

Interleukin-18 levels in induced sputum are reduced in asthmatic and normal smokers

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Summary

Background IL-18 is a cytokine which is known to have an important role in the development of a Th1 lymphocyte response. As such, it may have a regulatory role in asthma by modifying Th2 lymphocyte responses. Cigarette smoking may amplify the airway inflammation associated with asthma.

Objective This study investigated if IL-18 could be detected in induced sputum from asthmatics and normal subjects and if smoking altered IL-18 levels.

Methods Induced sputum was obtained from asthmatic (31 smokers, 35 non-smokers) and normal (20 smokers, 20 non-smokers) subjects. All smokers had a smoking history of ≥ 15 pack years. IL-18 levels in sputum supernatant were measured by ELISA. IL-18 mRNA expression and cellular localization were assessed by quantitative PCR and immunocytochemistry, respectively.

Results Smoking was associated with a significant reduction in IL-18 levels (median (interquartile range) – smokers 20 (0–102) pg/mL vs. non-smokers 358 (50–876) pg/mL, $P < 0.001$). This was more pronounced in asthmatics (smokers, 47 (40–64) pg/mL vs. non-smokers, 530 (30–1484) pg/mL; $P < 0.001$) than in normal subjects (smokers, 25 (0–78) pg/mL vs. non-smokers, 247 (50–656) pg/mL; $P < 0.01$). Within each of the smoking and non-smoking groups there was no significant difference in IL-18 levels between asthmatic and normal subjects. There was no correlation between sputum IL-18 levels and any specific cell type in the sputum samples nor serum IgE levels. IL-18 mRNA expression was reduced in asthmatic smokers compared with non-smokers. IL-18 production was localized to sputum macrophages by immunocytochemistry.

Conclusions IL-18 is detectable in induced sputum samples from both asthmatic and normal subjects. Cigarette smoking significantly reduces sputum IL-18 levels. This effect is more pronounced in asthmatics than in normal subjects.

Keywords asthma, cigarette smoking, IL-18, induced sputum

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Introduction

Atopic asthma is a chronic inflammatory condition of the airways associated with elevated serum IgE levels [1]. It is characterized pathologically by inflammatory infiltrates in the bronchial walls containing eosinophils, T lymphocytes and mast cells. Th2-type T helper lymphocytes and the cytokines they secrete, such as IL-4, IL-5 and IL-13, are thought to play a key role in the initiation and perpetuation of this airway inflammation [2, 3]. An alteration in the balance between a Th2 response and a Th1 response, where lymphocytes produce IFN- γ , may predispose to the development of allergic asthma [4].

IL-18, originally called IFN- γ inducing factor, is a pro-inflammatory cytokine secreted by activated macrophages. In

association with IL-12, it plays an important role in the development of a Th1 response and in the production of IFN- γ from T cells and natural killer (NK) cells [5]. However, IL-18 can also promote Th2 responses, depending on the surrounding cytokine environment [6, 7], and induce IgE production [8, 9]. It is able to induce IL-4, IL-13 and histamine production in basophils and mast cells in association with IL-3 [10], and IL-4 and IL-13 secretion from NK and naïve T lymphocytes in association with IL-2 [11]. IL-18 is expressed in the human airway epithelium [12] and is also produced by human alveolar macrophages [13]. As yet, there are conflicting reports on the actions of IL-18 in both human asthma [14–16] and animal models of this disease [17–21].

We therefore wished to assess further the role of IL-18 in airway inflammation in asthma. The objectives of the present study were to identify and quantify IL-18 levels in sputum samples from asthmatic and normal subjects and to assess its cellular distribution. Induced sputum is a non-invasive method of sampling airway cells and secretions which allows

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the identification of pro-inflammatory factors *ex vivo*. Cigarette smoking alters airway inflammation in asthma and changes the profile of cytokines secreted in induced sputum [22, 23], and so the effect of smoking on sputum IL-18 levels was also investigated.

Our results indicate that IL-18 can be detected in induced sputum samples in both asthmatic and normal subjects but that its levels are not significantly different between these groups. IL-18 expression was identified in sputum-derived macrophages. Cigarette smoking significantly reduced sputum IL-18 levels in both asthmatic and normal subjects. In asthmatic smokers, this was associated with a decrease in IL-18 mRNA expression. These data provide the first evidence that cigarette smoking alters airway IL-18 production in both asthmatic and normal subjects. This may have important implications for the control of the balance of Th1/Th2 cytokine production in asthmatic airways.

Materials and methods

Subjects

Thirty-five asthmatic non-smokers and 31 asthmatic smokers were studied. Twenty normal non-smokers and 20 normal smokers were studied as controls. Smokers aged over 55 years were excluded to prevent patients with possible co-existing chronic obstructive pulmonary disease (COPD) being included in the study. Asthma was defined according to the American Thoracic Society definition [24]. Asthmatic patients gave no history within the preceding 6 weeks of respiratory infection, antibiotic use or exacerbations of asthma. Each asthmatic group was subdivided into those using β_2 -agonist alone and those taking regular inhaled corticosteroid for at least 3 months and β_2 -agonist as required, but no other asthma drugs in addition. Normal subjects were asymptomatic and gave no history of asthma or other allergic disease. Atopy was assessed by raised total serum IgE level (>120 International Units/mL). Forty-nine percent (17/35) of non-smoking asthmatics and 65 percent (20/31) of smoking asthmatics were atopic. Of the normal subjects, 15 percent (3/20) of non-smokers and 20 percent (4/20) of smokers were atopic. The study was approved by the West Ethics Committee, West Glasgow Hospitals University NHS Trust. All volunteers gave informed consent.

Sputum induction

Sputum induction was performed following assessment of reversibility to salbutamol, using a modification of the method described by Pin et al. [25]. Briefly, following salbutamol inhalation, sputum induction was commenced using hypertonic (3%) saline administered via an ultrasonic nebulizer (Sonix 2000, Medix Ltd, Harlow, UK) over a period of 20 min. The subjects were encouraged to expectorate at any time throughout the procedure, and in addition inhalation was stopped every 5 min to allow expectoration, and to allow spirometry to be performed. The sample was collected in a sterile container and transferred to the laboratory on ice within 2 h.

Sputum cell preparation

All samples were processed blind. Sputum samples were transferred to a petri dish and the volume and macroscopic characteristics of the whole sample recorded. Sputum plugs were separated from contaminating saliva using sterile forceps. The plugs were placed in a pre-weighed tube and the weight recorded. The sputum was then mixed 2.5 w/v with freshly prepared 0.1% dithiothreitol solution made by mixing dithiothreitol powder (Sigma Chemical Co., Poole, UK) with Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Paisley, UK) plus 10% fetal calf serum. The sample was vortexed briefly and incubated at 37 °C for 15 min with constant agitation. The suspension was filtered through monofilament filter cloth (Cadish Precision Meshes Ltd, London, UK) to remove mucus and then centrifuged at 300 *g* for 10 min. The cell pellet was washed four times in DMEM before analysis. The supernatants were further centrifuged at 600 *g* for 10 min to remove debris and stored at -20 °C for future analysis.

Total cell counts were determined with a Neubauer haemocytometer (VWR International Ltd, Poole, UK) using trypan blue exclusion to determine cell viability; dead cells and epithelial cells were excluded. The cell suspension was then mixed with DMEM to obtain a count of 1.0×10^6 cells/mL and cell smear preparations were made using a cytocentrifuge at 270 *g* for 5 min (Cytospin-3, Shandon Inc., Pittsburgh, PA, USA). These cytospin slides were fixed using 50% alcohol/acetone (v/v) and then stained with May-Grunwald-Giemsa stain. Differential cell counts were performed by counting 300 cells and expressed as percentages. In general, there was less than 20% squamous epithelial cell contamination and the viability was greater than 85%. Samples that had total cell count less than 0.5×10^6 cells or viability lower than 70% were insufficient for analysis and were excluded.

Measurement of IL-18

The IL-18 concentration in sputum fluid was determined by ELISA using a kit purchased from Calbiochem (Bescon, France) and developed with TMB peroxidase substrate (Insight Biotechnology Ltd, Wembley, UK) according to the manufacturer's recommendations. The sensitivity of the assay was 5 pg/mL.

Quantitative PCR

Quantitative reverse-transcription PCR [26] was performed using the ABI Prism 7700 Sequence Detector System (PE Biosystems, Warrington, UK). Total RNA was extracted using the Nucleospin[®] RNA II system (Machery-Nagel, Düren, Germany). cDNA was then generated using the Superscript II system (Invitrogen, Paisley, UK). The primers used for IL-18 mRNA analysis were forward 5'-GGCCTCTATTGAA GATATGACTGATT-3', reverse 5'-CCTCTAGGCTGGC TATCTTTATACATAC-3' and probe 5'-TGACTGTAGA GATAATGCACCCCGGACC-3'. Primers were used at a concentration of 300 nM and probe at 200 nM. PCR reactions were performed in triplicate and data analysed using the Sequence Detector v 1.6.3 (PE Biosystems) and Excel (Microsoft, Redmond, WA, USA) software, using the 'Comparative C_T ' method. Results were expressed as fold difference of the reference gene HPRT. All reactions were

checked to be >90% efficient at the threshold value used. The mean of each triplicate was taken as the level of IL-18 mRNA expression in each sputum sample. Sufficient cDNA for analysis by quantitative PCR was obtained from 21 asthmatic non-smokers, 23 asthmatic smokers, 10 normal non-smokers and 12 normal smokers.

Immunocytochemistry of IL-18 expression

IL-18 localization on sputum cells cytopreparation smears was performed as previously described [27]. Freshly isolated sputum cells were cytocentrifuged at 270 *g* for 5 min (Cytospin-3, Shandon Inc.), fixed with ice-cold dried acetone (VWR International, Lutterworth, UK) and stored at -70°C until analysis. Slides were thawed and then washed at room temperature with phosphate-buffered saline (PBS). Endogenous peroxidase activity was abolished by pre-incubation with 0.5% hydrogen peroxidase in methanol before incubation with blocking buffer (20% v/v normal human serum and 20% v/v horse serum in PBS). IL-18 was localized using mouse anti-human IL-18 anti-serum (monoclonal antibody clone D4D1, Dr Andrew Jackson, Cancer Research UK, London, UK). Specificity of staining of this antibody for human IL-18 was demonstrated by pre-incubation with recombinant human IL-18 (produced in Department of Immunology, University of Glasgow) which neutralized binding, or the equivalent concentration of mouse IgG1 as control (DAKO, Ely, UK). A mouse anti-human CD68 monoclonal antibody (DAKO) was used as a positive control. Bound antibody was detected with biotinylated horse anti-mouse IgG, washed in PBS buffer, and incubated with an alkaline phosphatase complex (Vector Laboratories Ltd, Peterborough, UK) for 30 min. Immunoreactive cells were visualized by the addition of diaminobenzidine substrate (Vector Laboratories Ltd) and lightly counter-stained with

Harris haematoxylin (VWR International). All incubations were conducted at room temperature except for the addition of primary antibody which was incubated at 4°C overnight.

Statistical analysis

Results are expressed as median (interquartile range (IQR)) unless otherwise specified. Non-parametric statistical analysis was used to compare cell counts, IL-18 concentrations and IL-18 expression levels (Mann–Whitney *U*-test) using a software package (Minitab Statistical Software; Minitab Inc., State College, PA, USA). Significance was accepted at the 95% level.

Results

Patient details and pulmonary function

Table 1 presents the age, gender and spirometry (forced expiratory volume in 1 s (FEV₁) and percentage predicted FEV₁) in the different study groups. Smoking asthmatics had a significantly lower percentage predicted FEV₁ than non-smoking asthmatics ($P < 0.01$). However, smoking asthmatics had a higher percentage reversibility after bronchodilators ($P < 0.01$). The daily dose of inhaled steroid taken by smoking asthmatics was not significantly different from that of non-smoking asthmatics (median (IQR): smokers, 800 (300–1300) μg vs. non-smokers, 800 (800–1600) μg , beclomethasone dipropionate or equivalent). There was no difference in the ages of all the groups.

Serum total IgE levels

Serum total IgE levels (Table 1) were significantly elevated in asthmatics (154 (69–434) kU/L, $n = 66$) when compared with

Table 1. Subject characteristics

		Asthma patients		
	Controls	All	Non-steroid treated	Steroid-treated
<i>Non-smokers</i>				
<i>n</i>	20	35	17	18
Age (years)	33 (26–45)	38 (31–48)	34 (30–44)	44 (31–52)
Sex (M/F)	10/10	19/16	10/7	9/9
Baseline FEV ₁ (L)	3.8 (2.4–4.6)	3.0 (2.3–4.0)	3.4 (2.4–4.1)	2.9 (2.2–3.4)
Baseline FEV ₁ (%)	95 (83–110)	90 (80–101)	89 (8.1–101)	95 (44–76)
Reversibility (%)	–	7 (1–11)	8 (1–10)	6 (0–18)
Serum total IgE (kU/L)	15 (0–54)	125 (51–225)	134 (63–220)	117 (32–387)
<i>Smokers</i>				
<i>n</i>	20	31	12	19
Age (years)	42 (35–45)	38 (34–42)	34 (32–39)	40 (37–45)
Sex (M/F)	8/12	22/9	7/5	15/4
Smoking pack years	28 (15–36)	22 (18–30)	23 (18–30)	20 (18–30)
Baseline FEV ₁ (L)	2.8 (2.2–3.9)	2.7 (2.2–3.5)	2.7 (1.8–3.5)	2.7 (2.2–3.7)
Baseline FEV ₁ (%)	88 (76–102)	74 (63–89)**	73 (63–83)**	84 (60–92)
Reversibility (%)	–	17 (8–25)**	16 (7–25)*	19 (8–28)
Serum total IgE (kU/L)	59 (26–112)**	234 (78–499)	230 (116–431)	262 (68–772)

Age, sex, smoking history, spirometry and serum IgE levels for the subject groups. Data are presented as median (interquartile range). One pack year equals 20 cigarettes smoked per day for one year. M, male; F, female; FEV₁, forced expiratory volume in 1 s.

* $P < 0.05$; ** $P < 0.01$ compared to corresponding non-smoking group.

controls (35 (13–88) kU/L, $n = 40$, $P < 0.001$). In the normal control group, smokers had significantly higher levels of serum total IgE (smokers, 59 (26–112) kU/L vs. non-smokers, 15 (0–54) kU/L, $P < 0.01$), but in the asthma group, the smokers did not have significantly higher total IgE levels than the non-smokers.

Sputum cells

The total cell number and differential cell count in the induced sputum samples of asthma patients and controls are shown in Table 2. There was no difference in total cell counts between asthmatic and normal subjects in both the smoking and non-smoking groups. Asthmatic smokers had a raised percentage of neutrophils (51.3% (35.8–58.4%)) when compared with non-smoking asthmatics (33.2% (22–50.5%), $P < 0.01$) and also a reduction in sputum eosinophilia (smokers, 0% (0–0.7%) vs. non-smokers, 0.5% (0–5.9%); $P < 0.05$). Smokers had lower lymphocyte proportions in both asthmatic subjects (smokers, 0% (0–0.2%) vs. non-smokers, 1% (0–3%); $P < 0.001$) and normal controls (smokers, 0% (0–0.2%) vs. non-smokers, 0.6% (0–3.3%), $P < 0.01$).

IL-18 levels in induced sputum fluid

IL-18 levels were significantly reduced in cigarette smokers (smokers, 20 (0–102) pg/mL, $n = 51$, vs. non-smokers, 358 (50–876) pg/mL, $n = 55$, $P < 0.001$). This was more pronounced in asthmatics (smokers, 47 (40–64) pg/mL vs. non-smokers, 358 (50–876) pg/mL; $P < 0.001$) than in normal subjects (smokers, 25 (0–78) pg/mL vs. non-smokers, 247 (50–656) pg/mL; $P < 0.01$) (Fig. 1). Within each of the smoking and non-smoking groups there was no significant difference in IL-18 levels between asthmatic and normal subjects. In the asthmatic groups, there was no difference between IL-18 levels in those treated with β_2 -agonist alone or with β_2 -agonist and inhaled corticosteroid (non-smokers: β_2 -agonist, 156 (23–

1522) pg/mL vs. inhaled corticosteroid, 671 (118–1137) pg/mL, $P = 0.72$; smokers: β_2 -agonist, 60 (0–120) pg/mL vs. inhaled corticosteroid, 0 (0–96) pg/mL, $P = 0.61$).

There was no correlation between sputum IL-18 levels and any specific cell type in the sputum samples or with serum IgE levels in any of the groups.

IL-18 mRNA expression in induced sputum cells

There was a reduction in the expression of IL-18, expressed as a percentage of HPRT mRNA, in asthmatic smokers when compared with asthmatic non-smokers (smokers, 258% (163–317%) vs. non-smokers, 385% (221–554%), $P < 0.05$) (Fig. 2). There was no significant difference in IL-18 expression

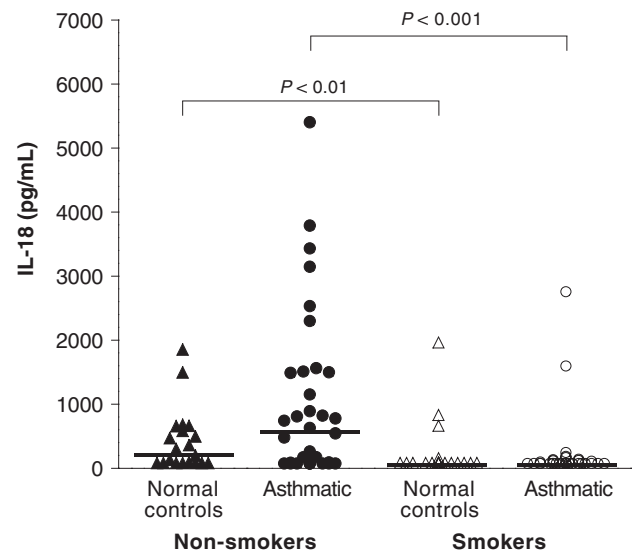


Fig. 1. IL-18 levels in the fluid phase of induced sputum of normal and asthmatic subjects, comparing non-smokers and smokers. Horizontal bars indicated the median value of each group.

Table 2. Sputum differential leucocyte profile

		Asthma patients		
	Controls	All	Non-steroid-treated	Steroid-treated
<i>Non-smokers</i>				
<i>n</i>	20	35	17	18
Total cell yield (× 10 ⁶)	2 (1.1–4.2)	2.7 (1–4.9)	2.6 (0.9–4.6)	3.1 (2–5)
Macrophages (%)	54.7 (26.3–69.6)	60.8 (38.7–69)	64 (45.3–74)	55 (18.2–65)
Neutrophils (%)	37 (26.4–60.4)	33.2 (22–50.5)	30.2 (17–36.3)	36 (26.2–60)
Lymphocytes (%)	0.6 (0–3.3)	1.0 (0–3)	0.8 (0–2)	2 (0–3.8)
Eosinophils (%)	0 (0–0.9)	0.5 (0–5.9)	0.5 (0–3.1)	1.2 (0–6.8)
<i>Smokers</i>				
<i>n</i>	20	31	12	19
Total cell yield (× 10 ⁶)	4 (2.5–4.7)	3.3 (1.9–4.8)	3.9 (1.9–7.1)	3.2 (1.9–4.8)
Macrophages (%)	53.6 (34.4–71.8)	47.2 (40.4–64)	48 (37.6–60)	47.2 (40.4–65.5)
Neutrophils (%)	46.2 (27.7–64.9)	51.3 (35.8–58.4)**	51.4 (40–50.8)**	51.3 (34.4–59.6)
Lymphocytes (%)	0 (0–0.2)*	0 (0–0.2)**	0 (0–0.2)**	0 (0–0.2)*
Eosinophils (%)	0 (0–0.5)	0 (0–0.7)*	0.1 (0–0.9)	0 (0–0.5)

Total and differential white cell profiles obtained from induced sputum samples from controls and asthmatic patients. Data are presented as median (interquartile range).

* $P < 0.05$; ** $P < 0.01$, when compared to the corresponding non-smoking group.

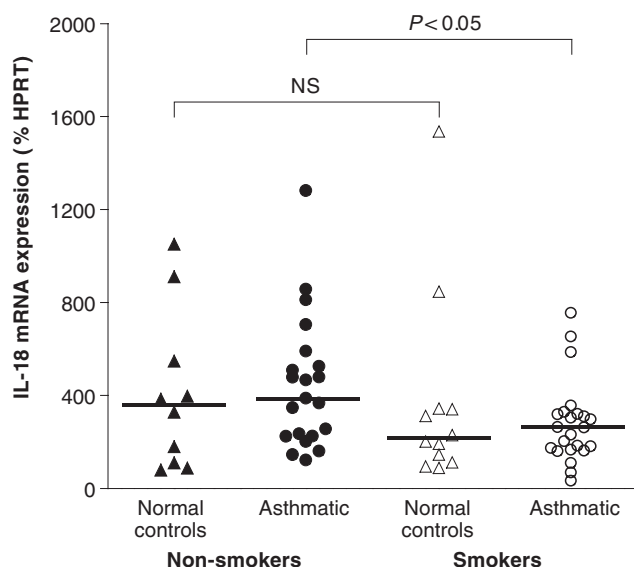


Fig. 2. IL-18 mRNA expression in the cell pellets of induced sputum samples from normal and asthmatic subjects, comparing non-smokers and smokers. Horizontal bars indicated the median value of each group. NS, not significant.

between normal subjects and asthmatics in both the smoking and non-smoking groups or between normal smokers and normal non-smokers (smokers, 214% (111–343%) vs. non-smokers, 356% (110–548)%, $P = 0.67$). Within each of the asthmatic therapy groups there was no significant difference in IL-18 mRNA levels.

IL-18 localization in sputum cells by immunocytochemistry

The cellular distribution of IL-18 synthesis was visualized in sputum cytospin preparations by immunocytochemistry (Fig. 3). Positive staining was observed in sputum macrophages. There was no positive staining for IL-18 in sputum neutrophils.

Discussion

Asthma is an inflammatory airways disease in which Th2 lymphocytes, and the cytokines they produce, have a key regulatory role [1–3]. In contrast Th1 cells, secreting IFN- γ , may attenuate the asthmatic response, as shown in experimental asthma [28]. However, there is also evidence from experimental models of asthma that Th1 cells may contribute to increased airway inflammation [29]. Therefore, the control of the extent of these two types of lymphocyte response and the balance between them is important in influencing the severity of airway inflammation.

IL-18 is a cytokine which has the ability to promote both Th1 and Th2 responses depending on the surrounding cytokine environment [6, 7]. There is increasing evidence that IL-18 may have a pro-inflammatory action in allergic disease. In murine models of asthma, IL-18 administered alone can promote allergen-induced eosinophil influx into the airways [19–21] and increase serum IgE levels [8]. In human studies, serum IL-18 levels were elevated in asthmatic patients during acute exacerbations [15], in stable asthmatics [14] and in those

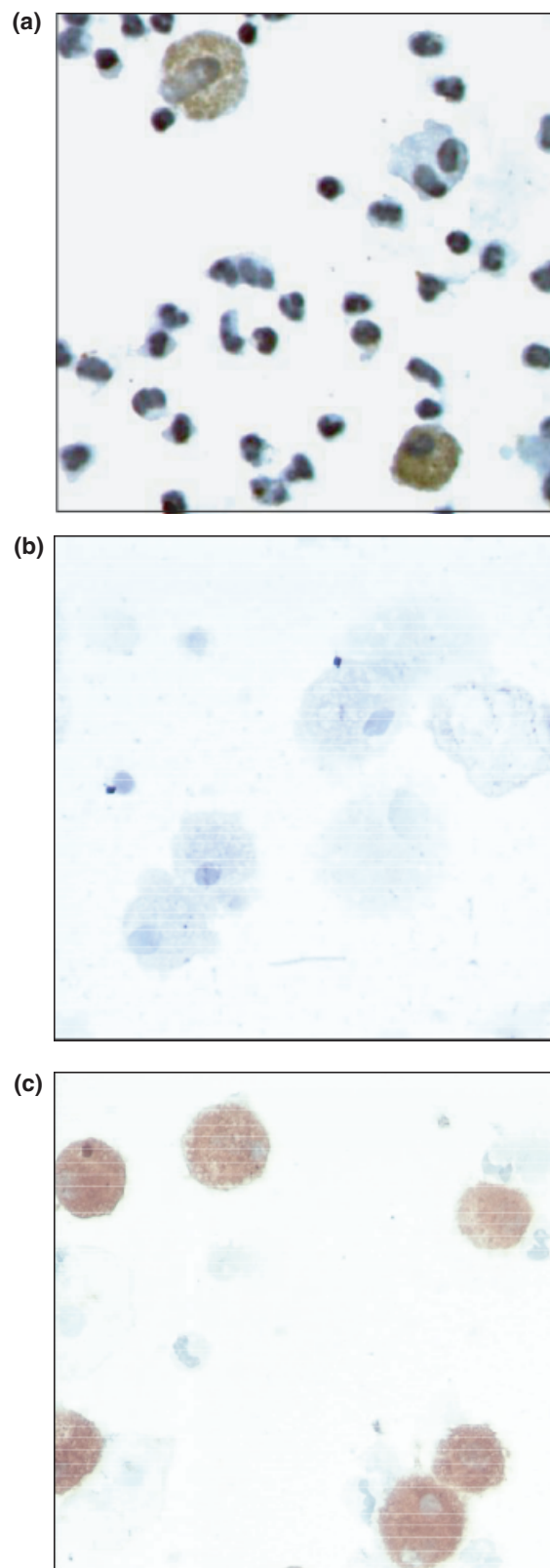


Fig. 3. Immunocytochemistry of IL-18 in sputum cells. Cells from an asthmatic subject were stained with anti-human IL-18 (a), normal murine IgG1 control (b) or anti-human CD68 (c). Positive staining was observed to be brown. Staining was observed only in sputum macrophages. Images are shown at magnification $\times 400$.

with a related allergic condition, atopic dermatitis [14, 30]. Also, IL-18 levels were found to be raised in nasal secretions from patients with allergic seasonal rhinitis [31]. Polymorphisms in the IL-18 gene have been associated with asthma [32]. In contrast, IL-18 had anti-allergic properties when administered with IL-12 in a murine asthma model [17], and IL-18-deficient mice had increased airway eosinophilia [18].

Here, we show the first evidence that IL-18 can be detected in induced sputum fluid. There was no significant difference in sputum IL-18 levels between asthmatic and normal subjects. However, in non-smokers, IL-18 levels tended to be higher in asthmatics than in normal subjects. Previous clinical studies have shown that IL-18 levels are reduced in bronchoalveolar lavage (BAL) fluid from asthmatics [16] and that IL-18 mRNA expression in the airway epithelium was decreased [12] compared with normal controls and patients with sarcoidosis. Since sputum induction provides a sample from the larger airways than BAL [33] and samples a different inflammatory compartment to bronchial biopsies [34], our result may reflect such differences.

As cigarette smoking alters airway inflammation in asthma [23], we investigated if smoking altered sputum IL-18 levels. The smoking asthmatics who participated in this study satisfied the criteria for the diagnosis of asthma [24], had a median age of 38 years and been symptomatic since their 20s or earlier, and so were felt to be distinct from patients with COPD. Cigarette smoking reduced IL-18 levels in both asthmatic and normal subjects. This was more pronounced in asthmatic smokers and was associated with a decrease in IL-18 mRNA expression. The reason for this is unclear. Smoking is known to suppress the ability of alveolar macrophages to secrete pro-inflammatory cytokines, such as TNF- α , in response to inflammatory stimuli [35] and has also been shown to reduce the number of cells spontaneously secreting Th1 cytokines in the airways [36]. A similar suppressive mechanism may be acting on sputum cell IL-18 production. Also, IL-18 is a cytokine highly dependent on proteolytic processing for its activity and extracellular secretion. It is produced initially as an inactive precursor, pro-IL-18, which is cleaved by IL-1 β converting enzyme (ICE or caspase-1) into the active protein [37]. Other proteases, such as neutrophil proteinase-3 [38], can also be involved in the production of active IL-18 whereas caspase-3 [39] can cleave active IL-18 into inactive fragments. Cigarette smoking promotes neutrophil influx into the airways [23] and increases protease activity in the airways [40]. This may contribute to the degradation of sputum IL-18 in smokers, especially in asthmatics.

IL-18 mRNA is highly expressed in cells from induced sputum samples. IL-18 mRNA levels are significantly lower in asthmatic smokers than in asthmatic non-smokers, and a reduction in IL-18 gene expression may therefore contribute to suppression of IL-18 production in smokers. The control of IL-18 gene expression is less well understood. IL-18 mRNA is highly stable and this may explain the high levels detected in sputum cells. The gene is constitutively expressed in human peripheral blood mononuclear cells [41] but its mRNA expression can be up-regulated by cytokines such as IFN- γ [42]. Cigarette smoke may contain compounds with the ability to alter IL-18 gene expression. As there was no significant change in IL-18 mRNA expression in normal smokers it is likely that there is some alteration of both IL-18

mRNA expression and protein processing causing the decrease in sputum IL-18 levels observed.

There was no significant difference in sputum IL-18 levels or sputum IL-18 mRNA expression between asthmatics treated with inhaled β_2 -agonist alone and those treated with inhaled β_2 -agonist and inhaled corticosteroid in either the smoking or non-smoking groups. Steroid treatment is known to affect the levels of several airway cytokines, including other macrophage-derived cytokines, such as IL-12 [43] and IL-15 [44]. Since our study was a cross-sectional study, further longitudinal studies would be necessary to assess fully if steroid therapy could alter IL-18 production.

Immunocytochemistry localized IL-18 production to sputum macrophages. In association with the IL-18 levels found in induced sputum fluid and the high levels of IL-18 mRNA expression in sputum cells, this suggests that IL-18 is produced directly by the cells in the sputum samples. However, other cells such as the bronchial epithelium, may also contribute to the IL-18 detected by ELISA in induced sputum fluid.

In conclusion, IL-18 can be detected in induced sputum fluid and IL-18 gene expression is also detectable in cells from induced sputum samples. There is no significant difference between sputum IL-18 levels in asthmatic and normal subjects but cigarette smoking significantly reduces levels of IL-18 in both these groups. This reduction may be mediated by alterations in the post-translational processing of IL-18 but direct suppression of IL-18 gene expression could also be involved. IL-18 is a pro-inflammatory cytokine which has the ability to induce Th1 and Th2 cytokine secretion, and as such, may have a pivotal role in the development of airway inflammation in asthma. The reduction in sputum IL-18 levels associated with cigarette smoking may alter the balance of Th1/Th2 cytokines secretion and contribute to increased airway inflammation. Further studies are necessary to clarify the mechanisms by which sputum IL-18 levels are reduced by cigarette smoking and to determine the precise effect of this reduction on Th1/Th2 cytokine secretion.

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