

Comparative study to elucidate the mechanism underlying the difference in airway hyperresponsiveness between two mouse strains

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Received 6 July 2007; accepted 6 July 2007

Abstract

The mechanism underlying airway hyperresponsiveness (AHR), a characteristic feature of asthma, remains obscure. We attempted to elucidate the mechanism responsible for the different degrees of AHR in two mouse strains, BALB/c and C57BL/6, following exposure to an anaphylactic trigger. When ovalbumin (OVA)-sensitized mice were challenged daily with OVA for up to three consecutive days, the BALB/c mice showed a higher degree of airway responsiveness to methacholine than did C57BL/6. Following the OVA challenge, eosinophils and macrophages in bronchoalveolar lavage fluid (BALF) from BALB/c increased significantly in number compared to those from C57BL/6. BALB/c mice also exhibited a higher serum IgE level than that of C57BL/6 after OVA challenge. The enhanced AHR and eosinophilic infiltration in BALF were significantly reduced by pretreatment with a selective cysteinyl-leukotriene type 1 receptor (cysLT₁R) antagonist, montelukast. In the *in vitro* study, cysLT production was significantly lower in the dissected lung tissue from BALB/c than in tissue from C57BL/6 when both groups were stimulated with saline. The lungs from BALB/c generated significantly larger amounts of cysLTs on incubation with OVA rather than with saline, while the lungs from C57BL/6 did not show any significant increase in cysLTs with antigen stimulation. Significant upregulation of cysLT₁R and cysLT₂R mRNA expression was induced by OVA challenge in the lungs of BALB/c, but not in those of C57BL/6. It is suggested that, after an anaphylactic reaction, the degree of AHR is dependent on the genetic background and that cysLTs play an important role in the mechanism involved.

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Keywords: Cysteinyl leukotriene; Ovalbumin; Airway hyperresponsiveness; Asthma; IgE; Receptors

1. Introduction

Asthma is one of the most prevalent disorders among industrialized nations, and is characterized by reversible

bronchoconstriction, increased mucous secretion, and complex airway inflammation [1,2]. Inhalation of non-specific air-borne pollutants by asthmatic subjects results in bronchoconstriction, an outcome not seen in normal individuals. This reaction is known as airway hyperresponsiveness (AHR). The precise mechanism for AHR remains unclear, particularly in terms of the relationship between the chronic form of AHR and airway inflammation [3].

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Basic and clinical studies have indicated that cysteinyl-leukotrienes (cysLTs) play an important role in the pathophysiology of bronchial asthma [4–6]. CysLTs have various effects on the airway system: induction of profound bronchoconstriction [7], enhancement of vascular leakage [8], enhancement of mucous secretion in the bronchi [9], and induction of the chemotactic activity of eosinophils [10,11]. The cellular origins of cysLTs in the lungs are considered to be mast cells, eosinophils, basophils, monocytes–macrophages, and products of cell–cell interactions, such as those between neutrophils and platelets [12,13]. CysLTs act out their physiological role through two G-protein-coupled receptors: cysLT₁ receptor (cysLT₁R) and cysLT₂ receptor (cysLT₂R) [14,15]. Homology between cysLT₁R and cysLT₂R is 38% in humans and 38.5% in mice [16]. The degree of homology between human and mouse cysLT₁R is 87.3%, and 73.4% for cysLT₂ R. CysLT₁R is distributed in lung smooth muscle cells, lung macrophages, peripheral blood eosinophilic leukocytes, mast cells, CD34+ granulocytes, monocytes, B lymphocytes and other tissue types [16,17]. The relative affinities of each cysLT to cysLT₁R are as follows: LTD₄>LTC₄>LTE₄ [13,14]. In the case of cysLT₂R, this receptor is distributed in lung macrophages, airway smooth muscle cells, heart Purkinje cells, adrenal medulla, peripheral blood leukocytes, and in the brain. The relative affinities of each cysLT to the receptor are LTC₄=or>LTD₄>LTE₄ [13,15].

In this study, we created an asthma model using two mouse strains, BALB/c and C57BL/6. Since the two strains showed markedly different degrees of AHR

following an anaphylactic response, we attempted to elucidate the mechanism underlying the difference while comparing the effects of montelukast, a cysLT₁R antagonist, on pulmonary resistance by examining the cyto-histopathological findings, as well as cysLT metabolism using chopped lung fragments.

2. Materials and methods

2.1. Materials

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Fukuoka University. Male C57BL/6 and BALB/c mice (CLEA Japan, Inc., Shiga, Japan) used in this study were 6–8 weeks old and weighed 20–25 g. The mice were kept on a 12/12 h light/dark cycle and allowed access to food and water ad libitum. The cysLT₁R antagonist (montelukast sodium) was donated by Merck and Co., Inc. (Rahway, NJ).

2.2. Sensitization of mice

Mice were actively sensitized on days 0 and 14 by intraperitoneal injection of 20 µg of ovalbumin (OVA, grade V, Sigma-Aldrich, St. Louis, MO) emulsified in 2.25 mg aluminum hydroxide (Nacalai Tesque, Kyoto, Japan) in a total volume of 100 µl saline. As shown in Fig. 1, the sensitized mice were challenged by inhalation of 1% OVA aerosol for 20 min using a pressurized nebulizer (PARI Turbo Boy, M.I.P.S., Osaka, Japan) on days 24, 25 and 26 while control animals received inhalation of saline aerosol only. Some groups of mice were orally administered 10 mg/kg of montelukast 1 h before challenge.

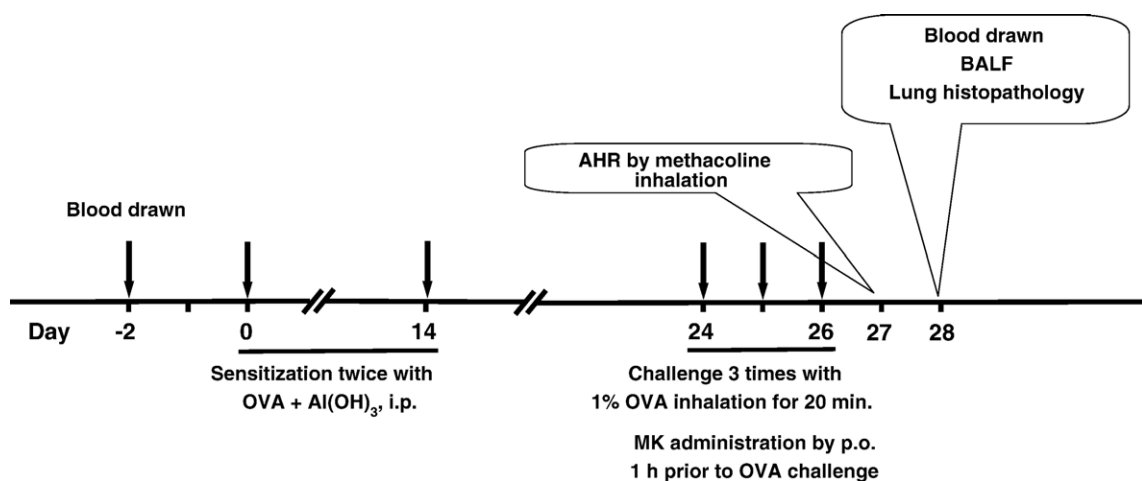


Fig. 1. Experimental schedules for sensitization and challenge. Sensitization to OVA was induced by i.p. injection of 20 µg OVA together with 2.25 mg Al(OH)₃. Fourteen days later, the same solutions were i.p. injected again. Sensitized mice were exposed to the antigen by the inhalation of 1% OVA aerosol for 20 min for three consecutive days (days 24–26). The next day (day 27), repeated measurements of airway resistance were examined following inhalation of methacholine at various concentrations. The following day (day 28), bronchoalveolar lavage was performed under anesthesia, and blood samples and lungs were obtained for histological examinations, as indicated in the experimental schedule.

2.3. Measurement of airway resistance

Airway responsiveness to methacholine was assessed on day 27 (AHR test). AHR to inhaled methacholine (Sigma-Aldrich) was measured with mice in a conscious and unrestrained state using a whole body plethysmograph (Win-PULMOS-1, Version 1.6, M.I.P.S.). Aerosolized saline or methacholine in increasing concentrations (3.125–50.000 mg/ml, [18]) was delivered through an inlet hole of the main chamber for 1 min, and airway resistance was measured as an average value for 5 min after each inhalation [19]. Specific resistance of the airway (sRAW) was reflected by changes in the wave forms of a rectangular pressure signal during both inspiration and expiration. sRAW was determined according to the manufacturer's instructions. The following day (day 28), histological and cytological examinations were performed.

2.4. Histological and cytological examination

On day 28, the mice were tracheostomized under anesthesia with 50 mg/kg i.p. of sodium pentobarbital (10% solution, Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan). Bronchoalveolar lavage (BAL) was performed twice via the tracheal cannula using 1.0 ml saline containing 1 mM ethylenediaminetetraacetic acid (EDTA). BAL fluid (BALF) was centrifuged at 300g for 5 min at 4 °C, and the cell pellet was resuspended in 1.0 ml of sterilized saline. Cells were counted with a hemocytometer under a light microscope. The differential cell count was obtained with a cytospin preparation stained with Diff-Quik (International Reagent Corp., Kobe, Japan) counting 200 cells at random under 200× magnification. The cells were identified by standard morphology.

The lungs were fixed in situ by i.t. administration of 10% neutral-buffered formalin and, the tissues were then stained with hematoxylin-eosin to assess the degree of inflammation.

2.5. Measurement of serum IgE

Serum immunoglobulin E (IgE) levels were measured by an enzyme-linked immuno sorbent assay (Mouse IgE ELISA kit, Morinaga Institute of Biological Science, Inc., Kanagawa, Japan), according to the manufacturer's protocol.

2.6. Estimation of cysLT production in isolated lung fragments

Under anesthesia, mice were perfused transcardially with saline through the right ventricle to replace circulating blood in the lung and the lung tissues were then removed. The large bronchi or blood vessels were dissected from the lung tissues and the remaining lung material was cut into small pieces with fine scissors. These pieces (150 mg) were preincubated with Tyrode's buffer for 5 min at 37 °C and further incubated for 30 min at 37 °C after the addition of OVA at various concentrations (10–1000 µg/ml). For incubation of the controls, saline was added instead of OVA. After terminating the reaction by adding cold ethanol, LTs were quantitated by means of a previously reported method [20]. Briefly, LTs were partially purified through a Sep-Pak cartridge (Waters Corporation, Milford, MA). After the evaporation of methanol and resuspension in high performance liquid chromatography (HPLC) solvent, LTC₄, LTD₄, and LTE₄ were separated on an HPLC system equipped with a Novapak C18 column (0.39×15 cm, Waters Corporation) and each fraction was collected. The LTC₄ and LTD₄ fractions were assayed using a CysLT-EIA kit (Cayman Chemical, Ann Arbor, MI), and LTE₄ fractions were assayed using an LTE₄-EIA kit (Cayman Chemical) according to the manufacturer's instructions. The amounts of LTC₄, LTD₄, and LTE₄ added together corresponded to the total cysLTs produced from the lung tissue fragments.

The HPLC system consisted of a Model 600 controller, a 717 auto sampler (Waters Corporation), and a Novapak C18 column. Solvent A (acetonitrile/methanol/water/acetic acid,

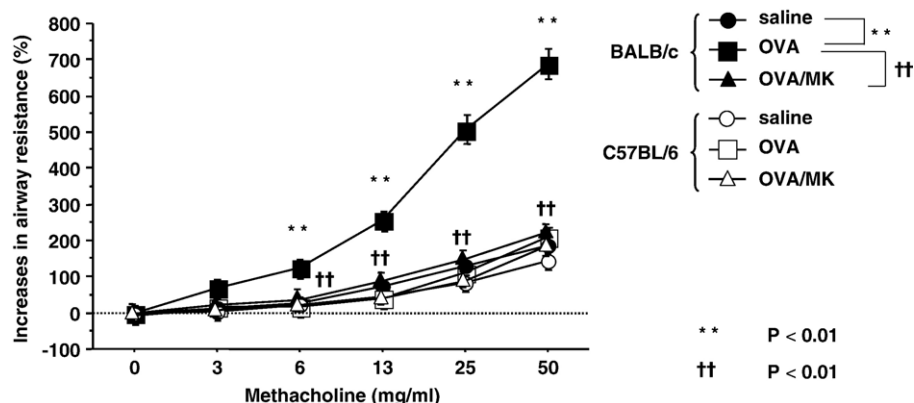


Fig. 2. Airway responsiveness to methacholine and effects of the cysLT₁R antagonist. Changes in airway resistance were measured by whole body plethysmography after inhalation of methacholine at different concentrations ranging from 0 to 50 mg/ml. Increases in airway resistance relative to that of the saline inhalation group (baseline value) are shown as a percentage. ***p* < 0.01, statistically significant difference between groups of BALB/c/saline and BALB/c/OVA; ††*p* < 0.01, statistically significant difference between of BALB/c/OVA and BALB/c/OVA/montelukast groups, by Tukey–Kramer analysis.

Table 1

Total white blood cell counts in BALF and their modulation by montelukast in the two strains

	Cell number ($\times 10^5$)	
Mouse	BALB/c	C57BL/6
Saline	0.45 ± 0.15	0.39 ± 0.18
OVA ^a	3.73 ± 1.16	1.10 ± 0.71
OVA + MK ^b	2.00 ± 0.55	0.66 ± 0.19

^aOvalbumin; ^b montelukast; * $p < 0.05$; # $p < 0.05$.

30: 12: 58: 0.03(v/v)) contained 0.03% EDTA-free acid (Dojindo, Kumamoto, Japan), and solvent B consisted of acetonitrile/methanol /water/acetic acid (60:12:20:0.01(v/v)) containing 0.001% EDTA. All solvents were adjusted to pH 5.6. The mobile phase was initiated with solvent A, and a gradient was applied for 20 min with solvent B at flow rate of 1 ml/min.

2.7. Changes in *cysLT₁R* and *cysLT₂R* expression in the lungs

Total RNA was extracted from the lung homogenates using an acid guanidine thiocyanate–phenol–chloroform method. The mRNA in the extracted RNA was then converted to cDNA by QuantiTect Reverse Transcription (Qiagen, Hilden, Germany). The resultant cDNA was subjected to polymerase chain reaction (PCR) analysis for LT₁R, LT₂R, and glyceraldehyde-3-phosphate dehydrogenase mRNAs, using the following specific primer pairs: the primer pair for LT₁R consisted of a sense primer, 5'-CAACGAACTATCCACCTTACC-3', and

an antisense primer, 5'-AGCCTTCTCCTAAAGTTTCCAC-3' [21]; the primer pair for LT₂R consisted of a sense primer, 5'-GTCCACGTGCTGCTCATAGG-3', and an antisense primer, 5'-ATTGGCTGCAGCCATGGTCC-3' [21]; the primer pair for glyceraldehyde-3-phosphate dehydrogenase consisted of a sense primer, 5'-CCATGGAGAAGGCTGGGG-3', and an antisense primer, 5'-CAAAGTTGTCATGGATGA-3'; and the product sizes were 162 bp, 180 bp and 195 bp, respectively.

The reaction mixture for PCR amplification consisted of 25 ng of cDNA, 0.1 μ M of each of the primers, 200 μ M of each of the four deoxynucleotide triphosphates and 0.625 U of GoTaq polymerase (Promega, Madison, WI) in 25 μ l of 1 $\times$ GoTaq Flexi Buffer (pH 8.5, Promega) with 1.5 mM MgCl₂. Each amplification cycle consisted of a denaturing step at 94 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, and was terminated by a final 7 min incubation at 72 °C. Controls were run without reverse transcriptase or without template cDNA to ensure that results were not due to amplification of any genomic or contaminating DNA. Each reaction mixture (10 μ l) was electrophoresed on a 1.5% agarose gel for 30 min and visualized by incubation for 10 min in a solution containing ethidium bromide (100 ng/ml).

The intensity and area of each visualized fragment were also measured using image analyzing software (Win ROOF, Version 5.6, Mitani Co. Ltd., Osaka, Japan).

2.8. Statistical analysis

Data are reported as means $\pm$ S.E.M. Statistical analysis was performed using the General Linear Models Procedure as part of the Statistical Analysis System. A p value of less than 0.05 was considered to be statistically significant.

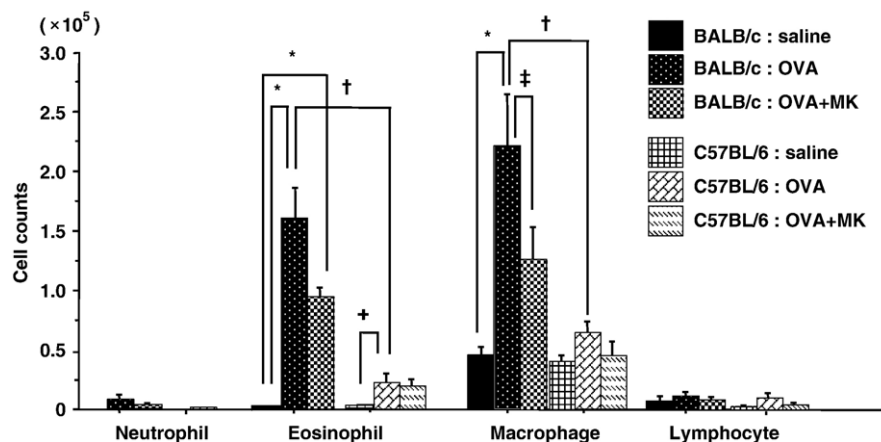


Fig. 3. Cytological study of bronchoalveolar lavage fluids (BALF). BALF was obtained on day 28, the day following the methacholine test for assessing AHR. Control group, saline injection instead of OVA followed by saline challenge; OVA group, OVA sensitization followed by OVA challenge; OVA + montelukast group, OVA sensitization and montelukast pretreatment 1 h before OVA challenge. Cellular differentiation observed in BALF was examined after Diff-Quick staining. The numbers of neutrophils (No), eosinophils (Eo), lymphocytes (Lo), and macrophages (Mφ) were calculated as a percentage of each cell type relative to the total cell number in BALF 6 h after saline or OVA challenge. Values are presented as means $\pm$ S.E.M ($n = 4-14$). * $p < 0.05$, statistically significant compared with those in the BALB/c/saline group; † $p < 0.05$, significance between BALB/c/OVA and C57BL/6/OVA groups; + $p < 0.05$, significance between C57BL/6/saline and C57BL/6/OVA groups; ‡ $p < 0.05$, significance between BALB/c/OVA and BALB/c/OVA/montelukast groups, by Tukey–Kramer analysis.

3. Results

3.1. Comparison of airway hypersensitivity and effects of the *cysLT₁R* antagonist

Airway responsiveness to inhalation of methacholine was evaluated in BALB/c and C57BL/6 mice after challenge with OVA or saline and the two strains were compared (Fig. 2). The BALB/c mice showed significant increases in airway resistance after OVA challenge compared with the sensitized group (saline-group). Pretreatment with montelukast significantly suppressed the increases in airway resistance to methacholine after the OVA challenge, as was also observed in the saline group.

In contrast, C57BL/6 mice did not show any significant increase in airway responsiveness following the OVA challenge (OVA group). The pretreatment with montelukast had no influence on the dose response relationship, which was almost the same as that of the saline group.

3.2. Comparison of the cytological changes in BALF and effects of the *cysLT₁R* antagonist

Total leukocyte counts in BALF of the two strains and their modulation by montelukast are shown in Table 1. Total cell counts of BALB/c mice in the OVA group were 8 times higher than those of the saline group. Pretreatment with montelukast significantly reduced the total cell count in the OVA-challenged mice. In C57BL/6, however, cell numbers recov-

ered after OVA challenge were 2.8 times higher than after saline challenge. Pretreatment with montelukast reduced the total cell number to nearly half, but this was not statistically significant.

The major leukocytes in BALF from control mice were macrophages, as shown in Fig. 3. In BALB/c mice, eosinophilic leukocytes markedly increased after OVA challenge, and pretreatment with montelukast reduced the number of eosinophils, but not to any statistically significant extent. The macrophage counts were significantly higher in the OVA group than in the saline group. Pretreatment with montelukast significantly reduced the macrophage count after OVA challenge. In C57BL/6 mice, the eosinophil count was significantly higher after OVA challenge. Although macrophages tended to increase after OVA challenge, this increase was not significantly different from that of the control groups (saline). The numbers of eosinophils and macrophages in the OVA-challenged group were significantly higher in BALB/c mice than in C57BL/6.

3.3. Histopathological correlations with the acquisition of airway hypersensitivity in the two strains with and without the *cysLT₁R* antagonist

Inflammatory cell infiltrations in the submucosal airway tissues of the two strains were compared using hematoxylin-eosin staining (Fig. 4). Cell infiltrations in BALB/c mice were more prominent than in C57BL/6, when both were subjected to OVA sensitization and had partially recovered by pretreatment with montelukast.

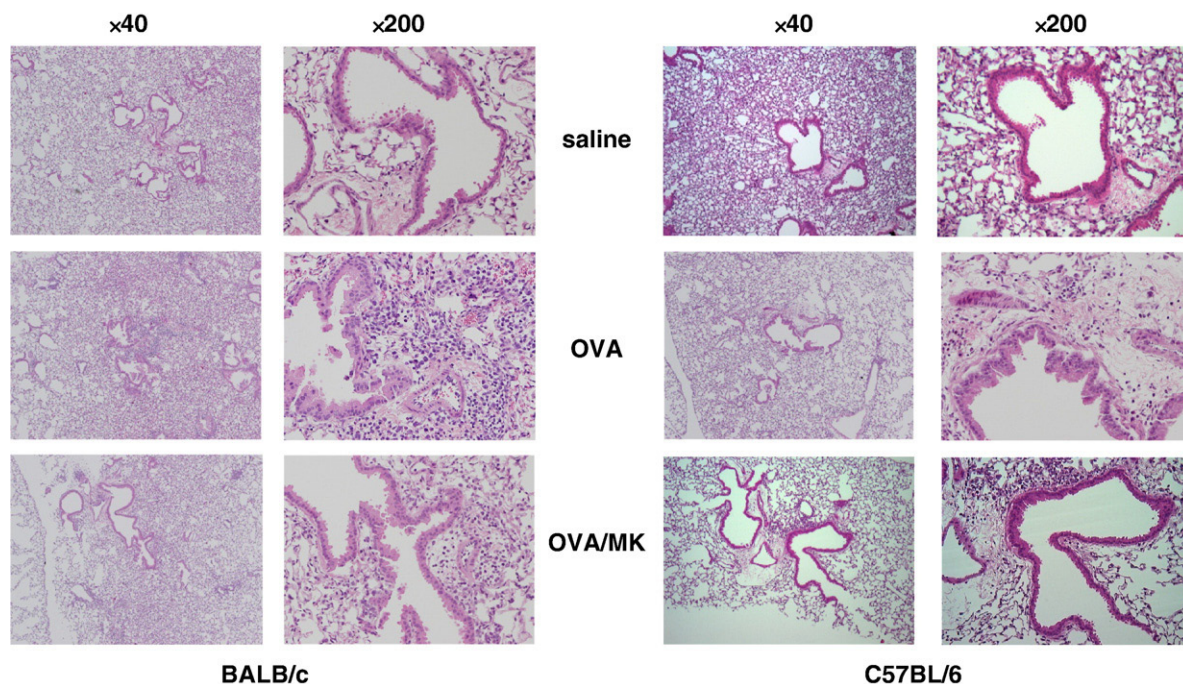


Fig. 4. Histopathology of the lungs. Histological findings in the lung on day 28 by hematoxylin-eosin staining. The treatment for each group was the same as in Fig. 3.

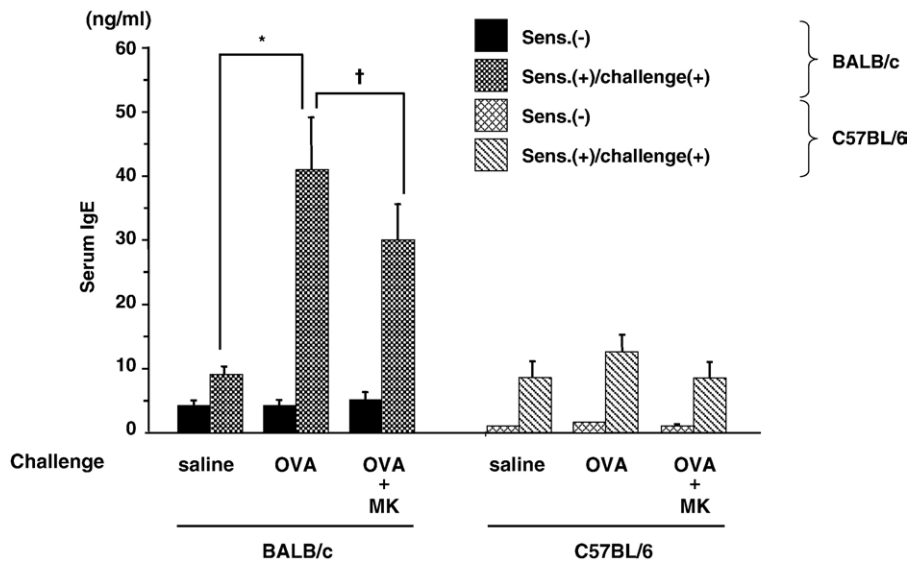


Fig. 5. Changes in serum IgE levels. Serum IgE was quantified by ELISA. Pre-sensitization samples were drawn 2 days prior to sensitization. Another blood sample was drawn after saline or OVA challenge on day 28. Values are presented as means $\pm$ S.E.M. ($n=4-8$). * $P<0.05$, statistically significant difference between BALB/c/saline and BALB/c/OVA groups; † $P<0.05$, significance between BALB/c/OVA and BALB/c/OVA/montelukast groups, by Tukey–Kramer analysis.

3.4. Serum IgE levels and effects of montelukast

In BALB/c mice, serum IgE levels were 4.40 ± 2.20 (ng/ml) before sensitization and 9.02 ± 2.32 (ng/ml) with OVA

sensitization and saline challenge, as shown in Fig. 5. However, serum IgE levels increased to 40.82 ± 23.35 (ng/ml) in mice who were OVA-sensitized and OVA-challenged. Pretreatment with montelukast reduced IgE levels to 29.85 ± 16.02 (ng/ml)

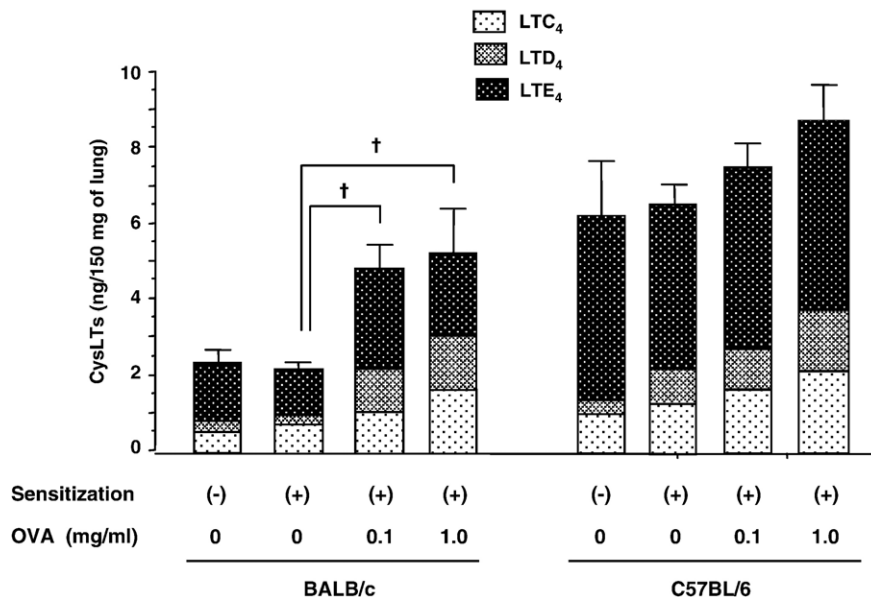


Fig. 6. CysLT production determined with dissected lung tissues. Lung tissue fragments (each 150 mg wet weight) from OVA-sensitized or non-sensitized mice were incubated after adding OVA (at a final concentration of 0.1 or 1 mg/ml) or saline. CysLTs in supernatants after centrifugation of the incubation mixtures were quantified by the method described. Values are presented as means $\pm$ S.E.M. ($n=3-18$). † $p<0.05$, statistically significant difference between BALB/c/saline and BALB/c/OVA groups by Tukey–Kramer analysis. No statistical significance was observed in the C57BL/6 groups.

after OVA challenge. In C57BL/6 mice, serum IgE levels were 0.70 ± 0.16 (ng/ml) before sensitization, and 8.39 ± 6.51 (ng/ml) with OVA sensitization and saline challenge. The OVA-sensitized mice showed 12.55 ± 7.05 (ng/ml) of IgE levels by OVA-challenge, but pretreatment with montelukast only suppressed these levels to 8.55 ± 6.25 (ng/ml), which was not statistically significant.

3.5. *In vitro* production of cysLTs from the chopped lung fragments

To compare the metabolism of cysLTs in the lung tissues of the two strains, the chopped lung fragments were incubated in Tyrode's solution with or without the allergen. After incubation, cysLT levels in tissues of non-sensitized mice that were challenged with saline were similar to those in tissues of OVA-sensitized mice that were also challenged with saline, as shown in Fig. 6. The lung tissues obtained from C57BL/6 mice produced more cysLTs than those from BALB/c. In contrast, the chopped lung fragments from BALB/c generated significantly higher amounts of cysLTs on incubation with OVA than with saline. However, this was not the case with lung fragments from C57BL/6.

In BALB/c mice, the ratios of cysLTs following stimulation with the allergen were increased relative to baseline levels resulting from stimulation with saline, and these were 2.25 and 2.44 with 0.1 mg/ml OVA and 1.0 mg/ml OVA, respectively. In C57BL/6, however, ratios were 1.20 and 1.42 with 0.1 mg/ml OVA and 1.0 mg/ml OVA, respectively.

3.6. Expression of cysLT₁R and cysLT₂R mRNAs in lungs

Expression of cysLT₁R mRNA significantly increased in lungs of BALB/c mice following OVA challenge compared with that in the saline-challenged mice, as shown in Fig. 7. CysLT₂R expression was also upregulated by the OVA challenge when compared with saline challenge. In contrast, expression of both receptors failed to increase in lungs of OVA-challenged C57BL/6.

4. Discussion

When we compared airway hyperresponsiveness (AHR) in BALB/c and C57BL/6 mice we found dramatic differences in these two strains, as previously reported by other groups [22–24]. BALB/c mice showed a prominent hyperresponsiveness to methacholine inhalation after allergen challenge, whereas that of C57BL/6 was rather weak. However, these two strains did show a similar degree of airway responsiveness after sensitization alone. The difference in AHR was associated with cytological and histological differences between the two strains; after allergen challenge, BALB/c mice showed a significant and greater infiltration of eosinophils and macrophages into the airway lumen than did C57BL/6 mice. These cytological changes were confirmed by the histological findings. OVA challenge of sensitized mice resulted in

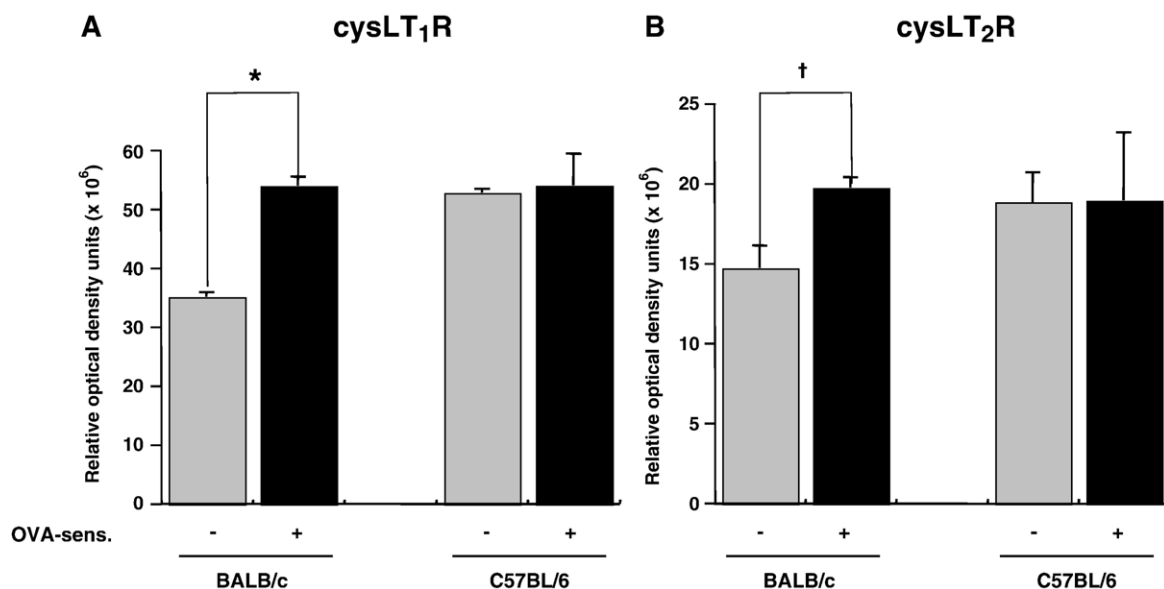


Fig. 7. Changes in cysLT₁R and cysLT₂R mRNA expression in lungs. CysLT₁R expression was significantly increased in lungs obtained from mice which were OVA-sensitized and-challenged in comparison with those exposed to saline. * $p < 0.05$, statistically significant difference between BALB/c/saline and BALB/c/OVA groups. CysLT₂R expression was significantly increased in lungs obtained from mice which were OVA-sensitized and-challenged in comparison with those exposed to saline. † $p < 0.05$, significance between BALB/c/saline and BALB/c/OVA groups, by Tukey–Kramer analysis.

eosinophilic infiltration into the bronchial submucosa, which was suppressed by pretreatment with montelukast. The increase in serum IgE which is characteristic of type I allergy, was also observed in blood samples of BALB/c, but only a weak increase was noted in C57BL/6 [25]. These findings appear compatible with previous reports in which BALB/c mice exhibited helper T type 2 (Th2)-prone immune responses, and C57BL/6 displayed a helper T type 1 (Th1)-prone diathesis. This difference in AHR suggested a genetic influence [26–28]. However, our results appear to be in contrast to the conclusions of a report describing a dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma [3].

Asthma is believed to be dependent on Th2-prone immune responses [1]. The pathogenesis of bronchial asthma is closely linked to various chemical mediators including leukotrienes, cytokines and chemokines released by the interaction between IgE antibody and antigen [25]. However, the exact kind(s) of intrinsic factors that would inevitably influence the acquisition of AHR following anaphylactic reaction remains to be determined. In view of its clinical benefit, we evaluated the effects of the cysLT₁R antagonist, montelukast, in these two mouse models. Pretreatment with montelukast significantly suppressed AHR in BALB/c mice following antigen challenge and this outcome paralleled suppression of cellular infiltration, especially that of eosinophils and macrophages, either in BALF or bronchial submucosal tissue. These results strongly suggest that cysLTs play an important role in AHR following an anaphylactic reaction in BALB/c mice [20,29,30].

We compared cysLT production from lung tissues of the two strains. Chopped lung fragments isolated from OVA-sensitized C57BL/6 mice produced higher amounts of cysLTs when saline was added to the incubation mixture than did those from BALB/c mice. However, tissues from BALB/c produced significantly higher amounts of cysLTs on adding the allergen, OVA, than by the addition of saline, and this response was dose-dependent. In contrast, tissues from C57BL/6 also showed a tendency towards a dose-dependent increase in cysLTs by adding OVA, but not to any significant extent. While lung tissues from BALB/c mice produced 2.44 times more cysLTs than baseline levels as a result of anaphylactic reaction, those of C57BL/6 produced amounts only 1.42 times more than baseline levels. It is speculated that the relative increment in the production of cysLTs due to anaphylactic stimulation compared to the baseline is more important in the acquisition of AHR than is the absolute amount of cysLTs in tissue. As for the metabolism of cysLTs, LTC₄ is synthesized

exclusively by LTC₄ synthase from LTA₄ [31], successively metabolized to LTD₄ by γ -glutamyl transpeptidase [32] or γ -glutamyl leukotrienase [33,34], and then metabolized to LTE₄ by dipeptidase [35] through the glutathione moiety of LTC₄. However, in this study, there was no statistical difference between each cysLT fraction of the two strains.

We also found that the increased level of IgE following antigen challenge was also suppressed by pretreatment with cysLT₁R antagonist. A recent study using mice deficient in LTC₄ synthase, a pivotal enzyme for cysLT production, found that a Th2 type inflammatory reaction which included IgE production and interleukin-4 (IL-4), and -13 generation was suppressed by antigen challenge in comparison with its wild type [30]. Mast cells may be directly exposed to antigens in the peripheral mucous membranes and generate large amounts of cysLTs [30]. Activated mast cells produce the cytokines IL-4, -5, and -13 which may be necessary for IgE isotype switching, and also express CD40 ligand by which mast cell interact with B cells [36,37]. Mast cells derived from LTC₄ synthase deficient mice do not produce cysLTs and do not show mast cell proliferation (comitogenesis) through autocrine/intracrine cysLT-induced signaling or by cysLT₁R binding [38]. The suppression of IgE levels by administration of cysLT₁R antagonist before antigen challenge would explain the blocking of signaling through CysLTs/cysLT₁R to generate IL-4 and other cytokines. The finding that cysLT₁ R expression, and expression of cysLT₂R mRNA significantly increased in lungs of BALB/c mice following OVA challenge compared with saline-challenged mice supports the notion of autocrine-type amplification of cysLTRs and cysLTs in allergic inflammation [38].

In conclusion, two mouse strains, BALB/c and C57BL/6, showed distinctly different forms of AHR following anaphylactic reactions, a difference in which cysLTs play a pivotal role.

Acknowledgements

We thank Dr. William Campbell for English editing of the manuscript. This manuscript is dedicated for Dr. Tony Hugli's retirement of the editor-in-chief of *International Immunopharmacology*. A relationship between Hugli and me (M. Abe) started when I had worked as a research associate for a few years in his laboratory of Research Institute of Scripps Clinic. Thereafter, we have continued collaboration with each other in studies for anaphylatoxins and eicosanoids. I believe that we will keep on challenging a newer subject concerning anaphylatoxins and human inflammatory disorders in future.

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