Expression of CysLT2 receptors in asthma lung, and their possible role in bronchoconstriction

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**Article Info**

**A B S T R A C T**

Background: The expression and functional role of CysLT2 receptors in asthma have not been clarified. In this study, we evaluated CysLT2 receptors expression, and effects of CysLT2- and CysLT1/CysLT2-receptor antagonists on antigen-induced bronchoconstriction using isolated lung tissues from both asthma and non-asthma subjects.

Methods: CysLT1 and CysLT2 receptors expression in asthma and non-asthma lung tissue preparations was examined in immunohistochemistry experiments, and their functional roles in antigen-induced bronchoconstriction were assessed using ONO-6950, a dual CysLT1/CysLT2-receptor antagonist, montelukast, a CysLT1 receptor antagonist, and BayCysLT2RA, a CysLT2 receptor-selective antagonist.

Results: CysLT1 receptors were expressed on the bronchial smooth muscle and epithelium, and on alveolar leukocytes in 5 in 5 non-asthma subjects and 2 in 2 asthma subjects. On the other hand, although degrees of CysLT2 receptors expression were variable among the 5 non-asthma subjects, the expression in the asthma lung was detected on bronchial smooth muscle, epithelium and alveolar leukocytes in 2 in 2 asthma subjects. In the non-asthma specimens, antagonism of CysLT2 receptors did not affect antigen-induced bronchial contractions, even after pretreatment with the CysLT1-receptor specific antagonist, montelukast. However, in the bronchus isolated from one of the 2 asthma subjects, antagonism of CysLT2 receptors suppressed contractions, and dual antagonism of CysLT1 and CysLT2 receptors resulted in additive inhibitory effect on anaphylactic contractions.

Conclusions: CysLT2 receptors were expressed in lung specimens isolated from asthma subjects. Activation of CysLT2 receptors may contribute to antigen-induced bronchoconstriction in certain asthma population.

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**Introduction**

Cysteinyl leukotrienes (CysLTs), LTC\textsubscript{4}, LTD\textsubscript{4} and LTE\textsubscript{4} are inflammatory mediators derived from the 5-lipoxygenase pathway of arachidonic acid metabolism.\textsuperscript{1} As these mediators are known to be deeply involved in bronchial asthma via activation of the CysLT1 receptor,\textsuperscript{2–4} selective antagonists of this receptor, including pranlukast,\textsuperscript{5,6} montelukast,\textsuperscript{7} and zafirlukast\textsuperscript{8} have widely been used as therapeutic agents for bronchial asthma. On the other hand, a number of studies have cloned and characterized another receptor subtype of CysLTs termed CysLT2 receptor.\textsuperscript{9–11} This receptor, like the CysLT1 receptor,\textsuperscript{12} is a G-protein-coupled receptor with an amino acid sequence 38% identical to that of the CysLT1 receptor.\textsuperscript{9} CysLT2 receptor mRNA has been detected in a number of human organ and tissues, including lung macrophages, airway smooth muscles, and peripheral blood leukocytes,\textsuperscript{9} and its expression has been identified on the nucus gland and nasal mucosal epithelium of patients with chronic rhinosinusitis, or allergic nasal vascular smooth muscles.\textsuperscript{13,14} As for endogenous
ligands binding, it is reported that CysLTs order of binding to human CysLT1 receptors is LTD₄ > LTC₄ > LTE₄, while that for CysLT₂ receptors is LTC₄ > LTD₄ > LTE₄.₁⁰ Based on these findings, it is expected that the CysLT₂ receptor is involved in the pathophysiology of bronchial asthma. However, it is unclear where CysLT₂ receptors are histologically expressed in airway tissues of asthma subjects. It is well known that CysLTs exert potent contractile action on human bronchial smooth muscles through activation of CysLT₁ receptors. Indeed, in vitro antigen-induced contraction of passively sensitized human bronchial tissue is markedly suppressed by a pretreatment with CysLT₁ receptor antagonists.₃,₁⁵,₁⁶ However, although CysLT₂ receptors are also expressed in bronchial smooth muscles, their functional role in bronchial contraction has not been clarified. In this study, we evaluated CysLT receptors expression and effects of ONO-6950, dual CysLT₁/₂ antagonists and BayCysLT₂RA,₁⁷ a CysLT₂ receptor-specific antagonist on antigen-induced bronchoconstriction using isolated lung tissues from both asthma and non-asthma subjects.

Methods

Subjects

Macroscopic normal portions of lung tissue were obtained from 21 non-asthma and 2 asthma subjects (Table 1) during lung cancer surgery at Ogaki Municipal Hospital (Ogaki, Japan). All subjects provided their informed consents for use of the collected materials in medical research under a protocol approved by the Ethical Committees of Ogaki Municipal Hospital and Kyoto Pharmaceutical University (Kyoto, Japan). Both asthma subjects were diagnosed with bronchial asthma at Ogaki Municipal Hospital in 2003. As shown in Table 1, asthma subject 1 and 2 were treated with inhaled corticosteroid (ICS)/long-acting β₂ receptor agonist (LABA) (fluticasone/salmeterol, Adoair®) at medium (250 μg, twice a day) and low (250 μg, once a day) doses, respectively. In addition, the asthma subject 1 had been treated with montelukast (Singulair®) for approximately 3 months from a day of approximately 1 year before the surgery.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Non-asthma subjects</th>
<th>Asthma subject 1</th>
<th>Asthma subject 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>66.1 ± 12.5</td>
<td>62</td>
<td>60</td>
</tr>
<tr>
<td>Age at asthma onset (yr)</td>
<td>54</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>18 male/3 female</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>ICS/LABA</td>
<td>Yes (medium dose)</td>
<td>Yes</td>
<td>(low dose)</td>
</tr>
<tr>
<td>(Fluticasone/salmeterol, Adoair®)</td>
<td>90.3</td>
<td>87.4</td>
<td></td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>8440</td>
<td>3920</td>
<td></td>
</tr>
<tr>
<td>Peripheral blood leukocytes (cells/μl)</td>
<td>39.7</td>
<td>40.7</td>
<td></td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>6.8</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>1.2</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>5.1</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>42.7</td>
<td>43.9</td>
<td></td>
</tr>
<tr>
<td>Platelets (cells/μl)</td>
<td>319000</td>
<td>201000</td>
<td></td>
</tr>
<tr>
<td>Total serum IgE (IU/ml)</td>
<td>838</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>No</td>
<td>Yes</td>
<td></td>
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</tbody>
</table>

In asthma subject 1, Adoair® therapy (250 μg, twice a day) had been continued until the lung cancer surgery, and the lung function and hematological data were collected approximately 6 months before and 1 week after the surgery, respectively. In asthma subject 2, Adoair® therapy (250 μg, once a day) had been continued until 2 years after the surgery when the lung function and hematological data were collected.

How the collected lung samples were used for various experiments is summarized in our online supplementary material (Supplementary Table 1).

Histological and immunohistochemical studies

The lung tissues were fixed in 10% formaldehyde, embedded in paraffin wax, and cut into 4 (4-μm thick) serial sections. Two of these sections were stained first with hematoxylin and eosin (HE), and then with alcian blue/periodic acid-Schiff (AB/PAS). The other 2 sections were used in immunohistochemistry for detection of CysLT₁ and CysLT₂ receptors expression. After antigenicity activation, the sections were treated with 3% hydrogen peroxide solution, washed with 50 mM Tris—HCl buffer (pH 7.4), and blocked with 1% bovine serum albumin. The sections were then stained with rabbit polyclonal anti-human CysLT₁ receptor antibody (LS-A1317, Life-Span Biosciences, Seattle, WA, USA at a dilution of 1:600) or with rabbit polyclonal anti-human CysLT₂ receptor antibody (120560, Cayman Chemical, Ann Arbor, MI, USA at a dilution of 1:300) overnight at room temperature. After washing with 50 mM Tris—HCl buffer (pH 7.4), the sections were treated with anti-rabbit biotinylated secondary antibody for 30 min at 4 °C, washed with 50 mM Tris—HCl buffer (pH 7.4) once again, and stained with streptavidin-horseradish peroxidase for 30 min. Coloring was then developed by soaking the sections in 3,3'-diaminobenzidine solution for 10 min, followed by counterstaining with a hematoxin solution.

CysLT receptor antagonists

Montelukast, a CysLT₁ receptor antagonist, BayCysLT₂RA,₁⁷ a CysLT₂ receptor antagonist, and ONO-6950, a dual CysLT₁/₂ receptor antagonist, were synthesized in Ono Pharmaceutical Co., Ltd. (Osaka, Japan) and used in this study as CysLT receptor antagonists. The chemical formula of ONO-6950 is 4,4'-[4-fluoro-7-(2-[4-[4-(3-fluoro-2-methylphenyl]butoxy]phenyl]ethynyl]-2-methyl-1H-indole-1,3-diyl]dibutanoic acid.

LTD₄-induced intracellular calcium mobilization

Human CysLT₁ (hCysLT₁) receptor- and human CysLT₂ (hCysLT₂) receptor-expressing cells were generated by transfecting each expression vector into Chinese hamster ovary (CHO)-K1 cells as described in Supplementary Methods.

Calcium mobilization assays were carried out using Fura-2 AM-loaded CHO-K1 cells stably expressing hCysLT₁ or hCysLT₂ receptor. The cells were cultured in Ham’s F-12 medium (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum and 0.5 mg/ml geneticin (Invitrogen) in 5% CO₂ at 37 °C. The cells were seeded into 96-well special optics flat bottom black polystyrene TC-treated microplates (Corning, Corning, NY, USA) at 3 x 10⁴ cells/well and then incubated in 5% CO₂ for 24 h at 37 °C. The cells were treated with 5 μM Fura-2 AM (Dojindo, Kumamoto, Japan) in Hanks’ Balanced Salt Solution (HBSS) containing 20 mM HEPES and 2.5 mM probenecid (Sigma—Aldrich, St. Louis, MO USA) at 1 h at 37 °C. After washing with HBSS containing 20 μM HEPES, various concentrations of montelukast, BayCysLT₂RA, ONO-6950 or vehicle (0.1% DMSO) were added to the cells, and the cells were incubated, protected from light, for another 30 min at room temperature. LTD₄ (100 nM for hCysLT₁, and 0.3 nM for hCysLT₂ receptor) was next added to the cells, and intracellular calcium mobilization was determined by the ratio of fluorescence intensity (F340/F380) measured at 500 nm every 3 s for 4.5 min using a Functional Drug Screening System (FDSS 3000, Hamamatsu Photonics, Hamamatsu, Japan).
Antigen- or LTD₄-induced contraction in isolated human bronchial strips

As described in our previous report,¹⁵ bronchi (outer diameter 2–6 mm) were isolated from macroscopic normal portions of the lung tissue and cut into 1.5 mm wide spirals. In the experiments for evaluation of the effects of CysLT antagonists on antigen-induced contraction, the bronchi were passively sensitized by incubation in human atopic serum (RAST score < 4) for 2 h at 37 °C. Bronchial strips (2 cm long, outer diameter 2–4 mm) were then cut and suspended under an isotonic resting tension of 300 mg at 37 °C in a Magnus bath containing Tyrode’s solution gassed with 95% O₂–5% CO₂. Before starting the experiments, acetylcholine (5 μM) and then histamine (10 μM) were repeatedly applied to the preparations until almost equal contractions were obtained.

When the resting tonus of the histamine-treated washed smooth muscle had stabilized, guinea pig serum albumin (Sigma-Aldrich) at final concentration of 0.1 or 1 mg/ml was added to prevent adsorption of ONO-6950 and montelukast to the inner wall of the Magnus bath. Just after addition of guinea pig serum albumin, ONO-6950 (100, 300 or 1000 nM), BayCysLT₂RA (100 or 300 nM), montelukast (30 or 100 nM), or DMSO (final concentration: 0.1%) was applied. Antigen (mite extract from Dermatophagoides farinae: 3 μg/ml) or LTD₄ (30 nM) challenge was initiated 30–35 min after treatment with each CysLT antagonist. As previously reported,²,¹⁸ a cyclooxygenase inhibitor, indomethacin (Sigma-Aldrich, 3 μM) and an anti-histamine drug, pyrilamine (Sigma-Aldrich, 1 μM) were applied 10 and 5 min before antigen challenge, respectively, to make antigen-induced contractile response dependent on CysLTs activity. Data, expressed as % of 10 μM histamine-induced contraction, were then calculated using height 30 min before the challenge as baseline.

In experiments using LTD₄ as a smooth muscle constrictor, passive sensitization of bronchial tissue was not performed, and neither indomethacin nor pyrilamine was used.

### Non-asthma

<table>
<thead>
<tr>
<th>HE staining</th>
<th>AB/PAS staining</th>
<th>CysLT₁ receptors</th>
<th>CysLT₂ receptors</th>
</tr>
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<tbody>
<tr>
<td>Subject 1</td>
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<td></td>
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<td>Subject 2</td>
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<td>Subject 4</td>
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<td>Subject 5</td>
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</table>

Fig. 1. Immunohistochemical micrographs of lung tissues from the 5 non-asthma subjects. The tissues were cut into 4 serial sections (4-μm thick). Two of the sections were stained with hematoxylin and eosin (HE), and then with alcin blue/periodic acid-Schiff (AB/PAS). The other two sections were used in immunohistochemical determination of CysLT₁ and CysLT₂ receptors expression. Arrows show representative immunoreactive sites for each receptor.
Results

Immunohistochemical detection of CysLT1 and CysLT2 receptors

As shown in Figure 1, the specimens isolated from all 5 non-asthma subjects revealed CysLT1-receptor immunoreactivity in the bronchial epithelium, bronchial smooth muscle layer, and alveolar leukocytes, although the degree of this receptor’s expression was relatively low in subject 2. On the other hand, CysLT2 receptors were expressed predominantly on the bronchial epithelium of non-asthma subjects. CysLT2 receptors expression on bronchial smooth muscle was observed in non-asthma subject 4 and 5, and that on alveolar leukocytes was in non-asthma subject 1, 4 and 5 (Fig. 1).

Like the specimens from the 5 non-asthma subjects, specimens from the 2 asthma subjects showed expression of CysLT1 receptor on the bronchial smooth muscle, bronchial epithelium, and alveolar leukocytes (Fig. 2). Regarding CysLT2 receptors as well, specimens from both 2 asthma subjects showed the expression on the bronchial smooth muscle, bronchial epithelium, and alveolar leukocytes (Fig. 2). In addition, CysLT2 receptor-positive sites were detected in mucus including leukocytes, which may have been released in the bronchial lumen of the 2 asthma subjects.

When asthma lung tissues were stained with rabbit IgG in place of anti-CysLT1 or anti-CysLT2 receptor antibody, no immunostaining sites were detected as shown in Supplementary Figure 1.

Effects of CysLT receptor antagonists on LTD4-induced intracellular calcium mobilization in CysLT1 or CysLT2 receptor-expressing cells

ONO-6950 inhibited both CysLT1 and CysLT2 receptors-mediated response in LTD4-induced calcium mobilization assay with IC50 values of 1.7 and 25 nM, respectively (Table 2). This inhibition was less than that of montelukast for CysLT1 receptor-mediated response (IC50 value of 0.46 nM), but comparable to that reported for BayCysLT2RA against CysLT2 receptor-mediated response (IC50 value of 14 nM).17 Montelukast showed very weak inhibition of CysLT2 receptor-mediated response (IC50 value of 1800 nM), while BayCysLT2RA inhibition of CysLT1 receptor-mediated response is reported to be even weaker (IC50 value of 2500 nM).17

Effects of CysLT receptor antagonists on LTD4- or antigen-induced bronchial smooth muscle contractions

As shown in Figure 3A, ONO-6950 concentration-dependently inhibited LTD4-induced contractions in the bronchi isolated from non-asthma subjects with almost complete inhibition at 1000 nM (Fig. 3A). This effect was comparable to that of montelukast (100 nM), suggesting that ONO-6950 antagonistic activity for the CysLT1 receptor is approximately 10-fold less than that of montelukast. As for BayCysLT2RA, this CysLT2 receptor antagonist at
300 nM had no effect on LTD4-induced contractions in the bronchi isolated from the non-asthma subjects (Fig. 3B).

Figure 4 represents effects on antigen-induced contraction of bronchi isolated from non-asthma subjects. Both ONO-6950 (300 nM) and montelukast (30 nM) clearly, but only partially, inhibited antigen-induced bronchial smooth muscle contractions, especially 30–90 min after antigen challenge (Fig. 4). BayCysLT2RA (100 nM), on the other hand, had no inhibitory or potentiating effect on such contractions, even at the high concentration of 1000 nM (data not shown). When the CysLT2 receptor-specific antagonist BayCysLT2RA was used in combination with montelukast, it neither potentiated nor weakened montelukast-induced inhibition of antigen-induced bronchial smooth muscle contractions (Fig. 4).

In the bronchi isolated from the asthma subject 1, both the CysLT1-specific antagonist montelukast and the CysLT2-specific antagonist BayCysLT2RA, partially inhibited antigen-induced airway smooth muscle contractions (Fig. 5A, Supplementary Fig. 2A). Interestingly, combination of montelukast and BayCysLT2RA produced additive strong inhibition of such contractions (Fig. 5A, Supplementary Fig. 2A). Consistent with this finding, the dual CysLT1/CysLT2 antagonist ONO-6950 inhibited antigen-induced airway smooth muscle contractions with a potency equivalent to that of montelukast and BayCysLT2RA combination treatment (Fig. 5A, Supplementary Fig. 2A). It is noteworthy to mention here that the baseline of montelukast-treated bronchus was relatively high at antigen challenge, probably due to unstable baseline during pre-treatment with montelukast, pyrilamine and indomethacin (Fig. 5A, Supplementary Fig. 2A).

As for the bronchi isolated from the asthma subject 2, only preparations treated with antagonists of the CysLT1 receptor (montelukast or ONO-6950) showed reduced contractions (Fig. 5B, Supplementary Fig. 2B).

### Discussion

In the present study, we showed that CysLT1 receptors were expressed on the bronchial smooth muscle and epithelium, and on alveolar leukocytes in all non-asthma and asthma subjects. On the other hand, CysLT2 receptors were expressed on these airway cells in 2 in 2 asthma subjects, whereas they were detected in only a part of non-asthma subjects. Furthermore, our results show that blockade of CysLT2 receptors suppressed antigen-induced bronchial smooth muscle contractions in lung tissue preparations from one of the two asthma subjects.

One of the limitations of our study is that we were only able to use 2 asthma samples. It is quite difficult to obtain sufficient lung tissues from asthma patients for research. However, it should be noted that we used serial tissue sections that allowed co-expression of CysLT2 and CysLT1 receptors at the same sites. To our knowledge, this is the first report demonstrating the concurrent expression of CysLT2 receptors with CysLT1 receptors in airway tissues of actual asthma subjects compared to non-asthma subjects. In addition, our findings suggest that CysLT2 receptors are functionally involved in asthma bronchoconstriction.

It is also interesting to note that the asthma subjects recruited in this study had mild symptoms compared to those of the so-called “severe asthma”, since asthma subject 1 and 2 were classified as “mild persistent” and “mild intermittent”, and were treated with a medium dose ICS (treatment step 2) and a low dose ICS (treatment step 1), respectively. It may therefore be speculated that expression of CysLT2 receptors is further up-regulated in subjects with severe asthma or asthma exacerbation. Indeed, it is reported that asthma exacerbation triggers CysLT2 receptors expression in eosinophils, a phenomenon not observed during stable periods. On the other hand, Negri et al. have demonstrated that fluticasone inhibited IL-
4-induced CysLT2 receptors protein expression, but not CysLT1 receptors, on monocytes, T cells, and eosinophils. Because the asthma subjects were treated with ICS/LABA, the CysLT2 receptors expression may have been negatively regulated. Regarding effect of LABA on CysLT receptors expression, there has been no study to our knowledge.

In agreement with our results showing the presence of CysLT2 receptors in alveolar leukocytes, both CysLT1 and CysLT2 receptors have been reported to be expressed not only in macrophages,9 but also in eosinophils,19,21 mast cells,22 basophils,23 and dendritic cells.24 The functional importance of CysLT2 receptors in these leukocytes was not addressed in this study. However, Jiang et al.22 have shown that both CysLT1 and CysLT2 receptors are expressed on the membranes and nuclei of a human mast cell line, and that knockdown of CysLT2 receptors increases CysLT1 receptors surface expression. These findings suggest that activation of CysLT2 receptors down-regulates CysLT1 receptors expression.

In addition to CysLT2 receptors activation negative feedback on CysLT1 receptors expression, a broader functional regulation between CysLT1 and CysLT2 receptors has also been reported: Knockdown of CysLT2 receptors was reported to increase CysLT1 receptor-dependent proliferation of human mast cells.22 Barrett et al.24 have shown that D. farinae sensitization and challenge in CysLT2 receptor-deficient mice results in a marked increase in eosinophilic pulmonary inflammation, serum IgE level, and Th2 cytokine level. Maekawa et al.25 have also reported that leukotriene-induced ear edema shows a delayed peak response in CysLT2 receptor-deficient mice compared to wild-type mice. These findings suggest that activation of CysLT2 receptors down-regulates not only CysLT1 receptors expression, but also CysLT1 receptor-mediated biological and inflammatory responses. However, as shown in this study, treatment with the CysLT2 receptor antagonist BayCysLT2RA did not reverse montelukast-induced inhibition of anaphylactic bronchoconstriction in specimens from both the non-asthma and asthma subjects. Therefore, CysLT1 receptors activation may be differently regulated by CysLT2 receptors expression, at least in human bronchial contractions. Alternatively, the observed discrepancy may be due to species difference.

It is now widely known that blockade of CysLT1 receptors strongly inhibits antigen-induced bronchial contractions in specimens isolated from non-asthma subjects. However, this blockade is not complete as shown in the present study and in other literature.4,15,16 The results of the current study suggest that CysLT2 receptors activation has no significant role in the bronchial contractions recorded in the non-asthma specimens. On the other hand, in one of the two asthma specimens, CysLT2 receptors blockade inhibited anaphylactic bronchoconstriction. This inhibition was potentiated by dual blockade of CysLT1 and CysLT2 receptors. These results suggest that there may be a certain asthma background, in which activation of CysLT2 receptors is involved in anaphylactic bronchoconstrictive response, and thus may play a significant role in asthma response in certain asthma population.

It is not clear why the involvement of CysLT2 receptor activation in anaphylactic response was different between the 2 asthma specimens, even though CysLT2 receptors were expressed in both specimens to a similar degree. Mechanisms other than increased...
expression of CysLT2 receptors, such as functional up-regulation, may also be involved in this response. It is intriguing to speculate that asthma background affects such functional up-regulation. On the other hand, it should be noted that one lung sample showing CysLT2 receptors contribution to bronchoconstriction was derived from an asthma subject (asthma subject 1), who had had a history of atopy, whereas the other subject (asthma subject 2) had not. In addition, asthma subject 1 had a relatively high percentage of eosinophils in peripheral blood leukocytes (6.8%). Moreover, asthma subject 1 was non-smoker, whereas asthma subject 2 was smoker. It is therefore suggested that these subjects backgrounds may have affected the function of CysLT2 receptors. However, further studies are needed to clarify the exact role of CysLT2 receptors in asthma pathogenesis.

Hennder et al.26 have demonstrated that montelukast blockade of CysLT1 receptors improves airway remodeling, including airway goblet cell metaplasia, smooth muscle cell layer thickening, and subepithelial fibrosis in a mouse model of asthma. The histological findings of this study revealed a thickened bronchial epithelium exhibiting epithelial cells filled with AB/PAS-positive mucus in the specimens prepared from the asthma patients. Interestingly, in these specimens, CysLT1 receptors tended to be expressed on the mucus-positive epithelium, suggesting that CysLT1 receptors activation is involved in the development of epithelial remodling in humans as reported in mice.26 It has also been reported that CysLTs mediate Th2 cell-dependent pulmonary inflammation through activation of CysLT1 receptors in mice.27,28 Considering the fact that both CysLT2 and CysLT1 receptors are highly expressed in the airway epithelium, it is possible that CysLT2 receptors also play a role in the development of airway remodeling. This hypothesis need to be further investigated both in vitro and in vivo.

In conclusion, we have shown in this study that CysLT1 receptors were expressed in lung specimens isolated from 2 asthma subjects. This CysLT1 receptors expression may contribute to antigen-induced bronchoconstriction in certain asthma cases. These results imply that CysLT1 receptor antagonists, including BayCysLT1/2RA and ONO-6950, may be useful for the treatment of the certain asthma population. However, because the present findings were led from only 2 asthma specimens, further preclinical and clinical studies on CysLT2- or CysLT1/2-receptor antagonists are required.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.allr.2015.04.008.

Conflict of interest

TS, MK and KK are employees of Ono Pharmaceutical Co., Ltd, Osaka, Japan. TN received a research grant from Ono Pharmaceutical Co., Ltd. The rest of the authors have no conflict of interest.

Authors’ contributions

TA and MH were involved in the recruitment of volunteers and in data interpretation. SK was involved in the design of the study and in data interpretation. KK was involved in the study design, data interpretation and manuscript preparation. MF contributed to data collection, analysis and interpretation. TS, MK and TN were involved in the study design, data collection, data interpretation, data analysis and writing the manuscript. TS and TN have full access to the data and are responsible for the integrity of data and final decision to submit this manuscript. All authors have approved the final version of this manuscript for submission.

References


