

LETTER

Cysteinyl-leukotriene and prostaglandin pathways in bronchial versus alveolar lavage in allergic asthmatics

To the Editor,

Increases in the eicosanoid-based arachidonic acid metabolism precede several pro-inflammatory activities and have been extensively reported to mediate many aspects of asthma.¹ Specifically, the cysteinyl-leukotriene pathway is involved in pro-inflammatory signalling, while the prostaglandin pathway can lead to either pro- or anti-inflammatory signalling.² Previously, we showed the fibroblasts from central airways of asthmatics have higher levels of leukotriene

B₄ receptors (BLT) and lower levels of cysteinyl-leukotriene (Cys-LT) receptors than the peripheral airway.³ However, the extent of the upstream enzyme cascade has not been investigated in bronchial and alveolar lavage cells nor in the context of an allergen bronchoprovocation test, as concluded in a recent review Reference S1.

Eighteen subjects with clinically stable mild-moderate allergic asthma (all non-smokers) underwent a bronchial and an alveolar lavage at baseline and 24 hours (h) after the bronchoprovocation test,

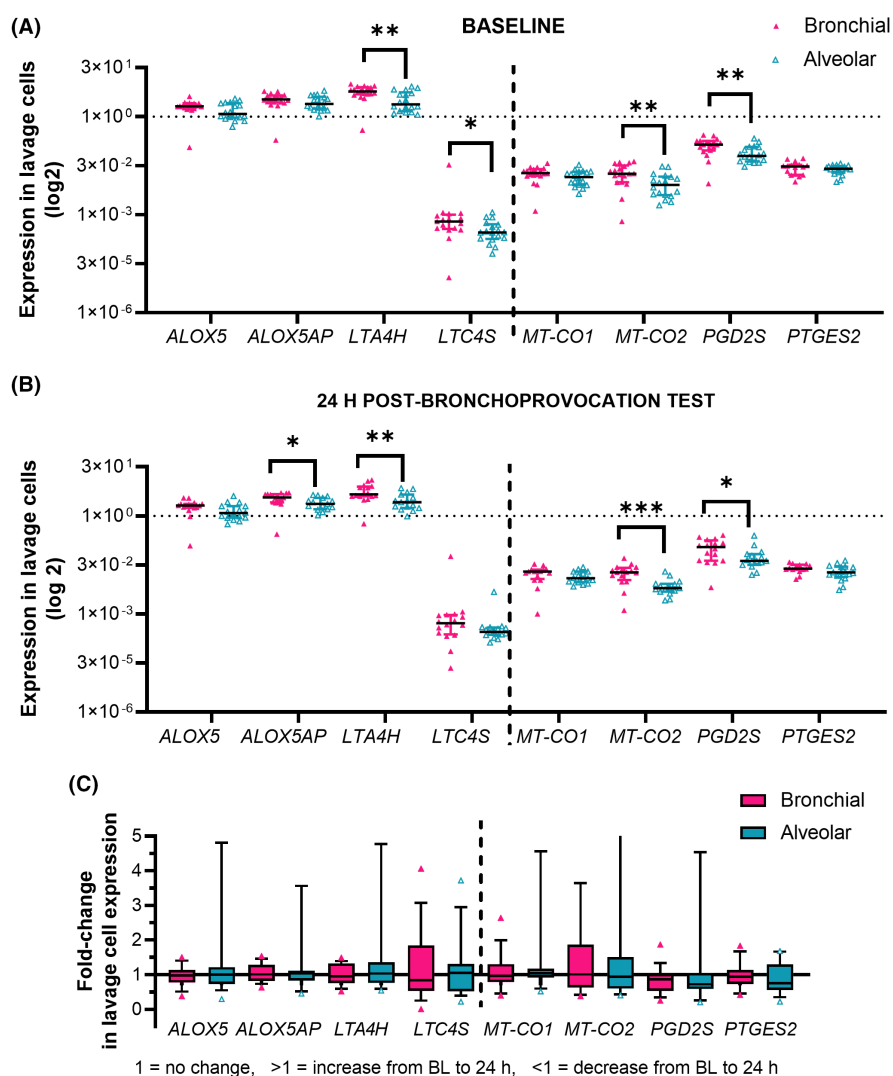


FIGURE 1 mRNA expression levels of the cysteinyl-leukotriene and prostaglandin pathways (relative to GAPDH) in bronchial (▲) and alveolar (△) lavage cells from allergic asthmatics, $n = 18$. Cysteinyl-leukotriene genes are placed left, and prostaglandin are placed right of the dashed line. Data are separated into sampling at (A) baseline and (B) 24 h after the bronchoprovocation test, and data are presented as median $\pm$ IQR. (C) Fold change in expression from baseline to 24 h post-test of bronchial (filled) and alveolar (open) fractions, and error bars are from 10th to 90th percentile. Statistics: paired analyses using the Wilcoxon signed-rank test, * $p < .05$, ** $p < .01$. See Calculations S1 for an example of expression and fold-change calculation

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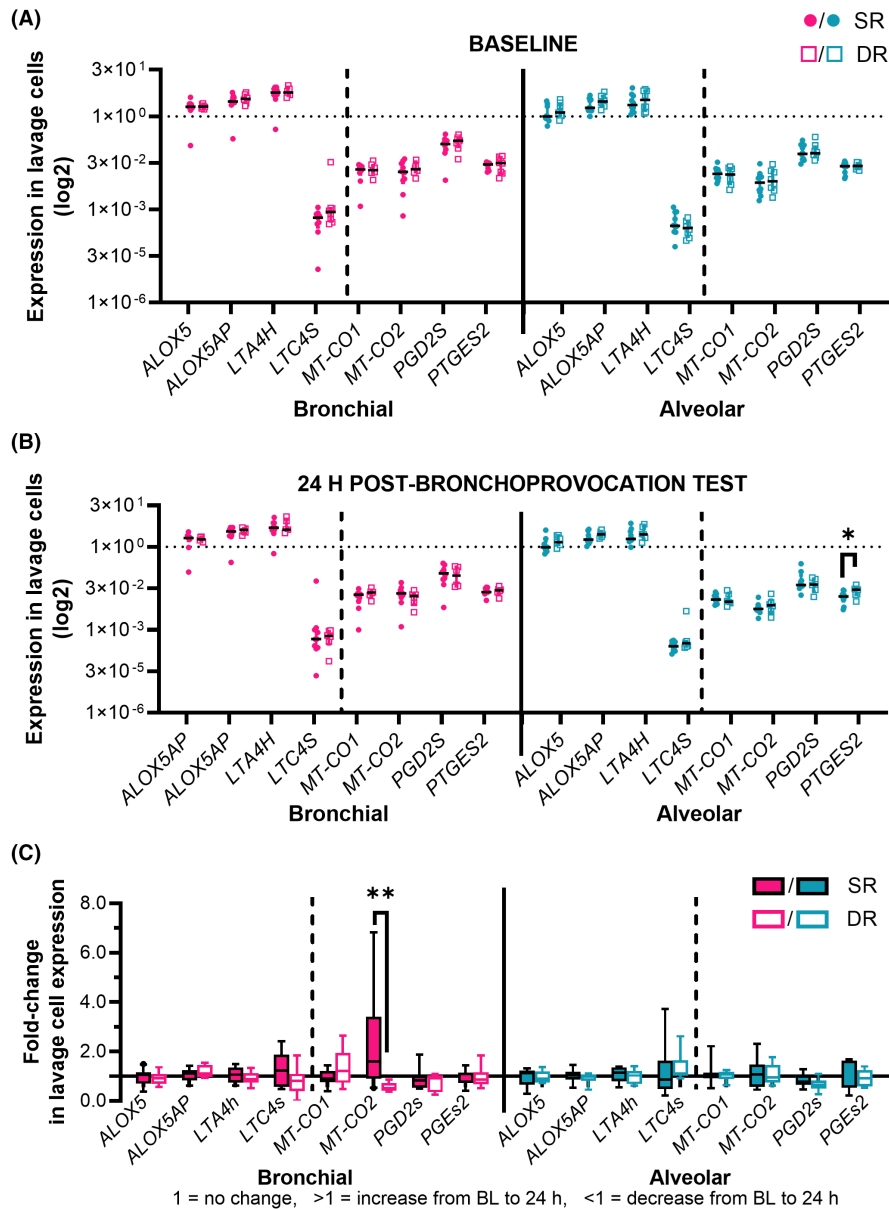


FIGURE 2 mRNA expression levels of the cysteinyl-leukotriene and prostaglandin pathways (relative to GAPDH) in lavage cells of single (●, $n = 10$) and dual (□, $n = 8$) responding allergic asthmatics. Cysteinyl-leukotriene genes are placed left, and prostaglandin are placed right of the dashed line for each fraction. Data are separated into sampling at (A) baseline and (B) 24 h after the bronchoprovocation test, and data are presented as median $\pm$ IQR. (C) Fold change in expression from baseline to 24 h post-test of bronchial (filled) and alveolar (open) fractions, and error bars are from 10th to 90th percentile. Statistics: unpaired analyses using the Mann-Whitney U-test, * $p < .05$. See Calculations S1 for an example of expression and fold-change calculation

as outlined in Figure S1 (all subjects signed a written informed consent form, and the Regional Ethics Review Board in Lund, Sweden, approved the study (2012/800)). Subject characteristics, methods and lung physiology data have been published previously, but the characteristics and allergens used in the subset of those who underwent bronchoscopy can be viewed in Table S1.⁴ Lavage was performed by instilling 3×50 ml sterile PBS into the middle lobe. The bronchial fraction was collected from the first 50 ml instillment, and the alveolar fraction was collected from the next 2×50 ml.⁵ Cells from each fraction were isolated for RNA, and levels of mRNA expression from 1 ng of RNA/sample were quantified using qPCR of pro-inflammatory 5-lipoxygenase (ALOX5), 5-lipoxygenase activating protein (ALOX5AP), leukotriene A4 hydrolase (LTA4H), LTC4 synthase (LTC4S), cytochrome c oxidase subunit 1 (MT-CO1), MT-CO2 and prostaglandin D2 synthase (PGD2S) and anti-inflammatory PGE synthase 2 (PTGES2) and presented as relative levels to housekeeping gene GAPDH (see Table S2 for primer sequences). The levels of cysteinyl-leukotriene metabolites, Cys-LT, and prostaglandin metabolites,

prostaglandin E metabolites (PGEM), were measured in the lavage fluids by the respective ELISA from Cayman Chemical (Ann Arbor, MI).

The mRNA levels were higher in the bronchial fraction for both the cysteinyl-leukotriene and prostaglandin pathways than the alveolar fraction at both baseline (Figure 1A) and 24 h post-test (Figure 1B). Leading to a higher anti-inflammatory response 24 h post-test in the bronchial than alveolar fraction is determined by increased PGEM levels ($p = .020$) but not by inflammatory Cys-LT levels (Figure S2). As both lavage fractions had a similar proportion of cell types (Table S3), and there was no relationship between expression and each cell type (data not shown), the increased response indicates that bronchial cells of allergic asthmatics show the strongest response (by increasing both inflammatory expression and an anti-inflammatory response) to inhaled allergen. Although eicosanoids have previously been shown to be upregulated in the first few hours in response to bronchoprovocation test Reference S2, here we show the levels in lavage at 24 h post-test were similar to baseline again (Figure 1C), comparable to results presented in urine

References S3,S4. This lack of response to allergen 24 h post-test of these particular enzymes suggests that the enzymes are not the rate-limiting factor for the lipid mediator release.

The allergic asthmatics were sub-grouped into single responders (SR) with only an early allergic reaction ($n = 10$) or dual responders (DR) with both an early and late allergic reaction ($n = 8$) based on the max FEV1% drop between 0–2 and 4–8 h post-test, respectively. Interestingly, there were no differences in the expression levels of the synthases between SR and DR at the baseline (Figure 2A) and 24 h post-test (Figure 2B), except for a higher expression of anti-inflammatory PGES2 in the DR at 24 h post-test (Figure 2C). The observed increase in PGES2 here matches our recent findings that the same DR patients have significantly less CD62L (to be published) of which PGES2 is a known inhibitor of.² This supports the notion that DR have a delayed inflammatory resolution, which may include imbalance of the lipid metabolism, as previously suggested.⁶

To conclude, lavage cell mRNA expression of cysteinyl-leukotrienes and prostaglandins is higher in the bronchial than in the alveolar airways of allergic asthmatics both before and 24 h after a bronchoprovocation test and, in general, does not differ between SR and DR allergic asthmatics. Therefore, we recommend that future studies regarding these metabolic pathways take the lavage location into careful consideration when assessing mixed-cell type lavage fractions.

KEYWORDS

Bronchial lavage, cysteinyl-leukotriene pathway, prostaglandin pathway, allergen bronchoprovocation test, asthma

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CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to this work.

AUTHOR CONTRIBUTIONS

ET: conceptualized the study and acquired fund for the study; NV, HS, LB and ET: designed the methodology; NV: formally analysed, curated, and visualized the data; NV, HS and ET: investigated the study, ET, HS and LB: contributed to resources for the study, NV and ZD: wrote the draft; ZD and ET: involved in project administration; NV, HS, LB, ZD and ET: revised the writing; and LB, ZD and ET: supervised the study.

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REFERENCES

- Diamant Z, Aalders W, Parulekar A, Bjermer L, Hanania NA. Targeting lipid mediators in asthma: time for reappraisal. *Curr Opin Pulm Med*. 2019;25(1):121-127. doi:10.1097/mcp.0000000000000544
- Insuela DBR, Ferrero MR, Coutinho DDS, Martins MA, Carvalho VF. Could arachidonic acid-derived pro-resolving mediators be a new therapeutic strategy for asthma therapy? *Front Immunol*. 2020;11:580598. doi:10.3389/fimmu.2020.580598
- Tufvesson E, Nihlberg K, Westergren-Thorsson G, Bjermer L. Leukotriene receptors are differently expressed in fibroblast from peripheral versus central airways in asthmatics and healthy controls. *Prostaglandins Leukot Essent Fatty Acids*. 2011;85(2):67-73. doi:10.1016/j.plefa.2011.04.025
- Stenberg H, Diamant Z, Ankerst J, Bjermer L, Tufvesson E. Small airway involvement in the late allergic response in asthma. *Clin Exp Allergy*. 2017;47(12):1555-1565. doi:10.1111/cea.13036
- Van Vyve T, Chanez P, Lacoste JY, Bousquet J, Michel FB, Godard P. Comparison between bronchial and alveolar samples of bronchoalveolar lavage fluid in asthma. *Chest*. 1992;102(2):356-361. doi:10.1378/chest.102.2.356
- Singh A, Yamamoto M, Kam SHY, et al. Gene-metabolite expression in blood can discriminate allergen-induced isolated early from dual asthmatic responses. *PLoS One*. 2013;8(7):e67907. doi:10.1371/journal.pone.0067907

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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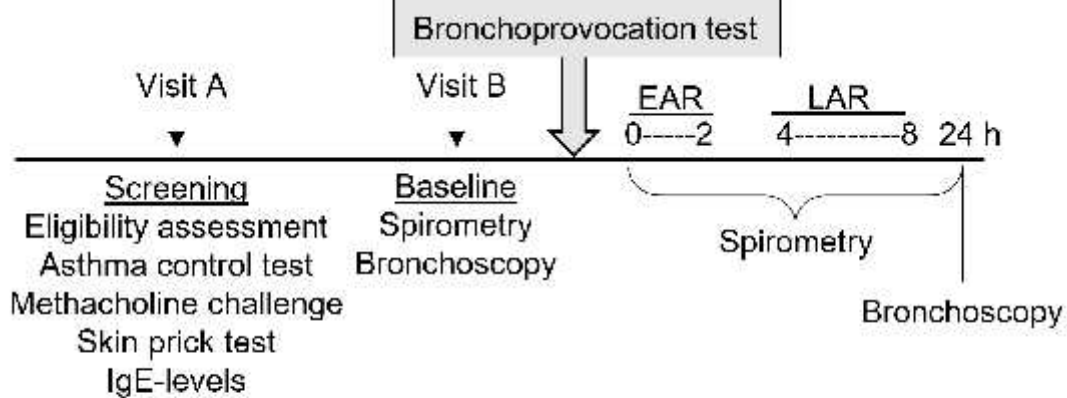
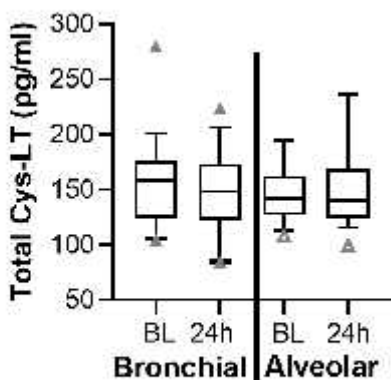


Figure S1_ van der Burg et al

(A)



(B)

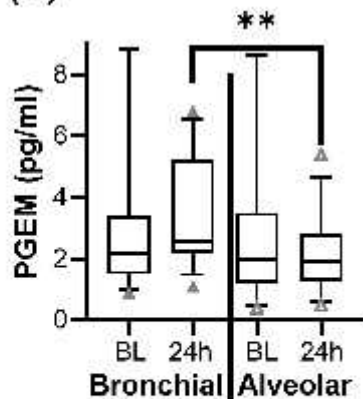


Figure S2_ van der Burg et al

Table S1

	SR (n = 10)	DR (n = 8)
Sex (Male/Female)	5/5	4/4
Age (years)	29	27
BMI (kg/m²)	23	24
Regular use of ICS (n)	4	6
Duration of asthma (years)	19	17
ACT (score)	22	22
Methacholine PD20 (µg)	137	225
Number of sensitisations	5	3
Specific IgE (kU/L)	2.9	2.2
Allergen dose given (SQ-U)	250	372
Allergen inhaled (n)		
○ Cat	7	4
○ Horse	2	1
○ Birch	0	2
○ D. pteronyssinus	1	1
FEV1 % max drop 0-2 h post-test	21.5	24.8
FEV1 % max drop 4-8 h post-test	6.9	20.2

Data is presented as median. Body mass index (BMI), inhaled corticosteroids (ICS), kilounits per litre (kU/L), standardised quality units (SQ-U), asthma control test (ACT), forced expiratory volume in one second (FEV1).

Table S2

Target	Forward 5' to 3'	Reverse 5' to 3'
<i>ALOX5</i>	CACGGGGACTACATCGAGTT	GTTTTTGCCGTGTTTCCAGT
<i>ALOX5AP</i>	GGAGAGAGAACGCAGAGCAC	GAGATGGTGGTGGAGATCGT
<i>LTA4H</i>	ACTGCTTGGAGGACCAGAGA	CCAGGCATTCCAATCAACTT
<i>LTC4S</i>	ACCATGAAGGACGAGGTAGC	GGGAAGTACTCGCTGCAGTT
<i>MT-CO1</i>	TACTCACAGTGCGCTCCAAC	GCAACTGCTTCTTCCCTTTG
<i>MT-CO2</i>	ATCACAGGCTTCCATTGACC	CAGGATACAGCTCCACAGCA
<i>PGD2S</i>	CAGATTTGGCTGGAAACACA	TGAGGCGCATTATACGTGAG
<i>PTGES2</i>	GGAAGGAGAAAGCTCGCAAC	GCCTCCTTCTCGTTGAGCAT

ALOX5, 5-lipoxygenase; *ALOX5AP*, 5-lipoxygenase activating protein; *LTA4H*, leukotriene A4 hydrolase; *LTC4S*, leukotriene C4 synthase; *MT-CO1*, cytochrome c oxidase subunit 1; *MT-CO2* cytochrome c oxidase subunit 2; *PGD2S*, prostaglandin D2 synthase; *PTGES2*, prostaglandin E synthase 2.

Table S3

Cell %	Macrophages	Neutrophils	Eosinophils	Lymphocytes
	Bronchial lavage			
Baseline	91.7 (85.1-95.3)	2.0 (1.2-5.1)	0.8 (0.2-2.0)	2.8 (1.5-6.9)
24 h post-test	90.9 (89.5-94.6)	3.8 (2.7-4.8)	0.5 (0.2-2.5)	3.0 (1.5-5.4)
<i>p</i>-value	0.9	0.4	0.7	>0.9
	Alveolar lavage			
Baseline	92.4 (89.7-96.6)	1.4 (0.3-1.9)	0.5 (0.1-1.8)	5.0 (1.3-6.9)
24 h post-test	92.3 (89.4-96.5)	1.5 (0.3-3.8)	0.5 (0.3-1.5)	4.2 (2.5-5.5)
<i>p</i>-value	0.7	0.4	0.9	0.9

Calculations S1

Well	Sample Name	Target Name	C _T	C _T Mean	ΔC_T : (C _T Mean gene) - (C _T Mean GAPDH)	2^{ΔC_T} ("Expression level") Fig1A,1B,2A,2B	$\Delta\Delta C_T$: (ΔC _T Mean BL) - (ΔC _T Mean 24h)	2^{ΔΔC_T} ("Fold- change") Fig1C,2C
A1	Pat01_Bronchial_BL	GAPDH	27.06669	27.11649				
A2	Pat01_Bronchial_BL	GAPDH	27.16629					
B1	Pat01_Bronchial_24h	GAPDH	26.93232	27.00061				
B2	Pat01_Bronchial_24h	GAPDH	27.0689					
A3	Pat01_Bronchial_BL	ALOX5	26.03938	26.10481	-1.011678696	2.016255817	-0.045297623	1.031896034
A4	Pat01_Bronchial_BL	ALOX5	26.17024					
B3	Pat01_Bronchial_24h	ALOX5	25.96617	25.94363	-1.056976318	2.080566381		
B4	Pat01_Bronchial_24h	ALOX5	25.92109					

Supplemental References

S1. Sokolowska M, Rovati GE, Diamant Z, Untersmayr E, Schwarze J, Lukasik Z, Sava F, Angelina A, Palomares O, Akdis CA, O'Mahony L, Sanak M, Dahlen SE, Woszczek G. Current perspective on eicosanoids in asthma and allergic diseases: EAACI Task Force consensus report, part I. *Allergy*. 2021;76(1):114-30. doi: 10.1111/all.14295

S2. Sladek K, Dworski R, Soja J, Sheller JR, Nizankowska E, Oates JA, Szczeklik A. Eicosanoids in bronchoalveolar lavage fluid of aspirin-intolerant patients with asthma after aspirin challenge. *American journal of respiratory and critical care medicine*. 1994;149(4 Pt 1):940-6. doi: 10.1164/ajrccm.149.4.8143059

S3. Diamant Z, Timmers MC, van der Veen H, Friedman BS, De Smet M, Depré M, et al. The effect of MK-0591, a novel 5-lipoxygenase activating protein inhibitor, on leukotriene biosynthesis and allergen-induced airway responses in asthmatic subjects in vivo. *The Journal of allergy and clinical immunology*. 1995;95(1 Pt 1):42-51. doi:10.1016/s0091-6749(95)70151-6

S4. O'Sullivan S, Roquet A, Dahlén B, Dahlén S, Kumlin M. Urinary excretion of inflammatory mediators during allergen-induced early and late phase asthmatic reactions. *Clin Exp Allergy*. 1998;28(11):1332-9. doi: 10.1046/j.1365-2222.1998.00368.x

S5. Gauvreau GM, Davis BE, Scadding G, Boulet L-P, Bjermer L, Chaker A, Cockcroft DW, Dahlén B, Fokkens W, Hellings P, Lazarinis N, O'Byrne PM, Tufvesson E, Quirce S, Van Maaren M, de Jongh FH, Diamant Z. Allergen Provocation Tests in Respiratory Research: Building on 50 Years of Experience. *European Respiratory Journal*. 2022:2102782. doi: 10.1183/13993003.02782-2021