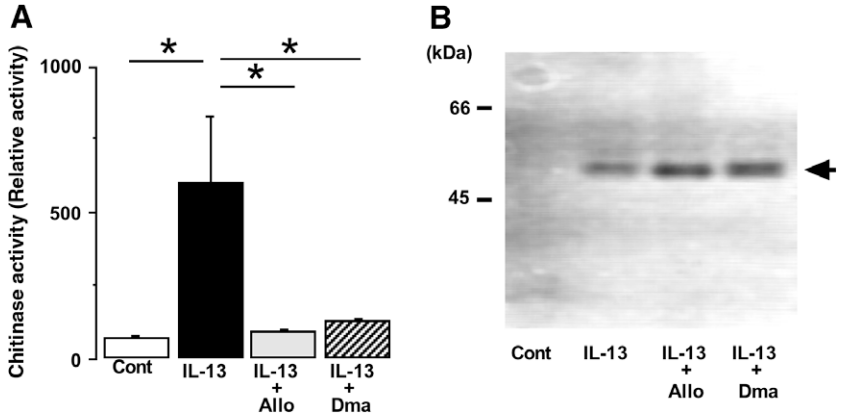

Fig. 2. Effect of treatment with Allo or Dma on chitinase activity in BAL fluid. (A) BAL fluids were obtained from vehicle (Cont), IL-13 instillation alone (IL-13), IL-13 plus 10 mg/kg Allo (IL-13 + Allo), or IL-13 plus 1 mg/kg Dma (IL-13 + Dma)-treated mice. The chitinase activity of each BAL fluid containing 0.5 μg of proteins was measured with 4MU-$(GlcNAc)_3$ as a substrate. The values of the relative chitinase activity were calibrated based on the activity of the control. The values are means ± SEM. $n = 5$. $^{*}p < 0.05$. (B) The chitinase in each BAL fluid containing 5 μg of proteins was analyzed by activity staining. The arrow indicates the band of the 50 kDa protein.

50 kDa was also detected in the samples of Allo or Dma, indicating that the elevation of the chitinase expression by IL-13 was not inhibited by treatment with Allo or Dma.

Inhibitory activity of Allo and Dma toward a recombinant AMCase

To determine the inhibitory activity of Allo and Dma toward AMCase, mouse AMCase was expressed with COS-7 cells. The IC_{50} values of Allo and Dma against chitinase showed that their inhibitory activities were not so different at a pH range of 2–7.5 (Table 1).

Effect of COX-2 inhibitor on IL-13-induced AHR

IL-13 has been shown to regulate the generation of arachidonic acid metabolites [20,21], and the interaction of chitinase inhibitors

Table 1
Inhibitory activity of Allo and Dma toward recombinant mouse AMCase at various pH values.

pH	IC_{50} (nM)	
	Allo	Dma
2.0	5900	9900
3.0	3000	4700
4.0	300	500
5.0	50	80
5.5	6.9	10
6.0	2.3	3.3
6.5	1.0	1.0
7.0	1.0	1.0
7.5	0.9	0.9

with lipid mediators may contribute to the differential regulation of IL-13 responses by Allo and Dma. We first studied the effect of a selective COX-2 inhibitor NS-398 on IL-13-induced AHR. Although treatment with NS-398 did not affect airway responsiveness in control mice with vehicle instillation, NS-398 enhanced IL-13-induced AHR (Supplementary Fig. S1A), suggesting that COX-2 product(s) predominantly exert a bronchoprotective function in IL-13 inflammation.

Effects of chitinase inhibitors on PGE_2 synthesis

Among lipid mediators in the COX-2 pathway, PGE_2 is bronchoprotective [22]. COX-2-derived mediators can modulate lipoxin biosynthesis, and lipoxin A_4 also inhibits AHR [23]. We next measured the levels of PGE_2 and lipoxin A_4 in BAL fluids using ELISA. Although there was no significant difference in the lipoxin A_4 levels among the groups (data not shown), IL-13 increased the PGE_2 concentration (Fig. 3A). Treatment with Allo significantly attenuated PGE_2 production induced by IL-13, whereas Dma did not affect the PGE_2 levels in BAL fluids compared with IL-13 alone (Fig. 3A). The levels of other eicosanoids in BAL fluids from mice with IL-13 plus Allo treatment were compared with those from IL-13 plus Dma-treated mice using a multiplex quantitation method [18]. The levels of LTB_4, LTC_4, PGF_{2a}, PGD_2, 6-ketoPGF_{1a}, and 11-HETE were comparable in IL-13 plus Allo-treated and IL-13 plus Dma-treated mice (data not shown).

The effects of chitinase inhibitors on PGE_2 pathway elements in lung tissue were evaluated. IL-13 enhanced the mRNA expression of COX-2 but not that of microsomal PGE synthase-1. Treatment with Allo, but not with Dma, significantly inhibited the expression of COX-2 and PGE synthase-1 in response to IL-13 (Fig. 3B). Allo or Dma alone had no effects on COX-2 or PGE synthase-1 expression in lung tissue. In the lipoxygenase pathway elements, neither Allo nor Dma affected mRNA expressions of 5-lipoxygenase (5-LO) or 5-LO-activating protein (FLAP) in lung tissue after IL-13 instillation (data not shown).

Airway epithelial cells predominantly express COX-2 in asthmatic airways [24], and we confirmed the effect of chitinase inhibitors on COX-2 mRNA expression in mouse airway epithelial cells *in vitro*. In TGMBE-02-3 cells, IL-13 induced the overexpression of

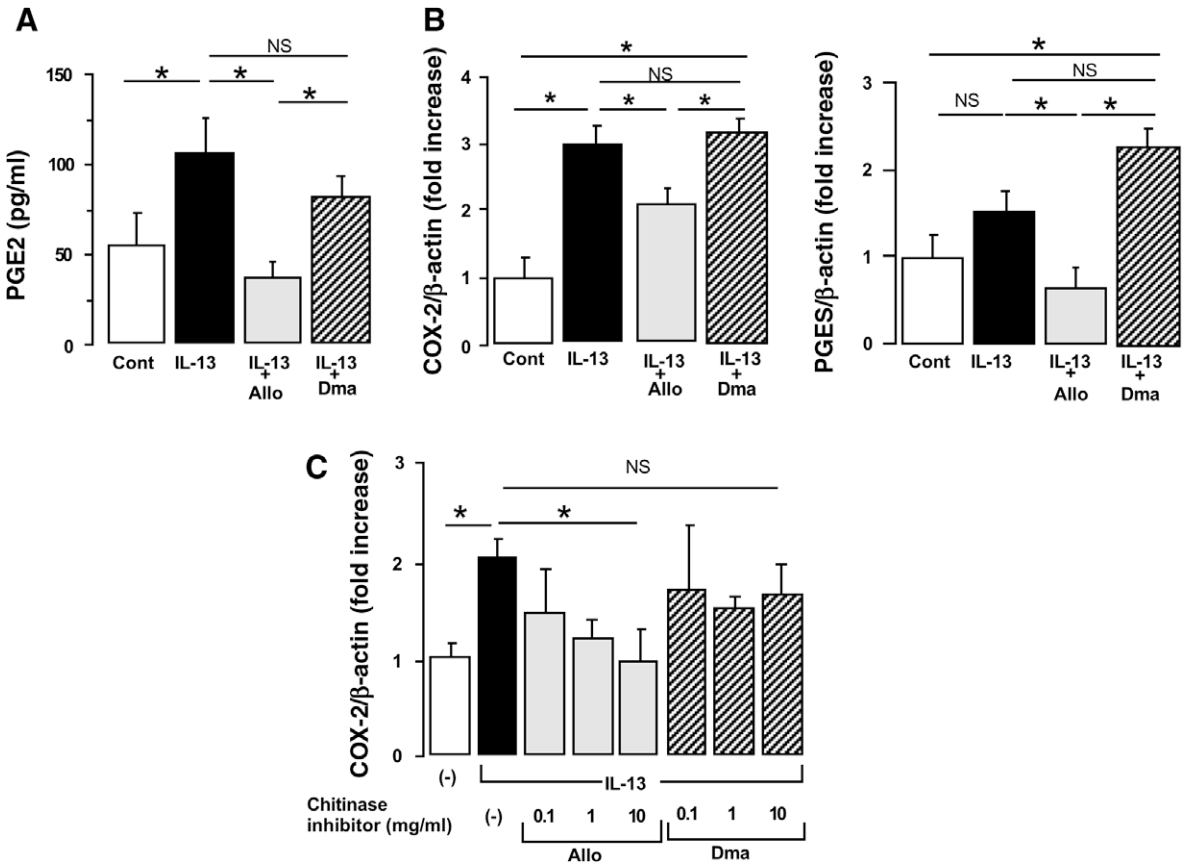

Fig. 3. Effects of chitinase inhibitors on lipid mediator synthesis. (A) Effect of Allo or Dma on the production of PGE_2. BAL fluids obtained from vehicle (Cont), IL-13 instillation alone (IL-13), IL-13 plus 10 mg/kg Allo (IL-13 + Allo) and IL-13 plus 1 mg/kg Dma (IL-13 + Dma)-treated mice. The level of PGE_2 was evaluated by ELISA. Values are means ± SEM. $n = 5$. $^{*}p < 0.05$. (B) Effect of Allo or Dma on mCOX-2 or mPGE syntase-1 expression in lung tissue. Whole lung RNA of vehicle-treated (Cont), IL-13 instillation alone (IL-13), IL-13 plus 10 mg/kg Allo (IL-13 + Allo), or IL-13 plus 1 mg/kg Dma (IL-13 + Dma) mice was analyzed by real-time RT-PCR and primers specially targeting mRNAs encoding COX-2 and microsomal PGE synthase-1 (PGES). The mRNA levels encoding COX-2 and PGES are normalized to β-actin, and the relative levels of transcripts are presented as the fold increase over the values of vehicle-treated control mice. $n = 5$. $^{*}p < 0.05$. (C) Effect of Allo or Dma on COX-2 expression in mouse airway epithelial cells *in vitro*. TGMBE-02-3 cells were incubated with IL-13 (10 ng/ml) plus Allo or Dma, as indicated. COX-2 expression was analyzed with real-time RT-PCR. Relative mRNA levels were normalized to β-actin and are presented as the fold increase over the control values. $n = 5$. $^{*}p < 0.05$.

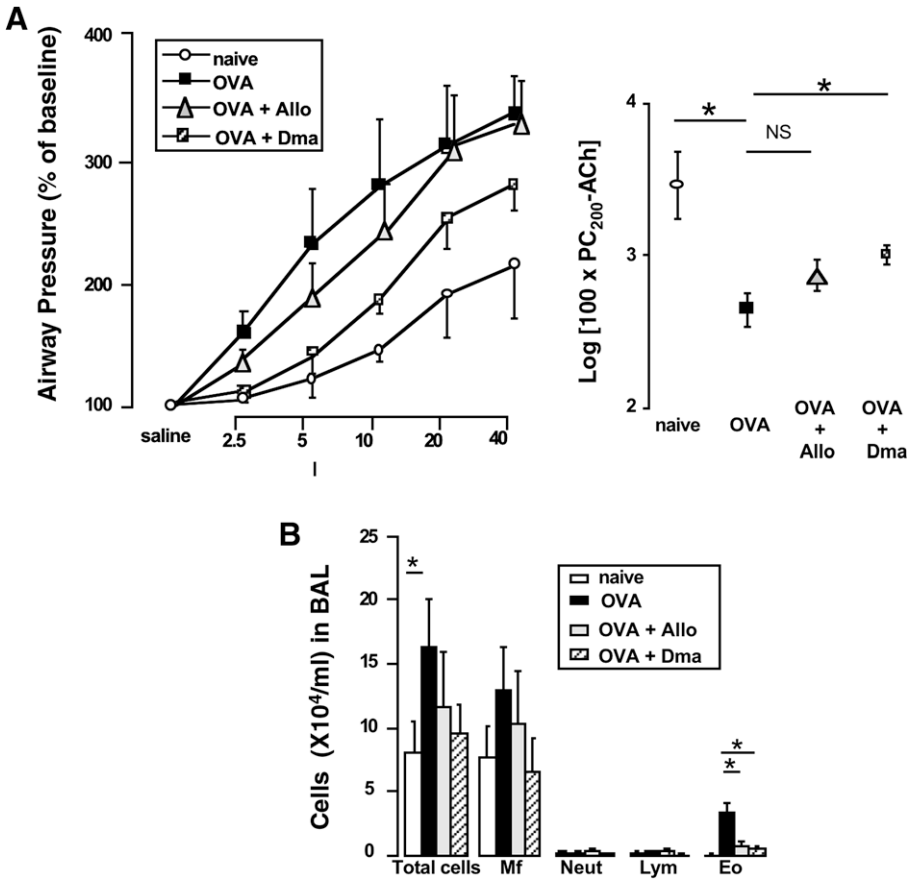

Fig. 4. Effect of Allo or Dma on allergen-induced asthma. (A) OVA sensitization and challenges resulted in airway hyperresponsiveness to inhaled acetylcholine. Treatment with Dma, but not Allo, significantly suppressed the decrease in PC_{200} values in an OVA asthma model. $n = 5–6$. $^{*}p < 0.05$. (B) OVA sensitization and challenges induced marked eosinophilia. Allo or Dma decreased eosinophil counts in BAL fluids after OVA sensitization and challenges. $n = 5–6$. $^{*}p < 0.05$.

COX-2 mRNA. Treatment with Allo, but not Dma, significantly suppressed COX-2 expression induced by IL-13 (Fig. 3C).

Effect of COX-2 inhibitor and chitinase inhibitor on IL-13-induced AHR

To assess the function of COX-2-derived bronchoprotective prostanoids following Dma treatment, we studied the effect of Dma on AHR under the NS-398-treated condition. After treatment with NS-398, Dma failed to inhibit IL-13-induced AHR (Supplementary Fig. S1B). These data suggest that Dma and Allo differentially regulate AHR induced by IL-13 through modulating COX-2-derived mediators, especially PGE_2.

Effects of chitinase inhibitors on allergen-induced responses

Finally, we examined the effect of Allo or Dma on allergic asthma. OVA sensitization and challenges resulted in AHR to acetylcholine and an increased number of eosinophils in BAL fluids. Treatment with Allo attenuated airway eosinophil accumulation but did not affect AHR after OVA sensitization and challenges. On the other hand, Dma suppressed both AHR and eosinophilia in allergic asthma (Fig. 4A and B).

Discussion

Upregulation of AMCase in the lung in mouse asthma models and results of experiments with anti-AMCase sera have suggested that AMCase plays an important role in mouse asthma [8]. However, it was unknown if the blocking of chitinase activity by a chitinase inhibitor is consistent with its anti-asthma activity. In this study, we focused on a chitinase inhibitor, Dma, because Dma has been reported to inhibit human chitotriosidase 20-fold more strongly than Allo. We demonstrated that treatment with Allo or Dma inhibited airway eosinophilic inflammation induced by IL-13 or an allergen. Although Allo did not affect AHR induced by exogenous IL-13, Dma attenuated IL-13-induced AHR even at a lower dose than that of Allo. Dma, but not Allo, also suppressed allergen-induced AHR. These findings suggest that Dma is a more effective inhibitor of asthmatic responses than Allo.

The expression of AMCase in BAL fluids was enhanced by IL-13 instillation. The chitinase activity and eosinophilia in BAL fluids were inhibited by treatment with Allo or Dma. A recent study demonstrated that chitinase stimulates chemokine production from pulmonary epithelial cells [9]. The inhibition of eosinophilia by these chitinase inhibitors may be due to a chitinolytic-dependent mechanism. In contrast, the decreased levels of chitinase activity did not coincide with the inhibitory activity of these two compounds on AHR. In this experiment, no difference between the metabolisms of Allo and Dma in the mouse was observed by LC–MS quantification of Allo and Dma in BAL fluids (data not shown). We tested the inhibitory activity of Dma toward a recombinant AMCase at a pH range of 2–7.5 because AMCase has two optimum pH values. Dma did not show stronger inhibitory activity than Allo at any of the pH values tested. These results suggested that the effective anti-asthma activity of Dma can not be explained by its inhibition of the chitinase activity of AMCase alone.

Cytokines may interact with arachidonic acid metabolites. In the present study, the PGE_2 levels in BAL fluids increased after

IL-13 instillation. Our findings regarding the PGE_2 upregulation by IL-13 are consistent with previous studies, in which PGE_2 levels were increased in BAL fluids from IL-13 Tg mice [21] and IL-13 enhanced COX-2 expression and the production of PGE_2 in brain microglia [25]. Allo, but not Dma, suppressed the overproduction of PGE_2 and the expression of COX-2 and PGE synthase-1 induced by IL-13. A COX-2 inhibitor, NS-398, enhanced IL-13-induced AHR, suggesting a bronchoprotective role of COX-2 product(s) in Th2 inflammation. Dma did not inhibit IL-13-induced AHR after treatment with the COX-2 inhibitor. These data suggest that the differential effect of Allo and Dma on AHR is linked to prostanoids, especially PGE_2.

In patients with asthma, it has been suggested that PGE_2 has a bronchoprotective effect [26]. PGE_2 prevents not only allergen-induced airway inflammation but also Th2 cytokine production in an OVA-induced animal model [27]. Additionally, excessive airway eosinophilia, IgE production, and AHR were found in both COX-1- and COX-2-deficient mice [28]. Therefore, PGE_2 production may be a negative-feedback regulator in asthmatic reactions [29], especially in AHR, and the present data suggest that Allo, but not Dma, failed to suppress AHR at least by limiting this feedback loop with PGE_2.

In conclusion, we provide evidence that Allo modulates bronchoprotective PGE_2 production in addition to chitinase inhibition and does not affect IL-13-induced AHR. Dma attenuates AHR induced by allergens or by IL-13 without affecting PGE_2 synthesis. A recent study reported that the inhibition of AMCase using a RNA-interference approach suppresses AHR in an OVA-induced animal model [30]. Because AHR in patients with asthma is less susceptible to inhaled steroids, further understanding of the regulatory mechanisms of IL-13-induced responses by Dma may have potential therapeutic implications for asthma.

Acknowledgments

We thank Ayako Hashizume, B.Sc., and Makiko Umemoto, M.Sc., for technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and in part by the National Institute of Biomedical Innovation, Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.09.075.

References

[1] W.R. Roche, R. Beasley, J.H. Williams, S.T. Holgate, Subepithelial fibrosis in the bronchi of asthmatics, Lancet 1 (1989) 520–524.
[2] J. Bousquet, P. Chanez, J.Y. Lacoste, G. Barneon, N. Ghavanian, I. Enander, P. Venge, S. Ahlstedt, J. Simony-Lafontaine, P. Godard, et al., Eosinophilic inflammation in asthma, N. Engl. J. Med. 323 (1990) 1033–1039.
[3] G. Grunig, M. Warnock, A.E. Wakil, R. Venkayya, F. Brombacher, D.M. Rennick, D. Sheppard, M. Mohrs, D.D. Donaldson, R.M. Locksley, D.B. Corry, Requirement for IL-13 independently of IL-4 in experimental asthma, Science 282 (1998) 2261–2263.
[4] M. Wills-Karp, J. Luyimbazi, X. Xu, B. Schofield, T.Y. Neben, C.L. Karp, D.D. Donaldson, Interleukin-13: central mediator of allergic asthma, Science 282 (1998) 2258–2261.
[5] A. Kibe, H. Inoue, S. Fukuyama, K. Machida, K. Matsumoto, H. Koto, T. Ikegami, H. Aizawa, N. Hara, Differential regulation by glucocorticoid of interleukin-13-induced eosinophilia, hyperresponsiveness, and goblet cell hyperplasia in mouse airways, Am. J. Respir. Crit. Care Med. 167 (2003) 50–56.
[6] R.G. Boot, E.F. Blommaart, E. Swart, K. Ghauharali-van der Vlugt, N. Bijl, C. Moe, A. Place, J.M. Aerts, Identification of a novel acidic mammalian chitinase distinct from chitotriosidase, J. Biol. Chem. 276 (2001) 6770–6778.
[7] R.J. Homer, Z. Zhu, L. Cohn, C.G. Lee, W.I. White, S. Chen, J.A. Elias, Differential expression of chitinases identify subsets of murine airway epithelial cells in allergic inflammation, Am. J. Physiol. Lung Cell. Mol. Physiol. 291 (2006) L502–L511.
[8] Z. Zhu, T. Zheng, R.J. Homer, Y.K. Kim, N.Y. Chen, L. Cohn, Q. Hamid, J.A. Elias, Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation, Science 304 (2004) 1678–1682.
[9] D. Hartl, C.H. He, B. Koller, C.A. Da Silva, R. Homer, C.G. Lee, J.A. Elias, Acidic mammalian chitinase is secreted via an ADAM17/epidermal growth factor receptor-dependent pathway and stimulates chemokine production by pulmonary epithelial cells, J. Biol. Chem. 283 (2008) 33472–33482.
[10] D. Hartl, C.H. He, B. Koller, C.A. Da Silva, Y. Kobayashi, C.G. Lee, R.A. Flavell, J.A. Elias, Acidic mammalian chitinase regulates epithelial cell apoptosis via a chitinolytic-independent mechanism, J. Immunol. 182 (2009) 5098–50106.
[11] T.A. Reese, H.E. Liang, A.M. Tager, A.D. Luster, N. Van Rooijen, D. Voehringer, R.M. Locksley, Chitin induces accumulation in tissue of innate immune cells associated with allergy, Nature 447 (2007) 92–96.
[12] B. Henrissat, A classification of glycosyl hydrolases based on amino acid sequence similarities, Biochem. J. 280 (Pt 2) (1991) 309–316.
[13] S. Sakuda, Y. Nishimoto, M. Ohi, M. Watanabe, S. Takayama, A. Isogai, Y. Yamada, Effect of demethylallosamidin, a potent yeast chitinase inhibitor, on cell division of yeast, Agric. Biol. Chem. 54 (1990) 1333–1335.
[14] F.V. Rao, D.R. Houston, R.G. Boot, J.M. Aerts, S. Sakuda, D.M. van Aalten, Crystal structures of allosamidin derivatives in complex with human macrophage chitinase, J. Biol. Chem. 278 (2003) 20110–20116.
[15] Z.Y. Zhou, S. Sakuda, M. Kinoshita, Y. Yamada, Biosynthetic studies of allosamidin. 2. Isolation of didemethylallosamidin, and conversion experiments of ^{14}C-labeled demethylallosamidin, didemethylallosamidin and their related compounds, J. Antibiot. (Tokyo) 46 (1993) 1582–1588.
[16] D. Laouini, A. Elkhal, A. Yalcindag, S. Kawamoto, H. Oettgen, R.S. Geha, COX-2 inhibition enhances the TH2 immune response to epicutaneous sensitization, J. Allergy Clin. Immunol. 116 (2005) 390–396.
[17] H. Inoue, R. Kato, S. Fukuyama, A. Nonami, K. Taniguchi, K. Matsumoto, T. Nakano, M. Tsuda, M. Matsumura, M. Kubo, F. Ishikawa, B.G. Moon, K. Takatsu, Y. Nakanishi, A. Yoshimura, Spred-1 negatively regulates allergen-induced airway eosinophilia and hyperresponsiveness, J. Exp. Med. 201 (2005) 73–82.
[18] Y. Kita, T. Takahashi, N. Uozumi, T. Shimizu, A multiplex quantitation method for eicosanoids and platelet-activating factor using column-switching reversed-phase liquid chromatography-tandem mass spectrometry, Anal. Biochem. 342 (2005) 134–143.
[19] M.J. Kuranda, P.W. Robbins, Chitinase is required for cell separation during growth of *Saccharomyces cerevisiae*, J. Biol. Chem. 266 (1991) 19758–19767.
[20] G.M. Nassar, J.D. Morrow, L.J. Roberts 2nd, F.G. Lakkis, K.F. Badr, Induction of 15-lipoxygenase by interleukin-13 in human blood monocytes, J. Biol. Chem. 269 (1994) 27631–27634.
[21] Y.M. Shim, Z. Zhu, T. Zheng, C.G. Lee, R.J. Homer, B. Ma, J.A. Elias, Role of 5-lipoxygenase in IL-13-induced pulmonary inflammation and remodeling, J. Immunol. 177 (2006) 1918–1924.
[22] I.D. Pavord, A.E. Tattersfield, Bronchoprotective role for endogenous prostaglandin E2, Lancet 345 (1995) 436–438.
[23] B.D. Levy, G.T. De Sanctis, P.R. Devchand, E. Kim, K. Ackerman, B.A. Schmidt, W. Szczeklik, J.M. Drazen, C.N. Serhan, Multi-pronged inhibition of airway hyperresponsiveness and inflammation by lipoxin A(4), Nat. Med. 8 (2002) 1018–1023.
[24] R. Taha, R. Olivenstein, T. Utsumi, P. Ernst, P.J. Barnes, I.W. Rodger, A. Giaid, Prostaglandin H synthase 2 expression in airway cells from patients with asthma and chronic obstructive pulmonary disease, Am. J. Respir, Crit. Care Med. 161 (2000) 636–640.
[25] M.S. Yang, K.A. Ji, S.B. Jeon, B.K. Jin, S.U. Kim, I. Jou, E. Joe, Interleukin-13 enhances cyclooxygenase-2 expression in activated rat brain microglia: implications for death of activated microglia, J. Immunol. 177 (2006) 1323–1329.
[26] G.M. Gauvreau, R.M. Watson, P.M. O'Byrne, Protective effects of inhaled PGE2 on allergen-induced airway responses and airway inflammation, Am. J. Respir. Crit. Care Med. 159 (1999) 31–36.
[27] J.G. Martin, M. Suzuki, K. Maghni, R. Pantano, D. Ramos-Barbon, D. Ihaku, F. Nantel, D. Denis, Q. Hamid, W.S. Powell, The immunomodulatory actions of prostaglandin E2 on allergic airway responses in the rat, J. Immunol. 169 (2002) 3963–3969.
[28] S.H. Gavett, S.L. Madison, P.C. Chulada, P.E. Scarborough, W. Qu, J.E. Boyle, H.F. Tiano, C.A. Lee, R. Langenbach, V.L. Roggli, D.C. Zeldin, Allergic lung responses are increased in prostaglandin H synthase-deficient mice, J. Clin. Invest. 104 (1999) 721–732.
[29] G.Y. Park, J.W. Christman, Involvement of cyclooxygenase-2 and prostaglandins in the molecular pathogenesis of inflammatory lung diseases, Am. J. Physiol. Lung Cell Mol. Physiol. 290 (2006) L797–L805.
[30] C.J. Yang, Y.K. Liu, C.L. Liu, C.N. Shen, M.L. Kuo, C.C. Su, C.P. Tseng, T.C. Yen, C.R. Shen, Inhibition of acidic mammalian chitinase by RNA interference suppresses OVA-sensitized allergic asthma. Hum. Gene The. (2009), in press.