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CysLT₁-R expression following allergen provocation in asthma and allergic rhinitis

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ABSTRACT

Cysteinyl leukotrienes (CysLTs) contribute to allergic and inflammatory diseases through CysLT₁-R. We aimed to assess CysLT₁-R mRNA expression in induced sputum of rhinitics with or without asthma before and following allergen challenges. Both groups underwent nasal and “low dose” lung allergen challenges. Asthmatics also underwent “standard” lung challenge. Sputum was obtained before and at different time-points following the challenges for CysLT₁-R, 5-lipoxygenase (5-LO), and eotaxin mRNA assessments. At baseline, there was no difference in mediator levels between groups. An increase in CysLT₁-R mRNA ($p=0.04$) and a trend towards an increase in 5-LO and eotaxin ($p=0.06$ for both) at 24 h post-nasal challenge were observed. Following “low dose” lung allergen challenge, there was a trend towards an increase in CysLT₁-R ($p=0.07$). In conclusion, CysLT₁-R gene expression changes can be detected in sputum following allergen challenges. No difference was observed between groups, suggesting that changes in CysLT₁-R expression occur whether or not the subject has concurrent asthma.

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1. Introduction

Atopy is considered to be a predisposing factor in the development of asthma [1,2]. Airway inflammation and remodeling may be observed in non-asthmatic subjects with allergic rhinitis, although in a less intense form than in asthmatic patients [3–5]. Airway inflammation has been associated with the increase in airway responsiveness observed during natural or laboratory exposure to allergens in atopic subjects without asthma [6–8]. Thus, it seems that asthma and allergic rhinitis result from a similar inflammatory process induced by allergens in the upper as well as in the lower airways of sensitised subjects. The nose and lung should therefore be seen as a continuum rather than as 2 distinct compartments, as proposed by the “united airways” concept. In keeping with this concept, it has been shown that challenging the nose with relevant allergens may induce bronchial inflammation [9].

Cysteinyl leukotrienes (CysLTs) play an important role in asthma. They are known as potent bronchoconstrictors [10] and are implicated in plasma exudation, mucus secretion, and eosinophil recruitment, all of which are well characterised in asthma.

The CysLTs exert their bronchoconstrictive and pro-inflammatory effects through activation of the CysLT₁-receptor (CysLT₁-R).

This receptor is blocked by antagonists of CysLT₁-R (e.g. montelukast). CysLT₁-R antagonists seem to play a role in the relief of allergic rhinitis symptoms [11]. CysLT₁-R antagonists have been shown to reduce allergic rhinitis and asthma symptoms [12–14]. Following allergen challenge, compared with placebo, CysLT₁-R antagonists attenuate the allergen-induced early and late responses [15,16], and decrease the eosinophil count and eosinophil progenitors at 24 h post-challenge [17–19]. Moreover, montelukast has significant inhibitory effects on airway structural cells influx into the airways following allergen challenge [20].

Induced sputum (IS) analysis is a standardized and reproducible method to non-invasively assess lower airway inflammation [21–23]. Inflammatory cell changes may be documented and supernatants may be used to measure various inflammatory mediators. It can be repeated frequently, making it possible to study the dynamics of airway inflammatory responses. CysLT₁-R expression increases in sputum cells in subjects with occupational asthma following exposure to isocyanates [24]. To our knowledge, the expression of CysLT₁-R has never been evaluated in sputum following allergen challenge.

As allergic rhinitis appears to be a predisposing factor in the development of asthma and as CysLT-receptors seem to be implicated in the first steps of asthma manifestations, it is of interest to look at the possible role of the CysLT₁-R in the transition from allergic rhinitis to asthma.

This exploratory study therefore aimed at assessing the expression of the CysLT₁-R (mRNA) in induced sputum of mild asthmatic subjects compared with non-asthmatic subjects with

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allergic rhinitis, at baseline and following different allergen provocations. We hypothesized that the expression of the CysLT₁-R in sputum would differ between both groups, at baseline and following the allergen challenges, asthmatic patients possibly demonstrating an increased expression compared to non-asthmatic rhinitic patients.

2. Patients and methods

2.1. Subjects

Mild allergic asthmatic subjects and non-asthmatic subjects with allergic rhinitis were recruited. All had a positive reaction to one or more allergen on prick tests. They were all non-smokers and did not have a respiratory track infection for at least one month prior to the study. Asthmatic subjects had a history of asthma for at least 6 months as defined by the ATS criteria [25]. They were using only inhaled β_2 agonists on an as needed basis for their asthma treatment. They had a PC₂₀ (the provocative concentration of methacholine inducing a 20% fall in forced expiratory volume in one second (FEV₁)) ≤ 8 mg/ml (tidal breathing method). Allergic rhinitic subjects had normal airway responsiveness (as shown by a PC₂₀ methacholine > 16 mg/ml) and they had never experienced any asthma symptoms or took any asthma medication in the past. All subjects signed an informed consent form and the study was approved by the institutional ethics committee.

2.2. Study design

The study design is presented in Fig. 1. On a baseline visit, subjects had allergy skin prick tests, spirometry and methacholine broncho-provocation. Induced sputum with differential leukocyte count was also obtained.

On subsequent visits, both groups underwent a nasal allergen challenge and “low dose” whole-lung allergen challenge (LDAC). Allergic asthmatic subjects also underwent an additional “standard” whole-lung bronchial allergen challenge. Challenges were done randomly, at three-week intervals. IS was obtained before each allergen challenge as well as at 24 h following nasal challenge, at 7 and 24 h following “standard” bronchial

challenges, and finally on days 2 and 4 of LDAC, 6 h following allergen inhalation.

Induced sputum was obtained for differential cell count. Total mRNA was extracted and used for PCR analyses.

The study was registered at www.ClinicalTrials.gov under NCT00631254.

2.3. Skin prick tests and titration

Atopy was determined from allergy tests with 26 common aeroallergens (trees, grasses, cat, house dust mite (*Dermatophagoides pteronyssinus*, *D. farinae*)). Normal saline and histamine were used as negative and positive controls, respectively. Skin wheal diameter was recorded for each allergen at 10 min as the mean of 2 perpendicular measurements. A positive response was defined as a skin wheal diameter of 3 mm or more.

To determine the allergen dose to be given on bronchial allergen challenge, skin titration was done with the allergen to which the subject reacted the most on prick tests. Serial 2-fold dilutions of the allergen were applied on the forearm and skin wheal diameter was measured at 15 min. The smallest concentration giving a 2 mm diameter in wheal response was defined as the end-point titration and was used to determine the allergen concentration to be inhaled.

2.4. Spirometry and methacholine inhalation test

Baseline FEV₁ and forced vital capacity (FVC) were measured according to the ATS criteria [26]. The predicted values were obtained from Knudson [27] and FEV₁ was defined as the best of 3 reproducible values.

Methacholine bronchial challenge was done as described by Juniper et al. [28]. Briefly, following a 2-min inhalation of 0.9% saline, increasing concentrations of methacholine were inhaled for 2 min via a Wright nebulizer (Roxon Meditech, Montreal, QC, Canada) delivering 0.13 ml/min. FEV₁ was measured at 30 and 90 s following inhalation or until FEV₁ had increased. The test was stopped when a $\geq 20\%$ fall in FEV₁ from the lowest post-saline value was obtained or when the last methacholine dose had been given. The response was expressed as the PC₂₀ methacholine obtained from the log dose-response curve.

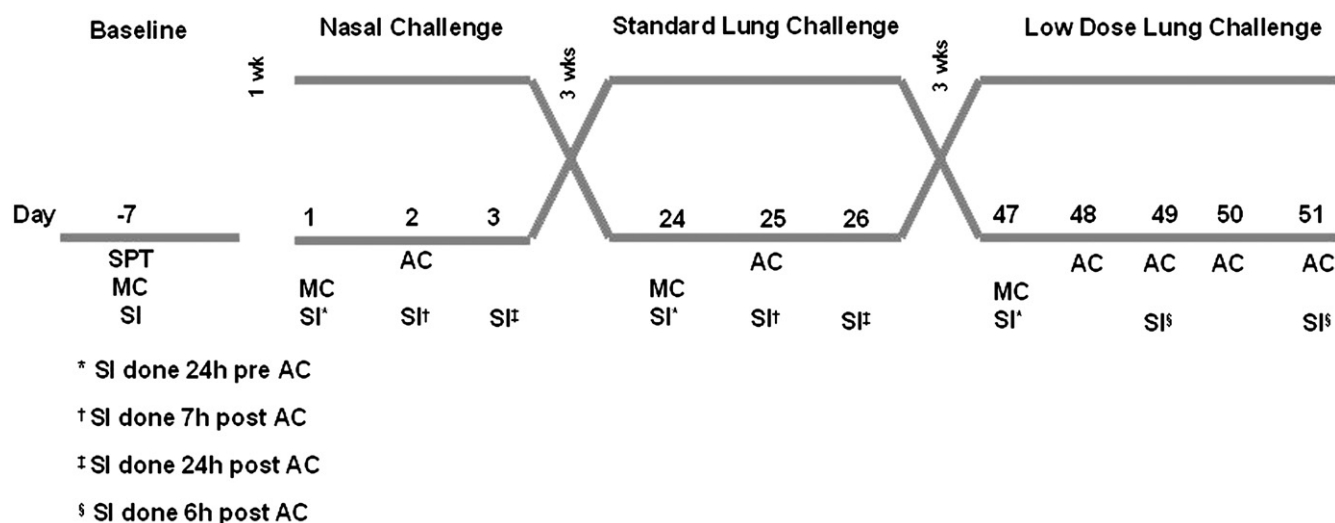


Fig. 1. Study design. Asthmatic subjects underwent nasal, whole lung, and low dose lung challenges in a randomized crossover fashion. Allergic rhinitic subjects underwent nasal and low dose lung challenges in a randomized crossover fashion. AC=allergen challenge, MC=methacholine inhalation challenge, SI=sputum induction.

2.5. Nasal allergen challenge

Nasal challenge was done by depositing 50 μ l drops of increasing doubling doses of allergen on the middle turbinate mucosa of one nostril. The starting dose of allergen was 4 doubling doses from the stock allergen. Itching, sneezing, nasal obstruction and rhinorrhea were recorded 10 min after the end of the allergen contact on a 0–3 scale as follows: 0=absent, 1=mild, 2=moderate, 3=severe. Challenge was stopped when at least 2 moderate or 1 severe symptom occurred, and the last inhaled concentration was considered to be the threshold dose. The maximum nasal symptom score within the first hour following allergen challenge was considered as the early response. Delayed nasal symptoms were also scored as above every hour following the challenge. The late response was defined as a symptom score of 2 or more for at least 2 h between 3 and 8 h following the allergen inhalation.

2.6. Bronchial allergen challenges

Aerosolized allergen was inhaled via a Wright nebulizer (Roxon Meditech) calibrated to deliver 0.13 ml/min. Inhalation lasted 2 min and FEV₁ was recorded 10 min later. The first allergen inhalation dose was determined using the Cockcroft equation which takes into account the end-point titration and the PC₂₀ [29].

“Low dose” lung allergen challenge was done as described previously [30]. Briefly, subjects came to the clinic on 4 consecutive mornings to undergo their allergen provocation. Three allergen doses were given for inhalation, corresponding to 9, 8 and 7 doubling doses below the calculated PD₂₀ (the provocative dose of allergen giving a 20% fall in FEV₁). Inhalation lasted for 2 min and FEV₁ was measured 10 min following inhalation. If FEV₁ had not fallen more than 5% from the highest baseline FEV₁, the next concentration was given, otherwise, the test was stopped. FEV₁ was measured at 10-min intervals for 30 min following inhalation of the last allergen concentration.

“Standard” lung allergen challenge was done as described by Cockcroft et al. [29]. The starting inhalation dose was 4 doubling doses below the calculated PD₂₀. Increasing doses were administered until FEV₁ had fallen by 20% or more from the highest baseline FEV₁. Following allergen inhalation, FEV₁ was recorded at 10, 20, 30, 45, 60, 90 and 120 min, and then hourly until the late asthmatic response (LAR) occurred or for a maximum of 7 h. The early asthmatic response (EAR) was defined as the $\geq 20\%$ fall from the highest post-baseline FEV₁ during the first hour following allergen inhalation. The LAR was defined similarly as a $\geq 15\%$ fall in FEV₁ between 3 and 7 h after the challenge.

2.7. Induced sputum

Sputum was induced and processed using the method described by Pin et al. [31] and modified by Pizzichini et al. [23] by inhalation of hypertonic saline. Sputum was processed within 2 h following induction. Briefly, mucus plugs were selected from saliva and treated with 4 times their volume of dithiothreitol (DTT). An equal volume of Dulbecco's phosphate buffered saline (D-PBS) 1 $\times$ was then added and the suspension was filtered. Total cell count and viability were determined using the trypan blue exclusion method. Slides were prepared and stained with Diff-Quik for differential cell count. The remaining cells were placed in RLT buffer (Qiagen, Toronto, ON, Canada) until RNA extraction.

2.7.1. RNA isolation and real-time PCR

Sputum total cellular RNA was extracted using the Qiagen RNeasy Total RNA kit (Qiagen, Valencia, CA, USA) and quantified by fluorescence through use of Ribogreen (Molecular Probes, Inc., Eugene, OR, USA) according to the manufacturers' instructions. 100 ng of total RNA per sample was synthesized into total cDNA using RT-MMLV and first strand buffer (Invitrogen). Amplification was carried out with the DNA Engine Opticon (MJ Research, Waltham, MA, USA). Total RNA was extracted from each sputum and samples were run on formamide/formaldehyde denaturing agarose gel. Quality of RNA was determined by 28S/18S ribosomal RNA ratios.

CysLT₁-R was amplified with the following primers: 5'-AAAACCTATACAAGAAGTCAGCCT-3' as sense and 5'-CAAGAAGTCACCAAGAGCCAAATG-3' as antisense, 5-lipoxygenase (5-LO) was amplified with the following primers: 5'-TACATCGAGTCCCTGCTAC-3' as sense and 5'-GTTCTTACGTCGGTGTGCT-3', eotaxin was amplified with the following primers: 5'-CCCAACCACCTGCTGCTTAACCTG-3' as sense and 5'-TGGCTTTGGAGTTGGAGATTTTGG-3', and GAPDH was amplified with the following primers: 5'-ATGCAACGATTGGTTCGTAT-3' as sense and 5'-TCTCGCTCTGGAAGATGGTG-3' as antisense. Primers were designed to amplify 150–200 bp fragments in the coding region of each genes and were compared to nucleotide databases to ensure primer specificity.

PCR reactions were carried in a final volume of 25 μ l containing 1 μ l cDNA template, 200 nM of each primer and 12.5 μ l of 2X B-R-SYBR Green Supermix Reagent (Quanta Bioscience, Gaithersburg, MD). PCR amplification included a denaturation step at 95 °C for 5 min followed by 40 cycles of 1-min denaturation at 95 °C, 30 s at 52 °C, and 1-min elongation at 72 °C. Data were acquired and analysed with the computer software Opticon Monitor (version 2.02.24). Each PCR reactions were migrated on a 1% agarose gel to verify non-specific binding. Normalized expressions were calculated using the calculated efficiency of each PCR reaction with a cDNA standard curve. No PCR products were obtained when DNA template was omitted, indicating that there was no DNA contamination. Real-time PCR were followed by melting curve analysis to ensure specificity.

2.8. Statistical analyses

Results are expressed as means and standard errors of the mean (SEM) for continuous variables. Categorical variables are expressed using the count of the observed event. The Fisher's exact test was performed to compare both groups. For continuous variables, subject's characteristics were compared between groups. The approach to analyse data from the sputum was done using an ANOVA design and values at baseline were defined as covariates. When the assumption of variances was not encountered, Satterthwaite's approximation was used for the degrees of freedom and probability level obtained with the Cochran and Cox approximation. This approach was performed when the normality assumptions were met. When normality and equality of variance assumptions were not encountered, Wilcoxon's rank sum tests were preferred to perform comparisons between groups. The results were considered significant if *p*-values were < 0.05. The data were analysed using the statistical package program SAS (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Characteristics of subjects

Twenty-two rhinitic subjects and twenty-two asthmatic subjects were included in the study. Baseline subject characteristics are presented in Table 1.

3.2. Baseline measurements

Fourteen rhinitic subjects and 10 asthmatic subjects produced sufficient sputum material for mRNA assessment. Levels of baseline CysLT₁-R, 5-LO and eotaxin mRNA were not significantly different between groups.

3.3. Nasal challenge

Twenty-one allergic rhinitic subjects and twenty-two asthmatic subjects underwent nasal challenge. For mRNA assessment, samples from 7 rhinitic subjects (6 for eotaxin) and from 8 asthmatic subjects (7 for 5-LO and eotaxin) were available at all time-points. When all subjects were analysed together, there was a significant increase in CysLT₁-R levels at 24 h compared with pre-challenge ($p=0.04$) and a trend towards a significant increase in 5-LO and eotaxin levels at 24 h compared with pre-challenge ($p=0.06$ for both mediators, Fig. 2). Six samples from the rhinitic subjects and 4 samples from the asthmatic subjects showed an increase in levels of CysLT₁-R at 24 h post-challenge compared

with pre-challenge. In rhinitic subjects, the mean CysLT₁-R/GAPDH ratio was 0.99 pre-challenge and 1.88 at 24 h post-challenge, whereas in asthmatic subjects, the mean CysLT₁-R/GAPDH ratio was 0.99 pre-challenge and 1.75 at 24 h post-challenge. Data from sputum differential counts in each group are presented in Table 2. When the 2 groups were analysed together, there was a trend towards a statistically significant increase in sputum eosinophil percentages ($p=0.08$) and in eosinophils per ml of selected sputum ($p=0.06$) at 24 h post-challenge compared with pre-challenge (data not shown).

3.4. Bronchial challenges

3.4.1. "Low dose" lung allergen challenge

For this analysis, sputum material was obtained from 18 rhinitic subjects and 10 asthmatic subjects. For 7 rhinitic subjects and for 5 asthmatic subjects, mRNA could be assessed at all time-points. When all subjects were analysed together, there was a trend towards a significant variation in CysLT₁-R at day 4 of challenge compared with pre-challenge ($p=0.07$; Fig. 3). In rhinitic subjects the CysLT₁-R/GAPDH ratio was 1.00 pre-challenge compared to 2.70 at day 4, and in asthmatic subjects, CysLT₁-R/GAPDH ratio was 1.01 pre-challenge compared to 2.28 at day 4. No significant changes in 5-LO or eotaxin levels were observed in any group at any time-point. Sputum eosinophils increased significantly at days 2 and 4 of the challenge compared to pre-challenge in rhinitic subjects ($p<0.0001$; Table 2), although there was no fall in FEV₁. No statistically significant variation in sputum eosinophils was observed in asthmatic subjects.

3.4.2. "Standard" lung allergen challenge

The "standard" lung allergen challenge was performed in 13 asthmatic subjects. The mean fall in FEV₁ at EAR was 22.7%. Only 2 subjects experienced a LAR. Since this was not an exclusion criteria, the 11 remaining subjects were still included in the study. Assessment of mRNA was available at all time-points for 7 of the 13 subjects. Allergen did not induce a significant variation in

Table 1
Baseline characteristics of subjects.

	Rhinitic subjects	Asthmatic subjects
Gender (M/F)	10/12	5/17
Age (years)^a	24 (22–29)	25 (24–28)
FEV₁ (L)^a	4.11 (3.52–4.50)	3.34 (2.92–3.72)
FEV₁ (% Pred.)^a	104 (96–112)	100 (91–109)
PC₂₀ (mg/ml)^b	76.4 (17.6–128)	2.0 (0.17–12.14)
Allergens used (n)		
Grasses	5	2
<i>Dermatophagoides pteronyssinus</i>	3	6
Ragweed	1	0
Birch	4	2
<i>Dermatophagoides farinae</i>	2	1
Cat pelt	2	1
Cat hair	4	10

^a Median (25–75 percentile).

^b Geometric mean (range).

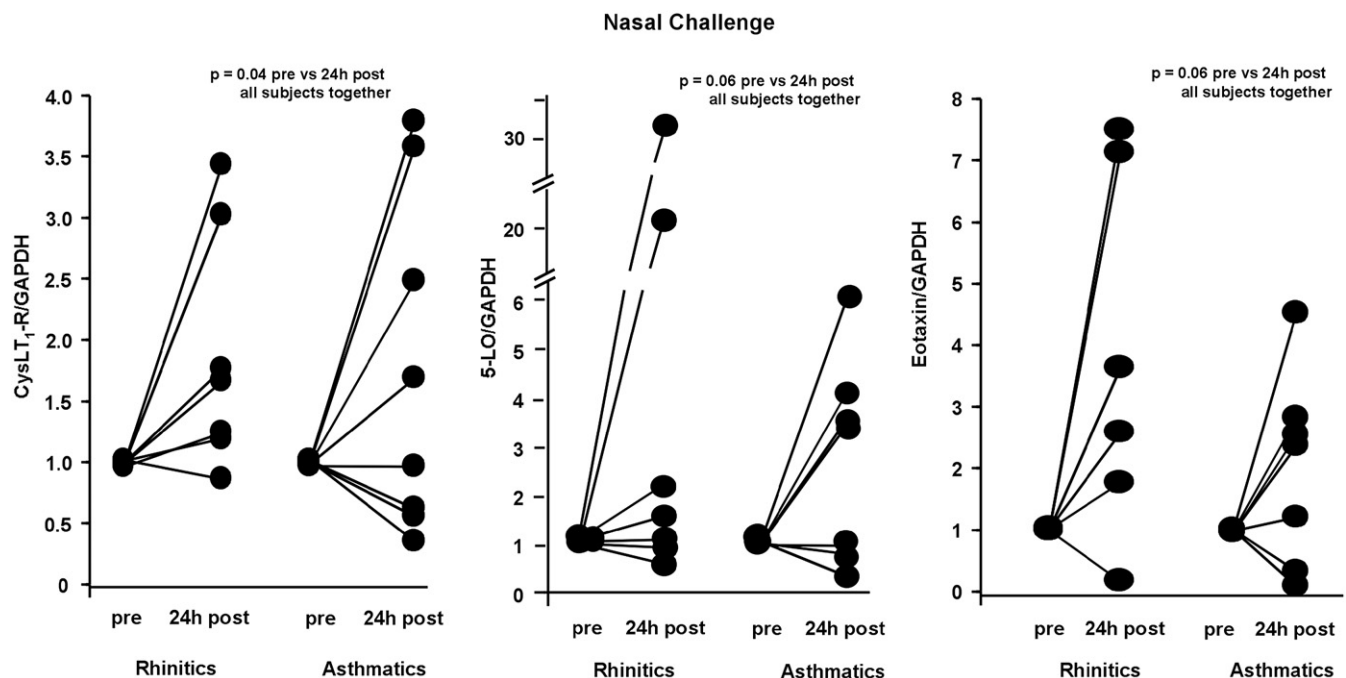


Fig. 2. Changes in CysLT₁-R, 5-LO and eotaxin mRNA from pre-allergen to 24 h post-nasal allergen challenge in rhinitic and asthmatic subjects.

Table 2
Results for sputum differential cell counts.

	N	TCC (× 10 ⁶)	Cells/ml selected mucus	Lymphocytes (%)	Macrophages (%)	Neutrophils (%)	Eosin ophils (%)	Bronchial cells (%)
Baseline	R: 22 A: 22	R: 1.2 (0.3) A: 1.6 (0.5)	R: 4.1 (0.7) A: 4.2 (0.7)	R: 2.1 (0.3) A: 2.1 (0.4)	R: 69.7 (4.5) A: 61.3 (4.4)	R: 25.6 (4.4) A: 31.5 (4.7)	R: 0.9 (0.4) A: 3.3* (1.8)	R: 1.7 (0.4) A: 1.7 (0.4)
High dose	A: 13							
Pre		A: 1.3 (0.4)	A: 3.8 (0.8)	A: 2.1 (0.3)	A: 60.6 (4.1)	A: 30.1 (4.0)	A: 5.2 (3.1)	A: 2.0 (0.6)
7 h post		A: 1.3 (0.3)	A: 5.4 (1.1)	A: 1.9 (0.6)	A: 38.1 (3.3)**	A: 45.6 (3.5)**	A: 13.0 (4.7)**	A: 1.4 (0.3)
24 h post		A: 2.6 (0.6)	A: 9.0 (1.7)**	A: 2.1 (0.6)	A: 42.3 (3.3)**	A: 45.2 (4.4)**	A: 9.5 (2.5)**	A: 1.0 (0.3)
Low dose	R: 18 A: 10							
Pre		R: 1.5 (0.3) A: 1.3 (0.3)	R: 5.3 (1.0) A: 4.3 (0.6)	R: 2.0 (0.3) A: 5.1 (3.2)	R: 59.5 (5.0) A: 61.5 (6.3)	R: 36.1 (5.0) A: 32.6 (6.4)	R: 0.8 (0.4) A: 2.7 (1.1)	R: 1.6 (0.4) A: 1.3 (0.3)
Day 2		R: 1.1 (0.2) A: 2.5 (0.8)	R: 4.7 (0.6) A: 4.3 (0.8)	R: 3.7 (0.5) A: 2.6 (0.5)	R: 57.4 (4.2) A: 56.2 (6.1)	R: 29.9 (4.7) A: 33.5 (6.7)	R: 7.3 (2.0)† A: 5 (2.0)	R: 1.7 (0.3) A: 2.7 (0.6)
Day 4		R: 1.0 (0.2) A: 1.1 (0.3)	R: 4.2 (0.6) A: 3.7 (0.8)	R: 1.7 (0.3) A: 2.5 (0.5)	R: 59.7 (5.4) A: 63.8 (4.8)	R: 25.1 (5.9) A: 28.5 (5.0)	R: 12.3 (4.0)† A: 3.5 (1.0)	R: 1.1 (0.3) A: 1.8 (0.4)
7-day post		R: 1.0 (0.1) A: 1.5 (0.3)	R: 4.7 (0.6) A: 6.2 (1.5)	R: 2.1 (0.3) A: 2.1 (0.5)	R: 65.8 (4.0) A: 56.7 (4.2)	R: 28.1 (4.3) A: 36.4 (4.5)	R: 2.3 (0.7) A: 3.0 (1.0)	R: 1.7 (0.3) A: 2.4 (0.6)
Nasal	R: 21 A: 22							
Pre		R: 1.1 (0.3) A: 1.2 (0.5)	R: 3.8 (0.7) A: 3.8 (0.7)	R: 1.8 (0.3) A: 1.8 (0.3)	R: 65.1 (4.4) A: 67.1 (4.4)	R: 30.2 (4.3) A: 25.7 (4.5)	R: 1.5 (0.4) A: 4.0 (0.9)	R: 1.4 (0.5) A: 1.5 (0.3)
24 h post		R: 1.5 (0.4) A: 2.0 (0.7)	R: 5.0 (0.9) A: 6.3 (1.6)	R: 1.7 (0.2) A: 2.0 (0.2)	R: 55.1 (5.1) A: 49.0 (4.1)	R: 37.2 (5.2) A: 42.5 (4.3)	R: 5.2 (2.5) A: 4.6 (1.4)	R: 2.6 (1.3) A: 1.4 (0.4)

Results are presented as means ± SEM.

TCC: total cell count; R: rhinitic non-asthmatic subjects; A: rhinitic asthmatic subjects.

* $p < 0.0009$ vs R.

** $p < 0.0001$ vs pre-challenge.

† $p < 0.0001$ vs pre-challenge.

CysLT₁-R, 5-LO or eotaxin gene expression in sputum cells (Fig. 4). Samples used for these assessments only came from subjects without LAR. Even though LAR was not observed in the majority of subjects, there was a significant increase in sputum eosinophil percentages at 6 and 24 h post-challenge (Table 2).

3.5. Correlation between CysLT₁-R and sputum cells

At baseline, there were no correlations between the expression of CysLT₁-R and any of the sputum cells in either group. Throughout the different challenges, there were no correlations between the variation in CysLT₁-R and the change in sputum eosinophils (either percentages or number of eosinophils/ml of selected sputum) in both groups. No correlations were observed between CysLT₁-R expression and any of the other sputum cell types in both groups throughout any of the challenges.

4. Discussion

In this study, we showed that CysLT₁-R mRNA can be measured in induced sputum obtained from allergic rhinitic subjects and from mild allergic asthmatic subjects. We also demonstrated that baseline levels of CysLT₁-R in induced sputum are not different between allergic rhinitic subjects and mild asthmatic subjects.

To our knowledge, this is the first study looking at the variations in CysLT₁-R expression following allergen challenges. Following nasal allergen challenge, we observed a significant increase in CysLT₁-R mRNA at 24 h post-challenge compared with pre-challenge. However, this increase was not significant when groups were analysed separately. There are conflicting data regarding the effects of single dose nasal challenges on lower airway inflammation. While Braunstahl et al. [9] observed increased eosinophils in nasal and bronchial biopsies, others

failed to observe such an increase in sputum [32,33]. Single dose nasal provocation may not mimic the full spectrum of allergic rhinitis manifestations. It would therefore be of interest to use multiple nasal challenges, which may more adequately induce changes in sputum cells and inflammatory markers in addition to being closer to natural exposure. Nevertheless, we observed a significant increase in CysLT₁-R mRNA levels and a trend towards a significant increase in sputum eosinophils 24 h following the challenge.

Following “low dose” lung allergen challenge there was a trend towards an increase in CysLT₁-R when both groups were analysed together. Even though allergen provocation induced a significant increase in sputum eosinophils in these subjects, there was no correlation between those 2 parameters. However, the pattern of CysLT₁-R expression through the challenge days is similar to what we previously observed in sputum eosinophils [30], suggesting a link between those 2 parameters.

“Standard” lung allergen challenge in asthmatic subjects induced no variations in CysLT₁-R mRNA. Nevertheless, this may be due to the fact that none of the samples used for mRNA assessment came from asthmatic subjects that experienced a LAR. Indeed, subjects showing a late asthmatic response following allergen challenge may have more marked changes in bronchial inflammatory responses following allergen exposures.

Based on the results from these 3 different types of allergen challenges, allergen-induced CysLT₁-R increase seems to occur whether or not the subject has concurrent asthma. We could think that a possible increased expression of this receptor in some individuals could be a risk factor of developing asthma, particularly following allergen exposure, through an enhanced effect of leukotrienes on lower airways inflammatory and remodelling processes. We observed changes in the receptor's expression in some subjects and not in others. This may reflect the fact that some subjects respond very well to CysLT₁-R antagonists while others do not respond at all.

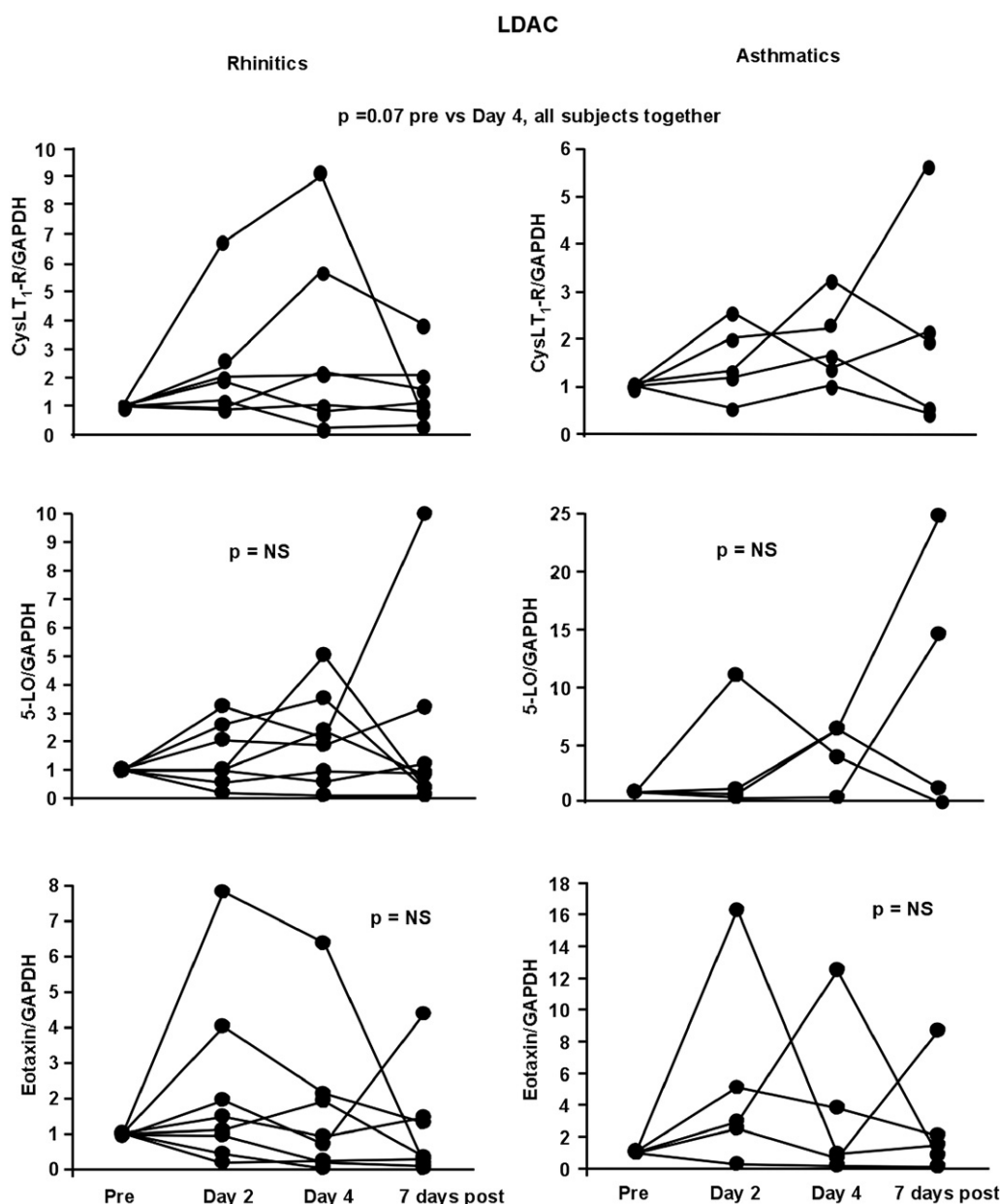


Fig. 3. Changes in CysLT₁-R, 5-LO and eotaxin mRNA from pre-allergen to 6 h post days 2 and 4 and 7 days post low dose lung allergen challenges in rhinitic and asthmatic subjects.

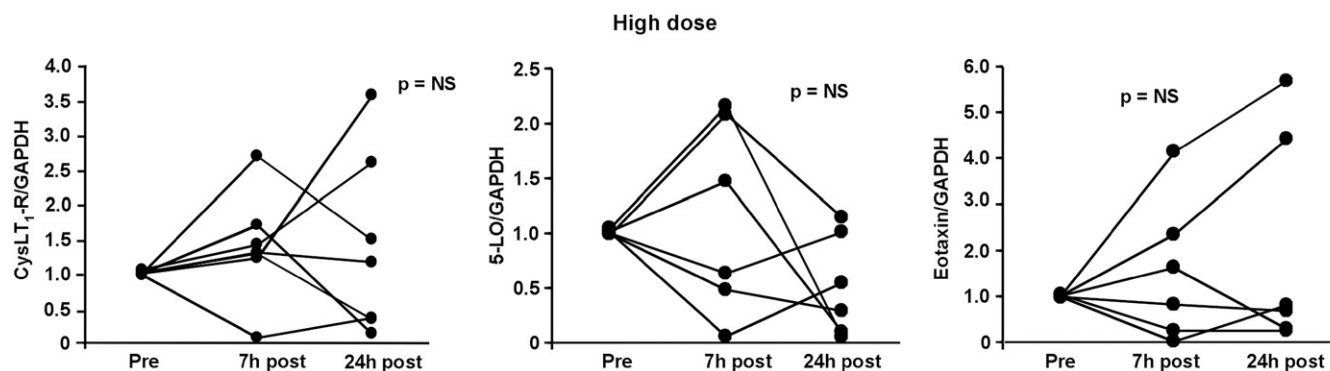


Fig. 4. Changes in CysLT₁-R, 5-LO and eotaxin mRNA from pre-allergen to 7 and 24 h post-whole lung allergen challenge in asthmatic subjects.

In a mouse model, it has been demonstrated that pre-treatment with a CysLT₁-R antagonist reduced the expression of the receptor following allergen challenge [34]. To our knowl-

edge, our study is the first to look at the expression of the receptor following allergen challenges both in rhinitic and asthmatic subjects. As mentioned above, CysLT₁-R antagonists attenuate

the early and late allergen-induced responses and prevent the changes in eosinophils and airway structural cells following allergen provocations in asthmatics [15–20]. However, the pathophysiological mechanisms involved are not well understood. By showing that changes in the receptor's expression can be measured in human following allergen provocation, our results open the door to future studies looking at the mechanisms of CysLT₁-R blockade in allergen-induced inflammation.

5-LO is a precursor in leukotriene biosynthesis while eotaxin is a major eosinophil chemoattractant. We thought that adding those measurements to the analyses would give us the opportunity to set the CysLT₁-R data in a wider context of inflammatory changes. We also observed a trend towards a significant change in 5-LO and eotaxin at 24 h post-nasal challenge when all subjects were analysed together. However, no changes in those mediators were observed following “low dose” lung challenge or “standard” lung challenge in either group. Those negative results are mainly due to the small number of samples available for analyses and to the great variability in such samples. Nevertheless, some subjects showed similar patterns in those CysLT₁-R, 5-LO and eotaxin expression and should therefore be further studied in order to determine the pathophysiology of rhinitis and asthma and in CysLT₁-R antagonist response.

Otherwise, during natural exposure, both the nose and lung come into contact with airborne allergens. However, the nasal obstruction associated with allergic rhinitis, limiting the filter function of the nose, is often associated with mouth breathing and increased penetration of allergens into the lower airways [35,36]. The pathophysiology of allergic reactions involves both local and systemic inflammation. It is therefore possible that nasal inflammation could influence lower airway inflammatory processes by the release of mediators into the circulation or through an effect on bone marrow progenitors or inflammatory cells [37]. CysLT₁-R mediates various pro-inflammatory effects, including mucus secretion, tissue oedema and eosinophil recruitment [38], which are characteristics of both allergic rhinitis and asthma.

We chose to study allergic rhinitic subjects with or without asthma to further explore the mechanisms that may lead to the development of asthma in subjects more at risk of this condition. Furthermore, this may help to determine if treatment of allergic rhinitis may reduce lower airway inflammation, therefore potentially preventing or postponing the development of asthma in non-asthmatic rhinitic subjects or help to keep asthma under control in those already suffering from this condition. This study helped us determine variations in the expression of the CysLT₁-R between asthmatic and rhinitic subjects, which to our knowledge, had never been done. We initially hypothesized that the expression of the CysLT₁-R in sputum would be increased in asthmatic patients compared to non-asthmatic rhinitic patients. However our findings do not point towards this direction and suggest that baseline levels of CysLT₁-R in induced sputum are not different between allergic rhinitic subjects and mild asthmatic subjects. This may therefore indicate that other factors such as non-allergic end-organ responsiveness, other immune mechanisms, possibly involving T regulators, or durations and intensity of exposure are more relevant as risks factors of developing asthma.

Throughout the study, we tried to link the variation in CysLT₁-R with the variation in sputum eosinophils. Following allergen provocations, the late phase response is mainly associated with an increase in eosinophils [31,39,40]. Furthermore, compared with placebo, CysLT₁-R antagonists decrease the eosinophil count and eosinophil progenitors at 24 h post-challenge [17–19]. We are aware that not only eosinophils express CysLT₁-R but that other sputum cells may also be involved in the receptor's variation. No correlations were observed between the variation in CysLT₁-R and sputum eosinophils in any of the challenges. The variation in

CysLT₁-R may therefore be attributed to an overall increase in expression of leukocytes and structural cells rather than to a specific cell population.

Although induced sputum is a valuable non-invasive technique that allows screening of inflammation in allergen challenge studies that are very demanding, this technique shows limitations to assess receptors and inflammatory mediators. As this study was very demanding, the number of subjects that could be included was modest, the small number of subjects studied being a limitation of this study. This is mainly due to the difficulty to obtain sputum samples containing a large number of cells in order to do mRNA analyses. Furthermore, once subjects were included, even if some sputum time-points were missing because of lack of material, they still completed the study. Even though we tried to minimize the number of subjects not providing quality samples for RNA extraction, after slides were prepared for differential cell count, there was often not enough material for the RNA analyses. Therefore, this contributed to the lack of power of the study and to the non-significant change in most analyses. Another limitation is the fact that we cannot assess changes in specific cell populations. However, as this was an exploratory study aiming only to look at the potential of sputum cells to express and modulate CysLT₁-R, this also explains the choice of looking only at the mRNA level instead of looking at both mRNA and protein levels. Nevertheless, this study provides new and hypothesis-generating results. We believe that it sheds more light on the mechanisms of allergen-induced airway inflammation.

In summary, this exploratory study shows that CysLT₁-R mRNA can be measured in induced sputum from allergic rhinitic and allergic asthmatic subjects. Furthermore, changes in the receptor's mRNA expression are detectable in sputum following allergen provocations. Future studies are necessary to analyse the protein expression of this receptor between allergic rhinitic subjects and allergic asthmatic subjects, and also following allergen provocations.

Conflicts of interest for Louis-Philippe Boulet

Advisory Boards: AstraZeneca, Altana, GlaxoSmithKline, Merck Frosst and Novartis.

Lecture fees: 3M, Altana, AstraZeneca, GlaxoSmithKline, Merck Frosst and Novartis.

Sponsorship for investigator-generated research: AstraZeneca, GSK, Merck Frosst, Schering.

Research funding for participating in multicenter studies: 3M, Altana, AsthmaTx, AstraZeneca, Boehringer-Ingelheim, Dynavax, Genentech, GlaxoSmithKline, IVAX, MedImmune, Merck Frosst, Novartis, Roche, Schering, Topigen, Wyeth.

Support for the production of educational materials: AstraZeneca, GlaxoSmithKline and Merck Frosst.

Organisational: Chair of the Canadian Thoracic Society Guidelines Dissemination and Implementation Committee. Laval University Chair on knowledge Transfer, Prevention and Education in Respiratory and Cardiovascular Health. Member of the asthma committee of the World Allergy Organisation.

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