

Leukotrienes and isocyanate-induced asthma: a pilot study

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Summary

Background The role of leukotrienes (LTs) in the pathophysiology of isocyanate-induced asthma is not well known.

Objective We sought to characterize the type of airway inflammation induced by exposure to isocyanates and to investigate whether exposure to isocyanates induced an increase in LT receptor cysteinyl leukotriene ((CysLT)₁, CysLT₂ and leukotriene B₄ receptor (BLT₁)) expression, as well as a release of LT (LTC₄ and leukotriene B₄ (LTB₄)) and IL-8 in both asthmatics with isocyanate-induced asthma and healthy subjects.

Methods We investigated eight subjects with isocyanate-induced asthma and eight healthy subjects. Both groups underwent specific inhalation challenges to isocyanates in the laboratory. Induced sputum was collected before and after exposure to isocyanates. CysLT₁, CysLT₂ and BLT₁ expression was assessed by flow cytometry, whereas LTC₄, LTB₄ and IL-8 were measured in the sputum supernatants by enzyme immunoassay.

Results Exposure to isocyanates induced an increase in sputum neutrophils only in subjects with occupational asthma. There was a significant increase in CysLT₁ and BLT₁ receptor expression, as well as a release of LTB₄ and IL-8 after exposure to isocyanates compared with the baseline, only in subjects with isocyanate-induced asthma, whereas there was no increase in LTC₄. Exposure to isocyanates did not induce any change in LT receptor expression nor in the levels of LTC₄, LTB₄ and IL-8, in healthy subjects.

Conclusion The neutrophilia observed after exposure to isocyanates is likely to be related to the release of LTB₄, probably enhanced by the increased expression of BLT₁ on neutrophils as well as by the release of IL-8. The significance of the increase of CysLT₁ receptor expression on neutrophils is unknown and needs further investigation.

Keywords induced sputum, isocyanates, leukotrienes, occupational asthma

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Introduction

Isocyanates are chemicals widely used in the automotive industry. They are the substances most frequently responsible for occupational asthma (OA) [1]. The pathophysiology of OA caused by isocyanates is not well known, but differs from OA caused by high-molecular-weight agents. Although there is an increase in sputum eosinophils in some instances after exposure to isocyanates [2], it seems that the principal cell type that increased after exposure to isocyanates is the neutrophil [3–5] as opposed to the eosinophilic type of inflammation found after exposure to high-molecular-weight agents.

Leukotrienes (LTs) are lipid mediators synthesized from arachidonic acid that play an important role in the pathophysiology of asthma. cysteinyl leukotriene (CysLT) (LTC₄, LTD₄) mimic many of the features of asthma such as bronchoconstriction, mucus secretion [6] and airway hyper-responsiveness [7]. LTB₄ signals mainly through the BLT₁ receptor

and acts as a chemoattractant for neutrophils in addition to increasing their survival and inducing their degranulation [8]. On the other hand, LTC₄ and LTD₄ signal mainly through the CysLT₁ receptor and can attract and activate eosinophils [9]. The number of sputum eosinophils has been shown to correlate with the level of CysLTs in atopic asthmatics after allergen challenge to common inhalants [10], whereas LTB₄ has been shown to increase after exposure to isocyanates inducing late asthmatic reactions [11]. Whether exposure to isocyanates *per se* can induce changes in LT receptor expression or release of LT in healthy subjects has never been investigated. The aim of this study was to investigate CysLT₁, CysLT₂ and BLT₁ receptor expression and to measure sputum concentrations of LTB₄, LTC₄ and IL-8 after exposure to isocyanates in subjects with isocyanate-induced asthma and in healthy subjects.

Methods

Subjects with isocyanate-induced asthma previously diagnosed by specific inhalation challenges (SICs) in Sacre-Coeur

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Hospital were enrolled. The subjects were no longer exposed to isocyanates and had not been exposed to these agents since they underwent SIC to isocyanates at the time of diagnosis.

Healthy subjects were randomly selected among apprentices who were regularly exposed to isocyanates in their daily lives but did not have asthma and did not report any respiratory symptoms. For obvious ethical reasons, it was not possible to expose to isocyanates healthy subjects who would not have been exposed to those agents otherwise. The study was approved by a research ethics committee of Sacre-Coeur Hospital and all subjects gave written informed consent.

Study design

On the first day (control day), the baseline characteristics of the subjects, including age, past medical and occupational history, respiratory symptoms, smoking habits, and treatment were recorded. The subjects who were treated with inhaled corticosteroids took their medication at bed-time only. Long acting β_2 -agonists were stopped 72 h before starting the SIC, whereas short-acting β_2 -agonists were stopped 12 h before the challenge.

Forced expiratory volume (FEV₁) was monitored for 7 h to ensure the stability of their asthma. Skin tests were performed with commercial extracts from 12 common inhalants as previously described [12]. A subject was considered as atopic, if at least one skin prick test to common inhalants was positive. Methacholine challenge followed by sputum induction and blood collection was performed at the end of the day as previously described [13]. On day 2, the subjects were exposed to a concentration of 15 p.p.b. of isocyanates until a 20% fall in FEV₁ occurred or for a maximum of 2 h using a close circuit apparatus [14] or in an exposure chamber as previously described [15]. Healthy subjects were exposed to 15 p.p.b. of hexamethylene diisocyanates (HDI) for 30 min. Seven hours after the end of exposure, methacholine challenge and sputum induction were performed and blood was collected. Sputum was induced using inhalations of increasing concentrations (3–5%) of hypertonic saline [16] and processed as previously described [17].

Measurement of leukotriene and interleukin-8 contents in sputum supernatants Measurement of contents of LTB₄ and LTC₄/LTD₄ in sputum supernatants were performed by enzyme immunoassay (Cayman Chemicals, Ann Arbor, MI, USA). IL-8 was measured by ELISA (BD Biosciences, San Jose, CA, USA), according to the manufacturer's instructions. The lower limits of detection for LTB₄, LTC₄/LTD₄ and IL-8 were 4, 10 and 0.8 pg/mL, respectively.

Flow cytometry The expression of LT receptors in neutrophils and other sputum cells was assessed using a monoclonal anti-BLT₁ antibody (Ab) [18] and polyclonal anti-CysLT₁ and CysLT₂ Ab (Cayman Chemicals) [19] for immunofluorescence analysis on a FAC Scan flow cytometer (BD Biosciences). The gate was set for the population of neutrophils, as determined by forward and side scatter characteristics and CD16 labelling. Results are expressed as mean fluorescence intensity (MFI) units $\pm$ standard deviation (SD).

Analysis of results Results are expressed as mean $\pm$ SD, except for data with non-normal distribution, which were expressed as median and interquartile range. Wilcoxon's signed-rank test was used to compare sputum cell counts, LT receptor expression, LT and IL-8 before and after exposure to isocyanates within subjects with OA and healthy subjects. Correlations between changes in sputum neutrophils, IL-8, LTB₄, and BLT₁, CysLT₁ and CysLT₂ receptor expression before and after exposure to isocyanates were examined using a Spearman's rank-order test. Significance was accepted at the level of 95%. The analysis was performed using the SPSS 10.0 statistical package (Chicago, IL, USA).

Results

Eight subjects with OA and eight healthy subjects were enrolled. Six subjects had OA caused by HDI, two of them had OA caused by methylene diphenyldiisocyanate (MDI). They were all car painters except one subject who was working in a foundry where he was exposed to MDI. The characteristics of the subjects are presented in Table 1.

Table 1. Characteristics of the subjects

	Occupational asthma (n = 8)	Healthy subjects (n = 8)
Age	46.3 $\pm$ 11.4	33.9 $\pm$ 15.6
Sex	8 M	8 M
Atopy	4 A	2 A
Time since last exposure to isocyanates (years)	4.5 $\pm$ 4.9	Ongoing
Duration of exposure (years)	13.9 $\pm$ 12.2	1.6 $\pm$ 0.7
Smoking habits (pack/year)	NS, 1; CS, 0; ExS, 7	NS, 4; CS, 1; ExS, 3
	13.6 $\pm$ 13.5	3.26 $\pm$ 8.4
Treatment	ICS, 7; LABA, 2	0
	Daily dose of ICS, 468.8 $\pm$ 28.15 μ g	
Agent	HDI, 6; MDI, 2	HDI, 8
FEV ₁ , % predicted	86.9 $\pm$ 13.4	104.5 $\pm$ 7.1
PC ₂₀ , pre-challenge (mg/mL)*	5.6 (44.7)	124.8 (5.6)
PC ₂₀ post-challenge (mg/mL)*	2.0 (39.0)	112.3 (83.0)

M, male; CS, current smoker; ExS, ex smoker; NS, never smoked; A, atopic; HDI, Hexamethylene diisocyanate; MDI, methylene diphenyldiisocyanate; ICS, inhaled corticosteroids; LABA, long-acting β_2 -agonists.

*PC₂₀ is expressed as geometric mean (range).

Three subjects with OA did not have a 20% fall in FEV₁ after exposure to isocyanates although one of them had a 16% fall in FEV₁. Both subjects who experienced a fall in FEV₁ less than 15% after exposure to isocyanates had a twofold decrease in their PC₂₀ after exposure to these agents. Three subjects experienced an immediate reaction while both subjects who were exposed to MDI had a late asthmatic reaction. None of the healthy subjects experienced a significant fall in FEV₁, nor a change in airway responsiveness after exposure to isocyanates.

Sputum cell counts before and after exposure to isocyanates in asthmatic and healthy subjects

There was a significant increase in the sputum neutrophil percentage (68.0 (54.8)%) after exposure to isocyanates compared with baseline (26.5 (43.9)%, $P = 0.03$) in subjects with OA, whereas no significant change in sputum neutrophil percentage was observed before and after exposure to isocyanates in healthy subjects. There was no significant change in sputum eosinophils after exposure to isocyanates in either asthmatic or healthy subjects compared with baseline. The results are presented in Table 2.

Leukotrienes C₄, leukotrienes B₄ and interleukin-8 levels in sputum supernatant of asthmatic and healthy subjects before and after exposure to isocyanates

There was no significant difference in LTB₄ levels at baseline between healthy subjects and subjects with isocyanate-induced asthma ($P = 0.2$). There was a significant increase in LTB₄ levels after exposure to isocyanates compared with baseline in subjects with isocyanate-induced asthma, whereas there was no increase in LTC₄ levels after exposure to isocyanates compared with baseline within the same subjects. We also found a significant increase in IL-8 in subjects with OA after exposure to isocyanates compared with baseline (Table 2). The subjects with OA because of MDI had a large increase in IL-8 after exposure to MDI (1297 and 1347 pg/mL, respectively).

There was no difference between LTB₄, LTC₄ or IL-8 levels in healthy subjects after exposure to isocyanates compared with baseline (Table 2).

There was a significant positive correlation between the increased expression of BLT₁ receptor and the increase in

LTB₄ levels after exposure to isocyanates compared with baseline ($\rho = 0.6$, $P = 0.046$), whereas we did not find any correlation between the increase in neutrophils and the levels of IL-8 ($\rho = 0.2$, $P = 0.4$) or LTB₄ ($\rho = 0.2$, $P = 0.6$). We did not find any correlation between the duration of exposure during the challenge, the time since last exposure and the changes in IL-8 ($\rho = -0.1$, $P = 0.9$; $\rho = 0.2$, $P = 0.7$, respectively) and LTB₄ ($\rho = 0.3$, $P = 0.3$ and $\rho = -0.1$, $P = 0.8$, respectively) after exposure to isocyanates.

There was a positive correlation between the ratio of PC₂₀ before and after challenge and the change in LTB₄ after exposure ($\rho = 0.6$, $P = 0.02$).

Cysteinyl leukotriene₁, cysteinyl leukotriene₂ and BLT₁ receptor expression on sputum neutrophils and macrophages of asthmatic and healthy subjects before and after exposure to isocyanates

Seven healthy subjects and seven subjects with OA had satisfactory sputum samples for performing this analysis. At baseline, the expression of CysLT₂ and BLT₁ receptor on neutrophils was higher in healthy subjects (Table 2). CysLT₁ and BLT₁ receptor expressions were significantly increased on neutrophils after exposure to isocyanates compared with the expression of the same receptors at baseline only in subjects with OA (Fig. 1). We did not find any significant difference in the expression of the CysLT₁, CysLT₂ and BLT₁ receptors on neutrophils from healthy subjects before and after exposure to isocyanates (Fig. 1, Table 2).

We did not find any significant change in CysLT₁, CysLT₂ and BLT₁ receptor expression on macrophages in either asthmatic or healthy subjects after exposure to isocyanates compared with baseline. Flow cytometry analysis of CysLT₁, CysLT₂ and BLT₁ expression by sputum neutrophils from an asthmatic subject and a healthy subject is illustrated in Fig. 2.

Cysteinyl leukotriene₁, cysteinyl leukotriene₂ and BLT₁ receptor expression on peripheral blood polymorphonuclear leucocytes and monocytes of asthmatic and healthy subjects before and after exposure to isocyanates

We failed to detect an increased expression of CysLT₁ (37.3 ± 21.1 MFI), CysLT₂ (3.1 ± 5.0 MFI) and BLT₁

Table 2. Inflammatory changes after exposure to isocyanates in asthmatic and healthy subjects

	Occupational asthma		Healthy subjects	
	Pre-challenge	Post-challenge	Pre-challenge	Post-challenge
TCC ($10^6 \times \text{mL}$)	1.4 (1.0)	3.4 (16.1)	2.6 (2.8)	3.3 (5.1)
Eosinophils (%)	0.8 (1.6)	1.4 (1.9)	0.3 (0.5)	0.6 (1.4)
Neutrophils (%)	26.5 (43.9)	68.0 (54.8)*	40.6 (40.2)	48.7 (58.6)
IL-8 (pg/mL)	481.7 $\pm$ 208.3	981.6 $\pm$ 586.5*	634.2 $\pm$ 264.8	715.3 $\pm$ 269.1
LTC ₄ (pg/mL)	94.4 $\pm$ 27.5	115.3 $\pm$ 44.7	106.9 $\pm$ 24.2	93.4 $\pm$ 23.5
LTB ₄ (pg/mL)	539.5 $\pm$ 532.4	990.2 $\pm$ 342.1*	934.5 $\pm$ 520.3	769.4 $\pm$ 611.2
CysLT ₁ MFI	10.8 $\pm$ 10.5	36.4 $\pm$ 22.2*	14.3 $\pm$ 14.5	20.2 $\pm$ 13.4
CysLT ₂ MFI	4.0 $\pm$ 5.2†	21.6 $\pm$ 18.9	19.8 $\pm$ 19.3†	20.8 $\pm$ 12.5
BLT ₁ MFI	20.0 $\pm$ 12.4†	47.9 $\pm$ 21.2*	40.2 $\pm$ 13.9†	48.5 $\pm$ 16.5

TCC, Total cell count; MFI, Mean fluorescence intensity; LT, leukotriene; Cys, cysteinyl.

*Comparison before and after specific inhalation challenge, $P \leq 0.05$.

†Comparison between healthy subjects and subjects with isocyanate-induced asthma at baseline, $P \neq 0.05$.

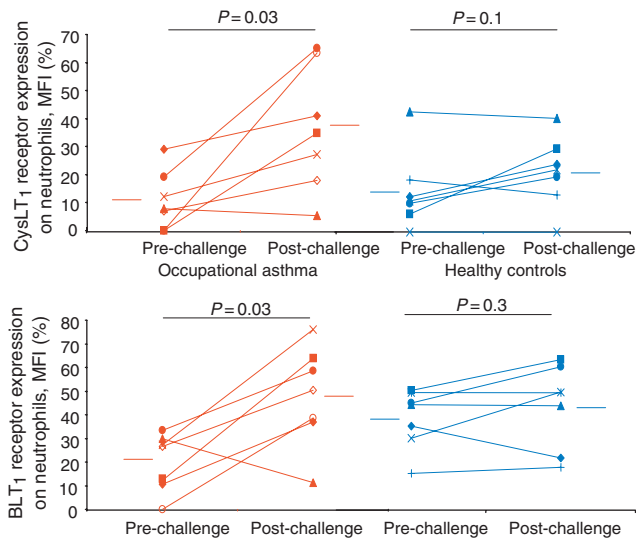


Fig. 1. Cysteinyl leukotriene (CysLT)₁ and BLT₁ receptor expression on sputum neutrophils of asthmatic and healthy subjects before and after exposure to isocyanates. Subjects with occupational asthma are represented with red symbols whereas healthy subjects are represented with blue symbols. The symbols x and ◇ in the group of subjects with occupational asthma (OA) identify the subjects who were exposed to methylene diphenyldiisocyanate. MFI, mean fluorescence intensity.

(34.6 ± 26.1 MFI) receptors on polymorphonuclear leucocytes after exposure to isocyanates compared with the expression of CysLT₁ (28.3 ± 22.1 MFI, $P = 0.2$), CysLT₂ (3.0 ± 5.1 MFI, $P = 0.5$) and BLT₁ (42.0 ± 24.6 MFI, $P = 0.3$) receptors at baseline. There was also no difference between the expression of the same receptors before and after exposure to isocyanates in healthy subjects. We did not find any change in CysLT₁, CysLT₂ and BLT₁ receptor expression on peripheral blood monocytes in either asthmatic or healthy subjects.

Discussion

Exposure to isocyanates induced a neutrophilic type of airway inflammation in subjects sensitized to these agents. Exposure to isocyanates also induced an increased expression of CysLT₁ and BLT₁ receptors as well as a release of LTB₄ and IL-8 in subjects with isocyanate-induced asthma, whereas these changes were not observed in healthy subjects.

The increase of sputum neutrophils after exposure to isocyanates in subjects with isocyanate-induced asthma is consistent with previous studies [3, 5]. The release of LTB₄ has also been shown previously in subjects experiencing late asthmatic reactions after exposure to isocyanates [11]. However, and to the best of our knowledge, this is the first time that an increased expression of LT receptors on neutrophils is found after exposure to isocyanates.

The majority of our subjects were treated with inhaled steroids. This may partly explain why we did not find sputum eosinophilia in those subjects. However, corticosteroids do not have a strong impact on neutrophilic inflammation nor on the LT pathway [20]. Therefore, treatment with inhaled steroids is likely to have had a limited impact on our findings related to neutrophilic inflammation and LT. Two of our

subjects with OA because of isocyanates were sensitized to MDI. They both had a late asthmatic reaction with a marked increase in the level of IL-8. Whether this increase is related to the type of isocyanates (MDI vs. HDI) or to the type of reaction induced (late vs. immediate) remains to be elucidated.

Exposure to isocyanates may induce an increased expression of BLT₁ on neutrophils, which subsequently enhances the release of LTB₄ by these cells. Conversely, increased synthesis of LTB₄ can enhance BLT₁ expression in neutrophils [18]. The two phenomena can occur concurrently and result in enhanced responsiveness of neutrophils to LTB₄ leading to augmented LTB₄ production and cell migration. The neutrophilic inflammation is also likely to be enhanced by the increased release of IL-8 [4], and LTB₄ may have played a role in the increased synthesis of this chemokine [21]. An alternative explanation for our observations could be that the migrating neutrophils are unique in that they are a subpopulation that has more LT receptors and is thus more sensitive to recruitment by LT. Consequently, they find themselves overrepresented in the migrating pool recovered in the sputum of asthmatics following isocyanate challenge.

More intriguing is the increased expression of CysLT₁ receptor on neutrophils after exposure to isocyanates. This increased expression seems to be independent of the release of CysLTs. Binding of CysLTs to their receptors on neutrophils may induce a release of some mediators such as nitric oxide by these cells as previously described [22]. However, this needs to be further investigated and confirmed. This finding is unlikely to be a non-specific effect of isocyanates since this increased expression of CysLT₁ on neutrophils was not observed in healthy subjects. Our controls were healthy subjects and did not have asthma. We do not know whether neutrophils of non-isocyanate-sensitive asthmatic subjects would react differently to the exposure to isocyanates. However, since exposure to isocyanates did not induce any change in the sputum cell counts of asthmatic subjects without OA [2], it is unlikely that this would induce an isolated increase in the expression of LT receptors on neutrophils. We found a higher expression of CysLT₂ and BLT₁ receptor on neutrophils in healthy subjects than in subjects with isocyanate-induced asthma at baseline. Although not statistically significant, there was also a trend towards higher neutrophil counts and higher levels of LTB₄ in healthy subjects than in asthmatic subjects that is not in accordance with previous results. Neutrophil counts and LTB₄ levels have not been reported to be higher in healthy subjects compared with asthmatics [23]. However, our control group was made of subjects exposed to isocyanates in their daily lives, whereas subjects with OA had been removed from exposure for several years. Although healthy subjects were asymptomatic, ongoing exposure to isocyanates may induce some airway inflammation. However, an acute exposure to isocyanates induced inflammatory changes only in the group of asthmatic subjects.

The increased expression of the LT receptors seems to be restricted to the neutrophils extracted from the airways. We did not observe any increased expression on peripheral blood neutrophils or monocytes, suggesting a local rather than a systemic effect. Even more intriguing is the lack of modula-

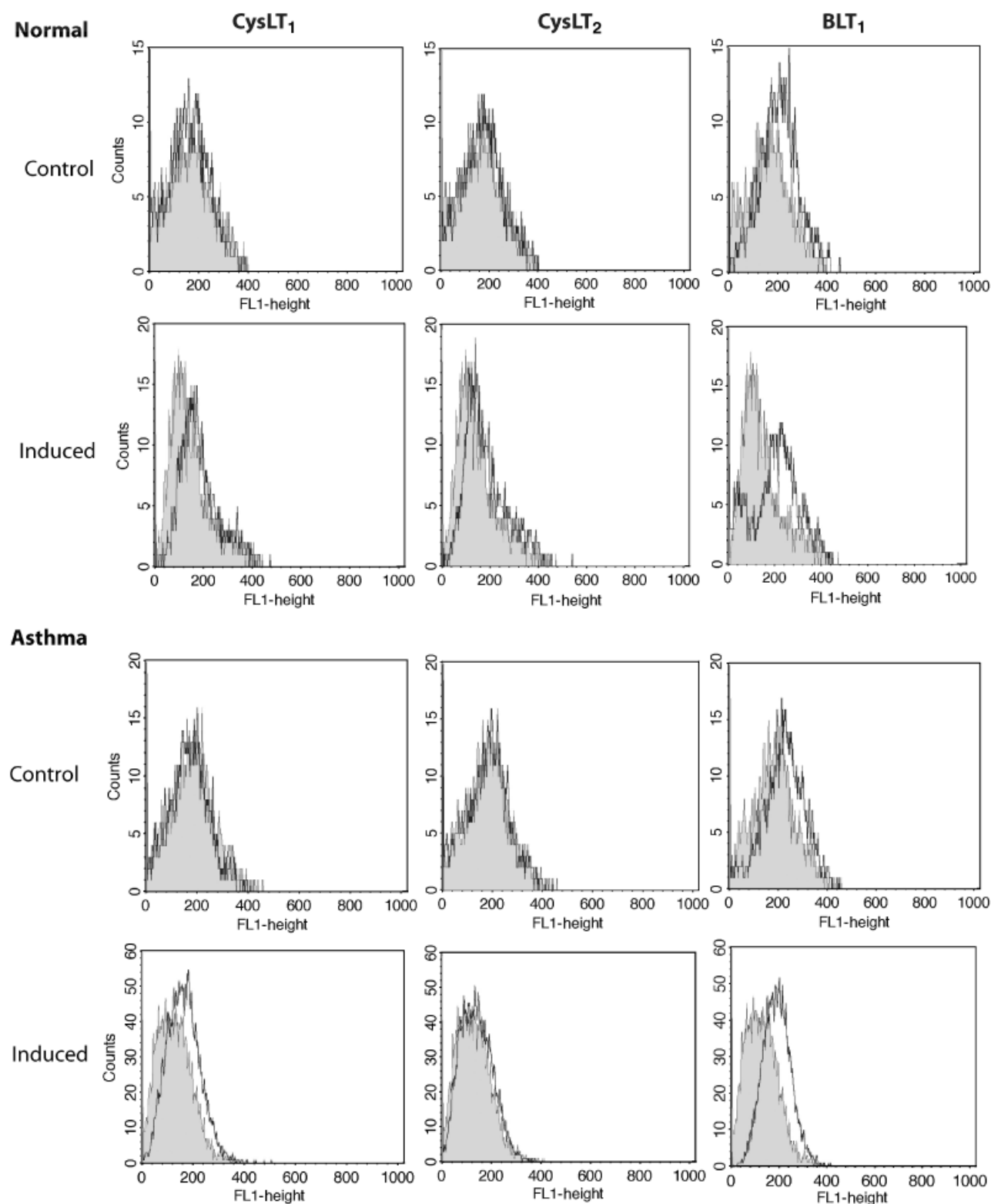


Fig. 2. Flow cytometry analysis of cysteinyl leukotriene (CysLT)₁, CysLT₂ and BLT₁ expression by sputum neutrophils from an asthmatic subject and a healthy subject obtained on the same day before (control) or 8 h after exposure to isocyanates (induced).

tion of LT receptor expression on alveolar macrophages. It is possible that the process of migration into the airways is essential for enhanced BLT₁ and CysLT₁ expression on neutrophils from asthmatics.

It must also be noted that the process of inducing sputum affects the neutrophil recovery from the airways [24, 25]. The effect of inducing sputum with saline on the levels of expression of LT and their receptors on neutrophils has never

been assessed to our knowledge. However, the increase in the neutrophil count and in the expression of LT receptor on neutrophils after exposure to isocyanates cannot be solely attributed to the effect of saline since this increase was restricted to the asthmatic subjects sensitized to isocyanates and was not seen in healthy subjects.

This pilot study suggests that LT may play a role in the pathophysiology of isocyanate-induced asthma. These observations need to be confirmed in a larger group of subjects and compared with asthmatic subjects without OA after exposure to isocyanates.

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