

Expression and localization of the cysteinyl leukotriene 1 receptor in human nasal mucosa

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Summary

Background The cysteinyl leukotrienes (CysLT) are lipid mediators that have been implicated in the pathogenesis of allergic diseases. Pharmacological studies using CysLTs indicate that two classes of receptors, named CysLT1 and CysLT2 receptor, exist. The former is sensitive to the CysLT1 antagonist currently used to treat asthma and allergic rhinitis. Recently, the cDNA for human CysLT1 and CysLT2 receptor have been cloned, making it now possible to study the gene expression of CysLTs receptors.

Objective We have used reverse transcription and polymerase chain reaction (RT-PCR) to study the gene expression of CysLT1 and CysLT2 receptor and *in situ* hybridization to determine the distribution of CysLT1 receptor mRNA in human nasal mucosa. In addition, the distribution of the CysLT1 receptor protein was studied by immunohistochemistry.

Methods Human turbinates were obtained after turbinectomy from six patients with nasal obstruction refractory to medical therapy. Total RNA was isolated from human nasal mucosa and both CysLT1 and CysLT2 receptor mRNA was detected in these tissues by using RT-PCR. For *in situ* hybridization study of human nasal mucosa, we used biotin-labelled oligonucleotides probes encoding human CysLT1 receptor cDNA. To identify the cells expressing the CysLT1 receptor protein, double immunostaining was performed by using anti-CysLT1 receptor antibody and monoclonal antileucocyte antibodies.

Results RT-PCR analysis of total nasal RNA demonstrated the expression of both CysLT1 receptor and CysLT2 receptor mRNA. *In situ* hybridization indicated high levels of CysLT1 receptor hybridization in blood vessels and the interstitial cells, but a sparse signal in airway epithelium and submucosal glands. The immunohistochemical studies revealed that anti-CysLT1 receptor antibody labelled eosinophils, mast cells, macrophages, neutrophils and vascular endothelial cells in the nasal mucosa.

Conclusion The results may have an important clinical implication and also promote further investigation of the regulation of CysLT1 receptor in health and disease.

Keywords CysLT1 receptor, CysLT2 receptor, nasal mucosa

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Introduction

The allergic response is a complex process involving the interaction of many mediators. It is well known that nasal challenge with histamine causes sneezing, rhinorrhea and nasal mucosal swelling, which are the major symptoms of allergic rhinitis. Similarly, a nasal challenge with cysteinyl leukotriene (CysLT) increases nasal blood flow and nasal mucosal swelling [1, 2]. Furthermore, CysLTs were recovered from the nasal lavage fluid of allergic rhinitis patients after allergen provocation [3, 4]. In addition, it has been shown that CysLT receptor

antagonist, zafirlukast [5], pranlukast [6] and montelukast [7] relieved the symptoms of allergic rhinitis. These reports suggest that not only histamine, but also Cys LT, may play an important role in the pathogenesis of allergic rhinitis. Pharmacological studies using Cys LTs indicate that at least two classes of receptors, named CysLT1 and CysLT2, exist. Recent cloning of the human CysLT1 [8, 9] and CysLT2 [10, 11] receptor genes allows an approach to study the gene expression of CysLTs receptors in nasal mucosa.

In the present study we used reverse transcription and polymerase chain reaction (RT-PCR) to demonstrate the presence of CysLT1 and CysLT2 receptors and *in situ* hybridization to determine the distribution of CysLT1 receptor mRNA in human nasal mucosa. In addition, immunohistochemistry was performed to confirm the CysLT1 receptor protein in these tissues.

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Materials and methods

Tissue preparation

Human turbinates were obtained after turbinectomy from six patients with nasal obstruction refractory to medical therapy. Informed consent was obtained from all patients and this study was approved by the ethics committee of the Sapporo Medical University. Donors ranged in age from 16 to 49 years: four male and two female. All were non-smokers and three patients had perennial allergy against mite as defined by history and CAP-RAST. All medications, including antibiotics, were prohibited for at least 3 weeks prior to the study. The nasal mucosal specimens were dissected from the cartilage, and: (i) immediately frozen in liquid nitrogen and stored at -70°C for RNA extraction; (ii) frozen in O.C.T. compound (Sakura Finetechnical Co., Tokyo, Japan) for *in situ* hybridization; and (iii) fixed in 10% formalin for immunohistochemistry.

RNA extraction and RT-PCR analysis

Total RNA from nasal mucosa was isolated in accordance with Chomczynski and Sacchi [12]. Some of the RNA samples ($1\text{ }\mu\text{g}/\mu\text{L}$) were treated with DNase I ($10\text{ }\mu\text{g}/\mu\text{L}$) for 30 min to avoid DNA contamination following the second RNA extraction. RT-PCR was carried out by using a commercial One Step RNA PCR Kit (Takara Biomedicals, Tokyo, Japan). RNA (50 ng) was reverse-transcribed into cDNA by incubating with 5 U AMV Reverse Transcriptase XL at 50°C for 30 min. After denaturation at 94°C for 2 min, cDNA were amplified by 30 or 40 cycles of PCR (94°C for 30 s, 60°C for 30 s, and 72°C for 1 min) with 5 U of AMV-Optimized Taq DNA polymerase to amplify genes for CysLT1 receptor, CysLT2 receptor and beta-actin. For the CysLT1 receptor, the forward primers (19–42): 5'-CTGACAGTATCTTCTGCCACATGC-3' and the reverse primers (155–178): 5'-CTTGAAGGCTGACTTCTTGAT-3' (160-bp fragment) were used; for CysLT2 receptor, the forward primers (510–532): 5'-AATAATGCTCCTGGACAGTGGCT-3' and the reverse primers (657–680): 5'-ACCCGAATGATCAGCAGATAACAG-3' (171-bp fragment) were used. PCR products were analysed by agarose electrophoresis in 1.5% gel and visualized by ethidium bromide staining. In addition, the purified PCR product was directly subjected to automated DNA sequencing by a dye terminator method (Bigdye: Applied Biosystems, Tokyo, Japan).

In situ hybridization

The oligonucleotide antisense probes used for the *in situ* studies of Cys LT1 receptor were 5'-GAATGCAGAAGTCCGTGGT-CATAACCTTGTCTCTGGC TGCATCCAATTG-3' (815–863), and their complementary sequence was used as sense probe. Tailing of oligonucleotides with biotin-16-dUTP (Roche Diagnostic GmbH, Germany) was carried out as described by the manufacturer except for substitution of digoxigenin-dUTP with biotin-16-dUTP in the tailing reaction. Prior to hybridization, the $6\text{ }\mu\text{m}$ cryostat sections were rinsed in phosphate-buffered saline (PBS) (pH 7.4) for 5 min and fixed in 4% paraformaldehyde/PBS for 15 min at 4°C . They were then permeabilized with 0.03% Triton X-100 for 10 min at room temperature (20°C) treated with a solution of proteinase K ($20\text{ }\mu\text{g}/\text{mL}$) in 10 mM Tris (pH 7.5) containing 1 mM EDTA for 10 min, refixed in 4% paraformaldehyde/PBS for 5 min, and dehydrated

through ethanol. Hybridization was performed with an anti-sense biotin-labelled Cys LT1 receptor probe ($10\text{ pmol}/\text{mL}$) diluted in hybridization buffer containing 50% formamide, 300 mM NaCl, 10 mM Tris (pH 7.5), 1 mM EDTA, 10% dextran sulphate, $1\times$ Denhardt's solution, 10 mM dithiothreitol (DTT), and $200\text{ }\mu\text{g}/\text{mL}$ yeast RNA in a humidified chamber for 20 h at 37°C . After hybridization, the slides were washed once in $2\times$ standard saline citrate (SSC)/50% formamide for 10 min at room temperature, twice in $1\times$ SSC for 15 min at room temperature, and twice in $0.5\times$ SSC for 15 min at room temperature. The signal of bound biotininated probe was amplified by using Tyramide Signal Amplification (NEN Life Science Products, Boston, MA, USA) following visualization with diaminobenzidine (DAB) according to the manufacturer's instructions. The colour reaction was stopped with 10 mM Tris HCl (pH 7.5), 1 mM EDTA, and the slides were lightly counterstained with haematoxylin, dehydrated, cleared, and mounted in Histomount (Zymed Laboratories, San Francisco, CA, USA).

Non-specific hybridization was determined by parallel incubation of adjacent sections with the sense biotin-labelled Cys LT1 receptor oligonucleotide probe (identical to the coding strand of human Cys LT1 receptor mRNA).

Antibodies

For immunohistochemistry of CysLT1 receptor, anti-CysLT1 receptor polyclonal antibody (Cayman Chemical, Ann Arbor, MI, USA) was used at 1:150 dilution. To identify the subsets of cells expressing the CysLT1 receptor, the following monoclonal antibodies were used: anti-CD68 (KP-1 clone, Dako) at 1:80 dilution for macrophage; antieosinophil cationic protein (EG2, Pharmacia, Stockholm, Sweden) at 1:40 dilution for eosinophil; antimast cell tryptase (AA1 clone, Dako) at 1:40 dilution for mast cell; anti-neutrophil elastase (NP57 clone, Dako) at 1:80 for neutrophil; and antipan T cell marker (MT1 clone, Bioscience Products AG, Emmenbrucke, Switzerland).

Immunohistochemistry

Deparaffinized sections were initially incubated with 3% H_2O_2 in methanol for 30 min to quench endogenous peroxidase activity, washed two times in PBS and treated with $10\text{ }\mu\text{g}/\text{mL}$ proteinase K for 10 min at room temperature. After microwave treatment (10 min at 500 Watt in citrate buffer), the sections were incubated in blocking solution (10% normal goat serum in PBS) for 30 min before incubation in primary antibody. Then, the sections were incubated with anti-CysLT1 receptor polyclonal antibody overnight at 4°C , washed, and incubated for 30 min with EnVision+, Peroxidase, Rabbit (Dako Corporation, Carpinteria, CA, USA). A further washing in PBS was followed by developing in DAB (Dako) as a chromogen for signal visualization. The slides were dehydrated, cleared and mounted in Histomount. To detect the phenotype of the leucocytes expressing CysLT1 receptor, some sections first were stained by using commercial streptavidin-biotin immunostain kits (alkaline phosphatase LSAB⁺ kit, Dako) prior to the microwave treatment. Using this method, CysLT1 receptor expressing cells were brown, and cellular phenotypical makers were stained red. Negative controls were obtained by replacing primary antibodies by either mouse IgG1 (Dako) or normal rabbit immunoglobulin (Dako).

Results

RT-PCR analysis

In order to detect human CysLT1 and CysLT2 receptor mRNA by RT-PCR, specific oligonucleotide primers were constructed in accordance with the sequence reported for human CysLT1 receptor cDNA [8,9] and human CysLT2 receptor [10,11]. Figure 1 shows the results of RT-PCR for 30 cycles using total RNA extracted from human nasal mucosa, which revealed the expression of CysLT1 receptor mRNA (Fig. 1, lane 1), but no PCR products of CysLT2 receptor with 30 cycles amplification (Fig. 1, lane 5). RT-PCR was carried out for 40 cycles using total RNA extracted from human nasal mucosa and the expected size products of CysLT1 receptor (Fig. 2, lane 1) and CysLT2 receptor (Fig. 2, lane 4) were obtained. As CysLT1 and CysLT2 receptors have only one exon, DNA contamination of the RNA preparation cannot usually be judged, because the PCR products will be similar in size. The control included cDNA reaction mixtures with RNA but no reverse transcriptase (Fig. 1, lanes 2 and 6; Fig. 2, lanes 2 and 5), without RNA (Fig. 1, lanes 3 and 7; Fig. 2, lanes 3 and 6), and with RNA but no Taq DNA polymerase (Fig. 1, lanes 4 and 8). These results indicated the existence of both CysLT1 and CysLT2 receptor mRNA in human nasal mucosa. Sequencing analysis of the PCR products of CysLT1 receptor and CysLT2 receptor revealed no mutated sequence in any of the PCR products of CysLT1 and CysLT2 receptor (data not shown).

In situ hybridization

Hybridization with biotin-labelled oligonucleotide probes encoding human CysLT1 receptor revealed the intense brown signal overlying the sections. The blood vessels and the interstitial cells were very densely labelled (Figs 3a and 4a). However, the epithelium and the submucosal glands were sparsely labelled (Figs 3a and 4a). The patterns of these signals in all six cases were remarkably similar. Specificity of the hybridization was shown by the absence of hybridization with a corresponding sense probe (Figs 3b and 4b).

Immunohistochemistry

CysLT1 receptor distribution in nasal mucosa was examined using a rabbit polyclonal antibody against a peptide corresponding to amino acid 318–333 at C-terminal of human CysLT1 receptor. Immunoreactivity for CysLT1 receptor was significantly detected in the interstitial cells and vascular endothelial cells (Figs 5a and 6). The interstitial cells expressed the highest level of CysLT1 receptor immunoreactivity in the nasal mucosa; however, the epithelial cells and the submucosal glands express little or no CysLT1 receptor immunoreactivity (Fig. 5a). Specificity of the staining was confirmed by the absence of labelling with normal rabbit immunoglobulin (Fig. 5b). In order to clarify the cell type of the interstitial cells expressing the CysLT1 receptor protein, we performed double staining for CysLT1 receptor and leucocyte phenotype marker. Figure 6 demonstrates the examples of CysLT1 receptor positive eosinophils, mast cells, macrophages, and neutrophils. The majority of these cells showed immunoreactivity for CysLT1 receptor. However, MT1-positive T cell (pan T cell) did not show immunoreactivity for CysLT1 receptor in allergic or non-allergic nasal mucosa (data not shown).

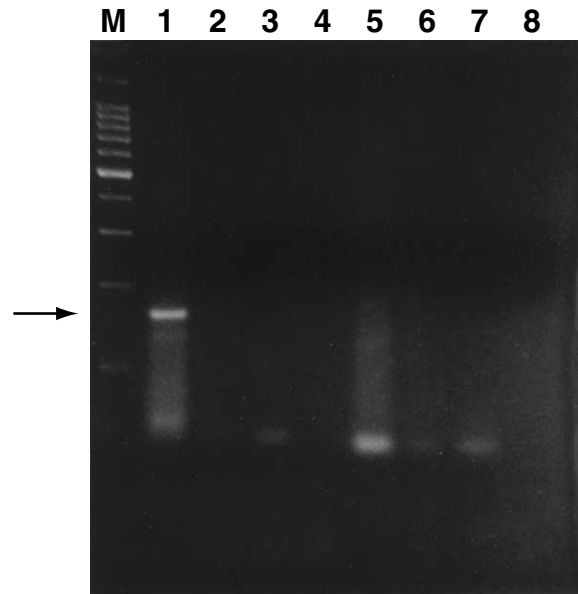


Fig. 1. Detection of Cys LT receptor mRNA by RT-PCR for 30 cycles of amplification from human nasal mucosa. Lane M: 100 bp ladder. Lane 1: PCR products of 30 cycles amplification using Cys LT1 receptor primers from total RNA of human nasal mucosa. Lane 2: negative control of lane 1 (no reverse transcriptase). Lane 3: negative control of lane 1 (without RNA sample). Lane 4: negative control of lane 1 (without Taq DNA polymerase). Lane 5: PCR products of 30 cycles amplification using Cys LT2 receptor primers from total RNA of human nasal mucosa. Lane 6: negative control of lane 5 (no reverse transcriptase). Lane 7: negative control of lane 5 (without RNA sample). Lane 8: negative control of lane 5 (without Taq DNA polymerase).

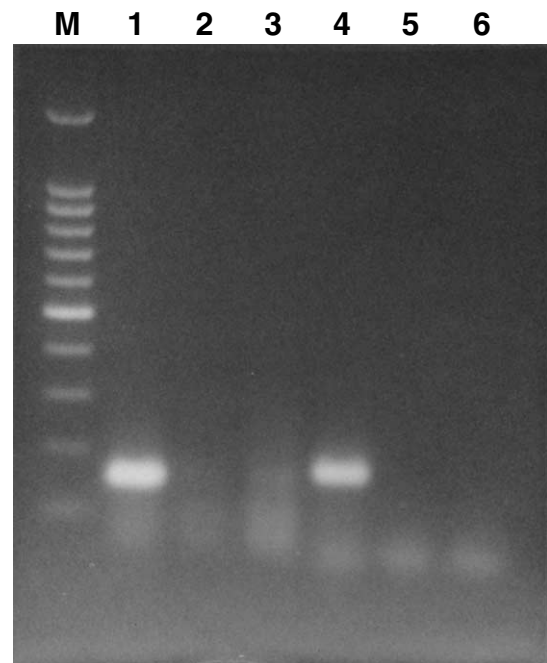


Fig. 2. Detection of Cys LT receptor mRNA by RT-PCR for 40 cycles of amplification from human nasal mucosa. RNA was extracted, RT-PCR performed using Cys LT1 receptor and Cys LT2 receptor primers, demonstrating 160 bp and 171 bp fragment, respectively. Lane M: 100 bp ladder. Lane 1: PCR products of 40 cycles amplification using Cys LT1 receptor primers from total RNA of human nasal mucosa. Lane 2: negative control of lane 1 (no reverse transcriptase). Lane 3: negative control of lane 1 (without RNA sample). Lane 4: PCR products of 40 cycles amplification using Cys LT2 receptor primers from total RNA of human nasal mucosa. Lane 5: negative control of lane 4 (no reverse transcriptase). Lane 6: negative control of lane 4 (without RNA sample).

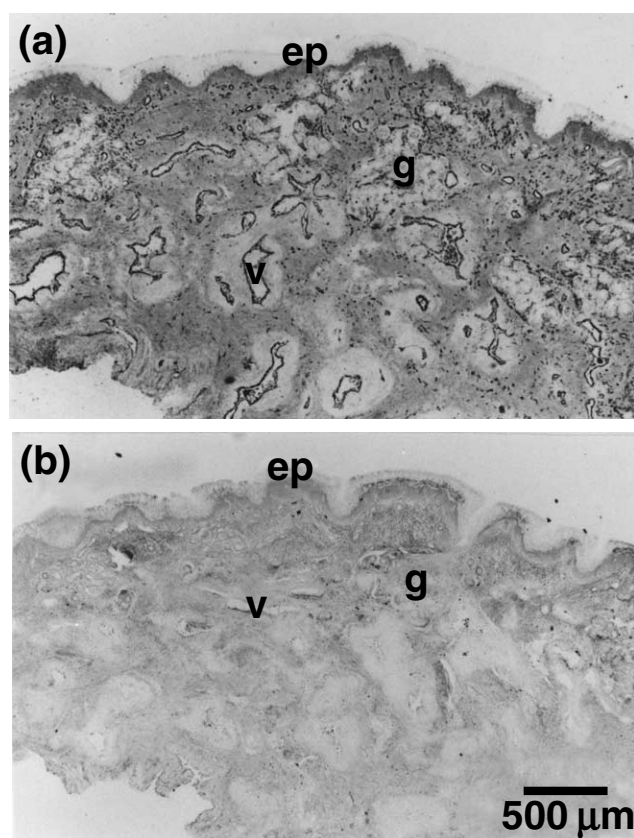


Fig. 3. *In situ* detection of Cys LT1 receptor mRNA in human nasal mucosa. Panel (a) hybridized with antisense Cys LT1 receptor biotin-labelled oligonucleotide probes. Panel (b) hybridized with sense Cys LT1 receptor biotin-labelled oligonucleotide probes. Specific hybridization is presented over blood vessels (v) but not over epithelium (ep) nor submucosal glands (g). Scale bar = 500 µm.

Discussion

In the present study, oligonucleotide primers were designed on the basis of the sequence reported for human CysLT1 receptor cDNA [8, 9] from regions of the molecule not homologous to human CysLT2 receptor [10, 11]. So, the single transcript that was amplified by these primers is likely to be the functional CysLT1 or CysLT2 receptor mRNA of human nasal mucosa. The present *in situ* hybridization study indicated CysLT1 receptor mRNA was densely expressed in the blood vessels.

LTD₄ induced rapid increase of cytosolic calcium concentrations in cultured human umbilical vein endothelial cells (HUVEC), which was inhibited by CysLT1 receptor antagonist SKF 104 354 (pobilukast), indicating the existence of CysLT1 receptor in HUVEC [13]. Recently, it was reported that IL-1 β , a primary pro-inflammatory cytokine, induces RNA expression for CysLT1 receptor in HUVEC, suggesting a potentially novel endothelial phenotype and/or role for this receptor in activated vascular tissue [14]. There are sufficient data concerning some vascular tissues to suggest that relaxations induced by CysLTs are via the stimulation of specific receptors present on the endothelium. Data derived from isolated human pulmonary arteries and veins indicate that stimulation of the endothelium by LTD₄-induced relaxations via the liberation of the nitric oxide (NO) pathway [15]. In contrast, LTD₄ and LTC₄

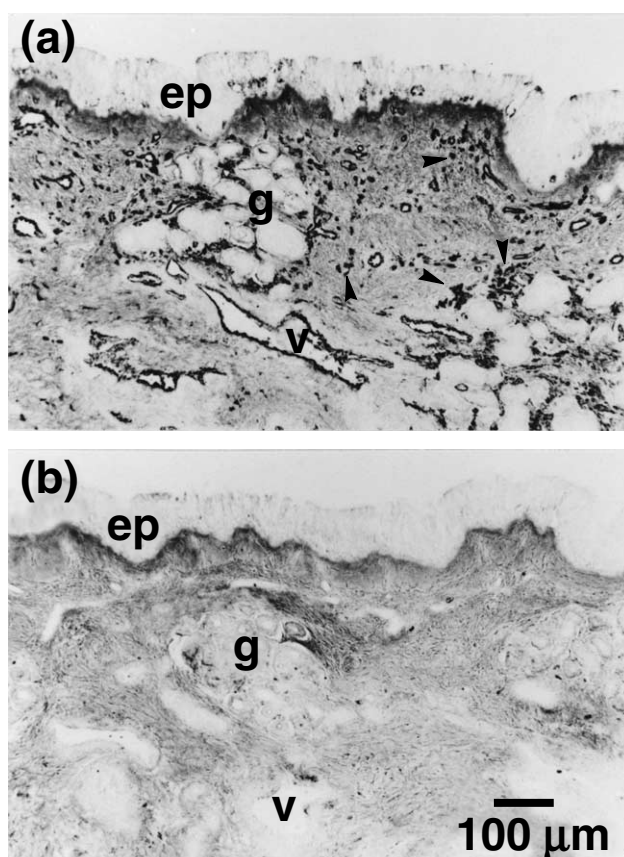


Fig. 4. Distribution of Cys LT1 receptor mRNA in human nasal mucosa. Panel (a) hybridized with antisense Cys LT1 receptor biotin-labelled oligonucleotide probes. Panel (b) hybridized with sense Cys LT1 receptor biotin-labelled oligonucleotide probes. Labelling of Cys LT1 receptor mRNA is present over blood vessels (v) and the interstitial cells (arrow) but not over epithelium (ep) nor submucosal glands (g). Scale bar = 100 µm.

relaxations of isolated human saphenous vein are dependent on both the NO and cyclooxygenase pathways [16]. While no prior studies of CysLTs effects on vessels in human upper airway have been made *in vitro*, there have been some investigations of the CysLTs effects on human nasal mucosa *in vivo*. In studies of allergic rhinitis patients, topical administration of LTD₄ has been shown to cause nasal mucosal swelling [2, 4, 17] and increased blood flow [1]. Together, these results suggest that LTD₄ effects in nasal vessels are predominantly relaxation, possibly via the NO pathway. In order to prove this hypothesis, a study using cultured nasal mucosa-derived microvascular endothelial cells is now in progress at our laboratory.

The expression of CysLT1 receptor mRNA was found not only on the blood vessels, but also on the interstitial cells, and immunohistochemical analysis showed CysLT1 receptor expressing cells were eosinophils, mast cells, macrophages and neutrophils in the present study. The initial molecular characterization of CysLT1 receptor by *in situ* hybridization made clear the presence of CysLT1 receptor mRNA in human lung smooth-muscle cells and lung macrophages [8]. In addition, the immunohistochemical characterization of the CysLT1 receptor protein in human lung and peripheral blood leucocytes revealed the expression of CysLT1 receptor not only on lung smooth-muscle cells and macrophages but also on eosinophils,

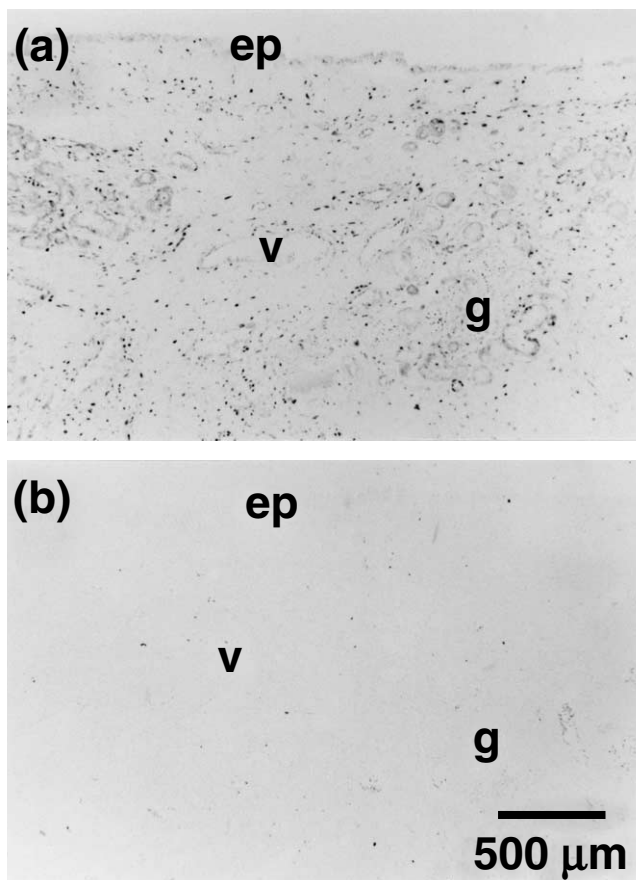


Fig. 5. Immunohistochemical staining for CysLT1 receptor in human nasal mucosa. Inferior turbinates were stained with antihuman CysLT1 receptor antibody (a) or normal rabbit immunoglobulin (b). Staining was observed mainly on interstitial cells. Blood vessels (v), epithelium (ep) and submucosal glands (g) expressed low or no levels of CysLT1 receptor immunoreactivity. Scale bar = 500 µm.

monocytes, B lymphocytes and CD34⁺ granulocytic precursor cells [18]. In Northern blot analysis of human tissues, the highest level of expression of the CysLT1 receptor was found in peripheral blood leucocytes, followed by spleen, lung, pancreas, small intestine, and a number of other tissues [8,9]. A recent RT-PCR study on peripheral blood leucocytes confirmed the higher expression of CysLT1 receptor mRNA in eosinophils and neutrophils, and the lower expression in monocytes and T lymphocytes [19]. In addition, it has been shown that human cord blood-derived mast cells expressed the CysLT1 receptor [20]. These reports may support our observations from immunohistochemistry. However, we could not confirm the CysLT1 receptor expression in local T lymphocytes by immunohistochemistry. Further study will be necessary to clarify whether local T lymphocytes express CysLT1 receptor or not. *In vivo*, CysLT1 receptor antagonists can modulate airway inflammation in that they can effectively reduce eosinophil chemotaxis [21], peripheral blood eosinophil counts [22] and monocyte/macrophage influx [23]. The expression of the CysLT1 receptor on monocytes and eosinophils, both of which are cell types capable of synthesizing CysLTs, suggests that autocrine and paracrine activation may occur after stimulation by the appropriate inflammatory signal [18]. Such a positive feedback mechanism may in part involve the exacerbations of allergic rhinitis.

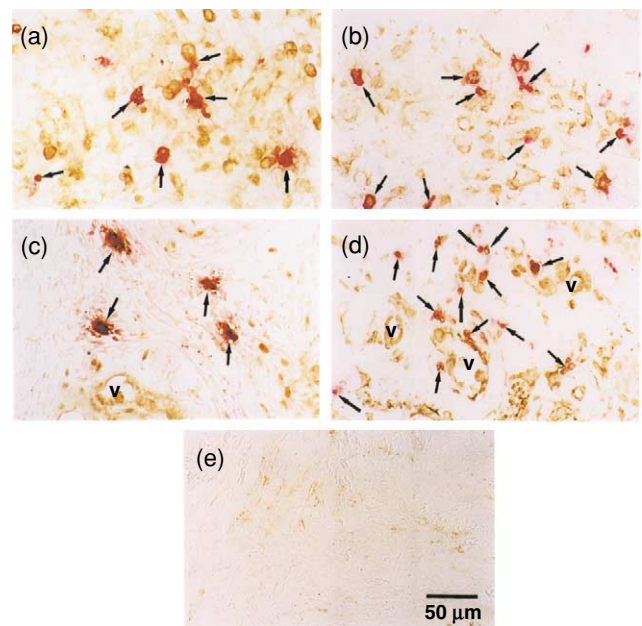


Fig. 6. Identification of subsets of cells expressing the CysLT1 receptor among interstitial cells in human nasal mucosa. Inferior turbinates were stained with antihuman CysLT1 receptor antibody (brown) and antileucocyte antibody (red). Identification markers for: eosinophil cationic protein (eosinophil) (a); mast cell tryptase (mast cell) (b); CD68 (macrophage) (c); neutrophil elastase (neutrophil) (d). The majority of these leucocytes express CysLT1 receptor (arrows). Vascular endothelial cells (v) also express CysLT1 immunoreactivity. Panel e: negative control obtained by replacing primary antibodies with either mouse IgG1 or normal rabbit immunoglobulin. Scale bar = 50 µm.

In the present study, we could not confirm the gene expression of CysLTs receptor on nasal epithelial cells by *in situ* hybridization and immunohistochemistry. The normal nasal epithelial cells consist of goblet cells, non-ciliated columnar cells with microvilli, basal cells and ciliated columnar cells. With respect to the effect of CysLT on ciliated cells of human airway, conflicting results have been reported regarding the effect of LTD₄ and LTC₄ on ciliary beat frequency (CBF) in human upper airway mucosa *in vitro*. Hisamatsu et al. demonstrated that LTD₄ decreased CBF of human paranasal sinus mucosa [24], while Saano et al. reported that LTD₄ increased CBF of human nasal mucosa [25]. Similarly, it has been reported that LTC₄ decreased the CBF in human nasal mucosa [26], whereas studies on human adenoid tissue found LTC₄ had no effect on CBF [27]. From these observations, further studies will be necessary to determine whether CysLT has an influence on CBF in human nasal mucosa or not. On the other hand, as to the effect of CysLTs on goblet cells that could release several types of mucin, no significant modification of basal release of MUC5AC was observed in bronchial preparations with an intact epithelium challenged with LTD₄ [28].

The present study indicated no evidence of CysLT1 receptor expression on the nasal submucosal glands by *in situ* hybridization. Human nasal submucosal glands consist of mucous and serous cells. Mucous cells and epithelial goblet cells contain acidic mucous glycoprotein, and serous cells contain lactoferrin. In lower airways, LTD₄ induced a dose-related increase in the release of glycoprotein, but not of lysozyme, from human bronchial mucosa *in vitro* [29]. On the other hand, there seem to be no reports describing CysLTs as the mucous secretagogues

of upper airway *in vitro*. *In vivo*, nasal challenge with LTD₄ failed to cause nasal secretion [2]. From the present results and these observations, it might be suggested that CysLTs could hardly increase nasal secretion in human. However, it has been clinically shown that CysLT1 receptor antagonists relieved the symptoms, including both sneezing and rhinorrhea, of allergic rhinitis [5,6]. It remains a possibility that CysLT1 receptor on macrophages, eosinophils and mast cells can modulate nasal allergic inflammation and clinical symptoms (especially sneezing and rhinorrhea).

In conclusion, using RT-PCR, *in situ* hybridization and immunohistochemical technique, we have demonstrated the expression and the distribution of CysLT1 receptor in nasal mucosa at gene and protein levels. The role of CysLT1 receptor on allergic rhinitis has not been well clarified; however, the precise knowledge of the identity and distribution of CysLT1 receptor should be of considerable interest for further investigation of gene regulation.

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References

- Bisgaard H, Olsson P, Bende M. Leukotriene D₄ increase nasal blood flow in humans. *Prostaglandins* 1984; 27:599–605.
- Naclerio RM, Baroody FM, Togias AG. The role of leukotrienes in allergic rhinitis: a review. *Am J Respir Dis* 1991; 143:S91–5.
- Shaw RJ, Fitzharris P, Cromwell O, Wardlaw AJ, Kay AB. Allergen-induced release of sulphidopeptide leukotrienes (SRS-A) and LTB₄ in allergic rhinitis. *Allergy* 1985; 40:1–6.
- Kojima T, Asakura K. The study of chemical mediators in the patients with allergic rhinitis. 2: Histamine, leukotrienes and kinins in the nasal secretion during dual phase response. *J Otolaryngol Jpn* 1991; 94:366–76.
- Donnelly AL, Glass M, Minkwitz MC, Casale TB. The leukotriene D₄-receptor antagonist, ICI 204, 219, relieves symptoms of acute seasonal allergic rhinitis. *Am J Respir Crit Care Med* 1995; 151: 1734–9.
- Numata T, Konno A, Yamakoshi T et al. Comparative role of peptide leukotrienes and histamine in the development of nasal mucosal swelling in nasal allergy. *Ann Otol Rhinol Laryngol* 1999; 108:467–73.
- Meltzer EO, Malmstrom K, Lu S et al. Concomitant montelukast and loratadine as treatment for seasonal allergic rhinitis: a randomized, placebo-controlled clinical trial. *J Allergy Clin Immunol* 2000; 105:917–22.
- Lynch KR, O'Neill GP, Liu Q et al. Characterization of the human cysteinyl leukotriene CysLT1 receptor. *Nature* 1999; 399:789–93.
- Sarau HM, Ames RS, Chambers J et al. Identification, molecular cloning, expression, and characterization of a cysteinyl leukotriene receptor. *Mol Pharmacol* 1999; 56:657–63.
- Heise CE, O'Dowd BF, Figueroa DJ et al. Characterization of the human cysteinyl leukotriene 2 receptor. *J Biol Chem* 2000; 275:30531–6.
- Takahashi J, Kamohara M, Matsumoto M et al. The molecular characterization and tissue distribution of the human cysteinyl leukotriene CysLT2 receptor. *Biochem Biophys Res Commun* 2000; 274:316–22.
- Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; 162:156–9.
- Heimbürger M, Palmblad JE. Effects of leukotriene C₄ and D₄, histamine and bradykinin on cytosolic calcium concentrations and adhesiveness of endothelial cells and neutrophils. *Clin Exp Immunol* 1996; 103:454–60.
- Gronert K, Martinsson-Niskanen T, Ravasi S, Chiang N, Serhan CN. Selectivity of recombinant human leukotriene D₄, leukotriene B₄, and lipoxin A₄ receptors with aspirin-triggered 15-epi-LXA₄ and regulation of vascular and inflammatory responses. *Am J Pathol* 2001; 158:3–9.
- Ortiz JL, Labat C, Norel X et al. Response to anti-human IgE in human pulmonary arteries: regulation by the endothelium. *Am Rev Respir Dis* 1993; 147:1029–33.
- Allen SP, Chester AH, Piper PJ et al. Effect of leukotriene C₄ and D₄ on human isolated saphenous veins. *Br J Pharmacol* 1992; 34:409–14.
- Konno A, Numata T, Terada N et al. Role of substance P in the vascular response of nasal mucosa in nasal allergy. *Ann Otol Rhinol Laryngol* 1996; 105:648–53.
- Figueroa DJ, Breyer RM, Defoe SK et al. Expression of the cysteinyl leukotriene 1 receptor in normal human lung and peripheral blood leukocytes. *Am J Respir Crit Care Med* 2001; 163:226–33.
- Mita H, Hasegawa M, Saito H, Akiyama K. Levels of cysteinyl leukotriene receptor mRNA in human peripheral leucocytes: significantly higher expression of cysteinyl leukotriene receptor 2 mRNA in eosinophils. *Clin Exp Allergy* 2001; 31:1714–23.
- Mellor EA, Maekawa A, Austen KF, Boyce JA. Cysteinyl leukotriene receptor 1 is also a pyrimidinergic receptor and is expressed by mast cells. *Proc Natl Acad Sci USA* 2001; 98:7964–9.
- Munoz NM, Douglas I, Mayer D et al. Eosinophil chemotaxis inhibited by 5-lipoxygenase blockade and leukotriene receptor antagonism. *Am J Respir Crit Care Med* 1997; 155:1398–403.
- Reiss TF, Chervinski P, Dockhorn RJ et al. Montelukast, a once-daily leukotriene receptor antagonist, in the treatment of chronic asthma. *Arch Intern Med* 1998; 158:1213–20.
- Calhoun WJ, Lavins BJ, Minkwitz MC et al. Effect of zafirlukast (Accolate) on cellular mediators of inflammation. *Am J Respir Crit Care Med* 1998; 157:1381–9.
- Hisamatsu K, Nakajima M. Pranlukast protects leukotriene C₄-and D₄-induced epithelial cell impairment of the nasal mucosa *in vitro*. *Life Sci* 2000; 67:2767–73.
- Saano JS, Koskela T, Toskala E, Bray MA, Nuutinen J. Effect of leukotriene D₄ on ciliary activity in human, guinea-pig and rat respiratory mucosa. *Pulm Pharmacol* 1996; 9:231–8.
- Bisgaard H, Pedersen M. SRS-A leukotriene decrease the activity of human respiratory cilia. *Clin Allergy* 1987; 17:95–103.
- Schuil P, Gelder J, Berg M, Graamans K, Heizing E. Histamine and leukotriene C₄ effects on *in vitro* ciliary beat frequency of human upper respiratory cilia. *Eur Arch Otorhinolaryngol* 1994; 251:325–8.
- Labat C, Bara J, Gascard JP et al. M1/MUC5AC mucin released by human airways *in vitro*. *Eur Respir J* 1999; 14:390–5.
- Coles SJ, Neill KH, Reid LM et al. Effects of leukotrienes C₄ and D₄ on glycoprotein and lysozyme secretion by human bronchial mucosa. *Prostaglandins* 1983; 25:155–70.