

Ozone-induced bronchial epithelial cytokine expression differs between healthy and asthmatic subjects

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Summary

Background Ozone (O₃) is a common air pollutant associated with adverse health effects. Asthmatics have been suggested to be a particularly sensitive group.

Objective This study evaluated whether bronchial epithelial cytokine expression would differ between healthy and allergic asthmatics after ozone exposure, representing an explanatory model for differences in susceptibility.

Methods Healthy and mild allergic asthmatic subjects (using only inhaled β_2 -agonists prn) were exposed for 2 h in blinded and randomized sequence to 0.2 ppm of O₃ and filtered air. Bronchoscopy with bronchial mucosal biopsies was performed 6 h after exposure. Biopsies were embedded in GMA and stained with mAbs for epithelial expression of IL-4, IL-5, IL-6, IL-8, IL-10, TNF- α , GRO- α , granulocyte-macrophage colony-stimulating factor (GM-CSF), fractalkine and ENA-78.

Results When comparing the two groups at baseline, the asthmatic subjects showed a significantly higher expression of IL-4 and IL-5. After O₃ exposure the epithelial expression of IL-5, GM-CSF, ENA-78 and IL-8 increased significantly in asthmatics, as compared to healthy subjects.

Conclusion The present study confirms a difference in epithelial cytokine expression between mild atopic asthmatics and healthy controls, as well as a differential epithelial cytokine response to O₃. This O₃-induced upregulation of T helper type 2 (Th2)-related cytokines and neutrophil chemoattractants shown in the asthmatic group may contribute to a subsequent worsening of the airway inflammation, and help to explain their differential sensitivity to O₃ pollution episodes.

Keywords air pollution, asthmatics, bronchial epithelium, cytokines, ozone

Submitted 8 July 2002; revised 13 February 2003; accepted 27 February 2003

Introduction

Ozone (O₃) is a common summertime air pollutant associated with adverse health effects. Epidemiological studies have suggested that asthmatics are a particularly sensitive group as illustrated by an increase in the number of hospitalizations and emergency room visits in asthmatic individuals during O₃ pollution episodes [1].

O₃ is known to elicit immediate impairment of lung function and an evolving pattern of airway inflammation. Human exposure chamber studies in healthy individuals have demonstrated O₃-induced inflammation with airway neutrophilia which increases up to 6 h and persists for 18–24 h post-exposure [2–4]. A range of inflammatory mediators and cytokines such as IL-6, IL-8, granulocyte macrophage colony stimulating factor (GM-CSF) and growth-related oncogene- α (GRO- α) have been shown to be elevated in airway lavage following O₃ [3–5].

Bayram and collaborators have recently demonstrated that exposure of human bronchial epithelial cells of asthmatic subjects exposed to 50–100 ppb of O₃ significantly increased the release of IL-8, GM-CSF, RANTES and sICAM-1 (intercellular adhesion molecule-1) from these cells after 24 h of incubation, whereas only IL-8 and sICAM were increased in non-asthmatics [6].

Certain studies using high-dose O₃ exposures have demonstrated that asthmatics exhibit a greater airway inflammatory response than healthy, non-atopic individuals [7–9]. This was not, however, observed in our recent study in which the airway inflammatory effects of 0.2 ppm of O₃ were studied in both healthy subjects and a group of subjects with mild atopic asthma [10]. Apart from the presence of allergic airway inflammation in the asthmatic subjects at baseline, both groups showed a neutrophilic response in airway lavage, without any discernible changes in eosinophil or lymphocyte numbers. In the healthy group, O₃ also induced upregulation of the vascular endothelial adhesion molecules P-selectin and ICAM-1 together with increases in tissue neutrophil and mast cell numbers, responses that were not seen in the asthmatic group. In contrast to the earlier studies, this investigation employed a lower O₃

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concentration, a shorter exposure time or lower minute ventilation. As a consequence, the total O₃ dose employed was more environmentally relevant and presumably accounts for the lack of difference between control and asthmatic subjects.

O₃ is a potent oxidant and reacts with molecules in the epithelial lining fluid. This causes the formation of secondary oxidation products, which are believed to be the source of the toxic signals sent to the pulmonary epithelium [11, 12]. The key target for the O₃ induced inflammatory response may thus be the pulmonary epithelium as, aside from being a physical barrier to inhaled particles, it also contributes to the local immune system. There is abundant experimental evidence suggesting that the epithelial cells influence airway inflammation through cytokine release [13]. In order to explore this, we have evaluated whether healthy and asthmatic subjects differ in their bronchial epithelial cytokine expressions after O₃ exposure, thereby providing an additional explanation for the increased susceptibility and response to oxidative air pollution of asthmatics.

Previous studies have often focused on a late time point post-exposure, usually 18 h, in addition to using a higher dosage of O₃ exposure. Because a neutrophilic inflammatory response was observed in the bronchial mucosa of healthy subjects at 6 h, but not in asthmatics, in our previous study, the principal hypothesis was that there might be an increased production of neutrophil chemokines such as IL-8, GRO- α and ENA-78 in the asthmatics at this time point, thus indicating a delayed inflammatory mucosal response within the asthmatic group. An alternative hypothesis to explain the epidemiological observation of increased sensitivity to O₃ among the asthmatic population, could be an effect of O₃ on the expression of T helper type 2 (Th2)-associated cytokines (IL-4, IL-5 and GM-CSF). As T lymphocytes are central cells in the pathogenesis of asthma, fractalkine was also stained as an important chemoattractant for T cells.

Methods

Subjects

Fifteen healthy, non-atopic subjects (six males, nine females; mean age 24 years, range 19–31 years) and 15 asthmatic subjects (nine males, six females; mean age 29 years, range 21–48 years) with intermittent to mild persistent disease according to GINA guidelines [14] were recruited. The asthmatic subjects had normal lung function with a mean forced expiratory volume of 1 s (FEV₁) of 90% of predicted (range 75–114%) and exhibited increased bronchial hyper-responsiveness to methacholine (geometric mean PC₂₀ 2.3 mg/mL). In addition, they had a positive skin prick test against at least one common allergen. Aside from inhaled β -agonists on demand, they did not require any additional antiasthma therapy. None of the subjects involved in the study had ever smoked and they were all free of airway infection for at least 6 weeks prior to the first exposure and throughout the study. No anti-inflammatory drugs or antioxidant supplements were allowed 2 weeks before or during the study.

Study design and exposures

All volunteers were exposed to filtered air and air containing 0.2 ppm of O₃ for 2 h on two separate occasions in an exposure chamber located at the National Institute for Working Life in

Umeå according to a previously described standard protocol [15]. The O₃ concentration of 0.2 ppm was chosen based on previous experimental studies and because it is a relevant O₃ level in urban areas during summertime [15]. The order of the two exposures was randomized and there was a minimum of 3 weeks between exposures. During exposures, the subjects alternated moderate exercise on a bicycle ergometer ($V_E = 20$ L/min/m² body surface) and rest at 15-min intervals. The O₃ concentration remained steady at 0.20 ± 0.01 ppm (mean $\pm$ SEM). The study was conducted outside pollen season. Levels of ambient O₃ and NO₂ were low in the Umeå area and thus not expected to affect the results. All subjects gave their written informed consent and Umeå University Ethics Committee approved the study.

Bronchoscopies

Six hours after the end of each exposure, bronchoscopy under local anaesthesia was performed to obtain endobronchial mucosal biopsies using a standardized procedure previously described [15]. Three or four endobronchial mucosal biopsies were obtained at each bronchoscopy. Preceding the bronchoscopy, the asthmatic subjects inhaled 0.2 mg of salbutamol dry powder.

Processing of samples

Endobronchial mucosal biopsies obtained during bronchoscopy were placed in ice-cooled acetone containing protease inhibitors and were processed in glycolmethacrylate (GMA) resin for immunohistological staining as previously outlined [16].

Immunohistochemistry

Cell markers and adhesion molecule expression were enumerated and have been reported elsewhere [10]. In order to evaluate epithelial cytokine expression, further analysis was performed on the same biopsies. From the original 30 subjects, one had to be omitted due to insufficient biopsy tissue, leaving paired biopsy specimens from 14 healthy and 15 asthmatic subjects. The biopsies were stained using monoclonal antibodies for a selection of cytokines and the streptavidin biotin-peroxidase detection system according to standardized protocol [17] (Table 1).

Table 1. Name and sources of monoclonal antibodies used for immunostaining of epithelial cytokines and chemokines

Name	Catalogue number	Clone	Source
GRO- α	MAB 275	20326.1	R & D Systems, Abingdon, UK
ENA-78	MAB 254	—	R & D Systems, Abingdon, UK
IL-8	BMS 136	NAP II	Boeringer Ingelheim, Heidelberg, Germany
IL-4	211-41396	4D9	Nordic Biosite, Stockholm, Sweden
IL-5	MAB 205	14611.3	R & D Systems, Abingdon, UK
GM-CSF	MAB 215	3209.1	R & D Systems, Abingdon, UK
TNF- α	MAB 610	28401.111	R & D Systems, Abingdon, UK
IL-6	MAB 206	6708.111	R & D Systems, Abingdon, UK
IL-10	MAB 217	23738.111	R & D Systems, Abingdon, UK
Fractalkine	MAB 365	51637.11	R & D Systems, Abingdon, UK

Quantification of immunostaining

The cytokine expression in the bronchial epithelium was determined using 3,3-diaminobenzidine tetrahydrochloride (DAB) staining techniques according to a previously described protocol [18]. Readings were done using a computerized image analyser system (Leica Microsystems Q500IW Image System, Leica, Cambridge, UK). The epithelial expression of each cytokine was expressed as the percentage of epithelial area with positive staining as compared with the total epithelial area. Only areas with intact epithelium with present cilia and basal membrane were included in the analysis. The complete set of readings was conducted by one investigator blinded to the exposure conditions and subject groups.

Statistical analyses

All statistical analyses were executed using SPSS® version 10.0 for Windows (SPSS Inc., Chicago, IL, USA). To evaluate paired observations within the same individual, Wilcoxon's non-parametric-signed test was used. The Mann-Whitney *U*-test was applied to assess between-group comparisons. A *P*-value < 0.05 was considered significant.

Results

Immunohistological staining for cytokines in the bronchial epithelium revealed several significant baseline differences between the two groups, as well as changes owing to O₃ exposure (Tables 2 and 3). The analysed epithelial area was 80 500 (46 000–126 000), µm² (median and IQR).

Table 2. Epithelial cytokine expression in bronchial biopsies

	Air	*	O ₃	†	‡
GRO-α					
Healthy	2.6 (1.8–3.7)	0.006	2.1 (1.2–3.5)	NS	0.011
Asthmatics	1.7 (1.5–2.2)		2.1 (1.3–4.3)	NS	
ENA-78					
Healthy	2.5 (1.8–3.0)	0.006	1.8 (1.3–2.5)	NS	0.045
Asthmatics	1.9 (1.2–2.1)		2.7 (1.5–3.6)	0.046	
IL-8					
Healthy	3.0 (1.9–3.5)	0.003	2.2 (1.9–2.7)	NS	0.004
Asthmatics	1.4 (0.7–2.5)		2.5 (1.2–3.5)	0.06	
IL-4					
Healthy	0.06 (0.03–0.16)	0.02	0.18 (0.09–0.47)	NS	NS
Asthmatics	0.3 (0.05–0.76)		0.51 (0.09–0.94)	NS	
IL-5					
Healthy	1.8 (1.4–2.2)	0.02	1.7 (1.3–2.0)	NS	0.025
Asthmatics	2.3 (2.0–3.2)		3.5 (2.3–8.6)	0.03	
GM-CSF					
Healthy	1.6 (1.3–1.8)	NS	1.3 (1.2–1.5)	NS	0.003
Asthmatics	1.7 (0.8–2.5)		2.3 (1.8–3.5)	0.03	

Values are given as percentage (%) staining of total epithelial area. All values are expressed as median (IQR). *Baseline (air) differences between groups (Mann-Whitney *U*-test). †O₃-induced effects within group (Wilcoxon's rank sum test). ‡O₃-induced effects, differences between groups (O₃-air within the healthy group vs. O₃-air within the asthmatic group; Mann-Whitney *U*-test).

Baseline results

In the biopsies obtained after air, the asthmatic subjects showed significantly higher expression of the Th2-related cytokines IL-4 and IL-5, compared to the healthy group (*P* = 0.02 and *P* = 0.02, respectively). In contrast, expression of the neutrophil chemoattractants GRO-α, ENA-78 and IL-8 was lower in the asthmatic group (*P* = 0.006, *P* = 0.006 and *P* = 0.003, respectively) as was the expression of TNF-α and IL-10 (*P* = 0.02 and *P* = 0.001, respectively). No significant baseline differences were found in GM-CSF, IL-6 or fractalkine.

O₃ effects

In the asthmatic group, exposure to O₃ increased the expression of IL-5 (*P* = 0.03) and GM-CSF (*P* = 0.03). An increase was also noted in the expression of the neutrophil chemoattractant ENA-78 (*P* = 0.046) and a trend towards an increase in IL-8 (*P* = 0.06; Fig. 1). Except for a small decrease in the expression of IL-10, no significant changes were detected in cytokine expression in the healthy group. No O₃-induced effects were detected in IL-4, TNF-α, IL-6 or fractalkine in either group.

To further examine these differences, the O₃-induced responses were compared between the two groups (O₃-air within the healthy group vs. O₃-air within the asthmatic group). The increase in the neutrophil chemoattractants GRO-α, ENA-78 and IL-8 in the asthmatic group was significantly different compared to the non-significant decrease observed in the healthy group (*P* = 0.011, *P* = 0.045 and *P* = 0.004, respectively). In similarity, the asthmatic group showed a significantly higher response for IL-5 and GM-CSF (*P* = 0.03 and *P* = 0.003, respectively).

Discussion

This study shows a pronounced difference in the baseline epithelial cytokine pattern between healthy and mild atopic asthmatic subjects as well as a striking difference in epithelial

Table 3. Epithelial cytokine expression in bronchial biopsies

	Air	*	O ₃	†	‡
TNF-α					
Healthy	2.3 (2.0–3.1)	0.02	2.7 (1.7–3.5)	NS	NS
Asthmatics	1.8 (1.3–2.3)		2.5 (0.9–3.6)	NS	
IL-6					
Healthy	2.1 (1.5–2.9)	NS	2.0 (1.8–2.5)	NS	NS
Asthmatics	1.6 (1.0–2.4)		1.4 (0.8–1.9)	NS	
IL-10					
Healthy	2.7 (1.9–3.1)	0.001	2.0 (1.6–2.6)	0.06	NS
Asthmatics	1.1 (0.7–2.0)		1.0 (0.6–2.0)	NS	
Fractalkine					
Healthy	1.3 (0.6–1.6)	NS	1.3 (0.7–1.9)	NS	NS
Asthmatics	1.5 (0.8–8.3)		0.6 (0.1–1.5)	NS	

Values are given as percentage (%) staining of total epithelial area. All values are expressed as median (IQR). *Baseline (air) differences between groups (Mann-Whitney *U*-test). †O₃-induced effects within group (Wilcoxon's rank sum test). ‡O₃-induced effects, differences between groups (O₃-air within the healthy group vs. O₃-air within the asthmatic group; Mann-Whitney *U*-test).

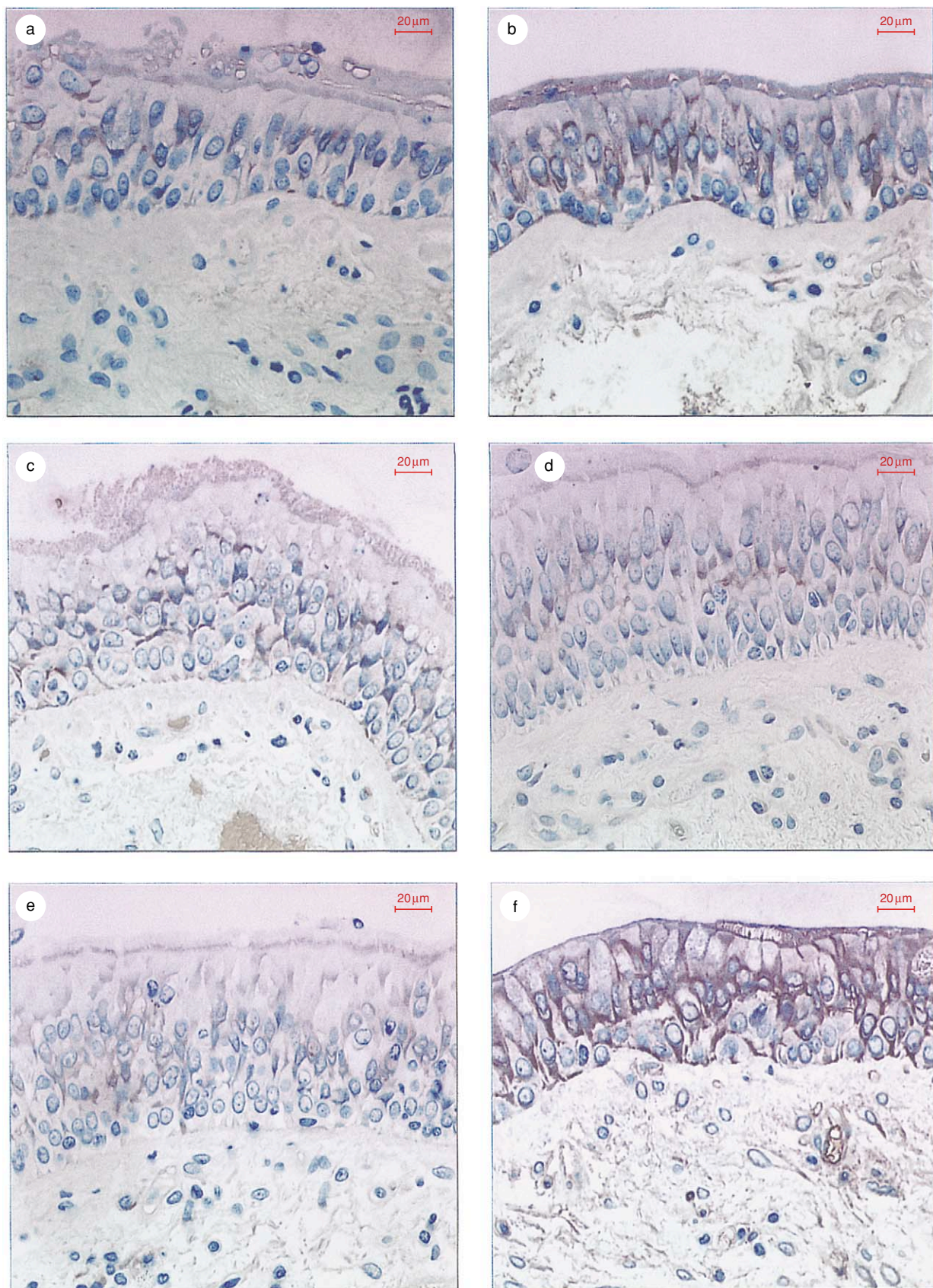


Fig. 1. Immunohistological staining of cytokine expression of ENA-78 (a and b), IL-5 (c and d) and GM-CSF (e and f) in airway epithelium of asthmatic subjects after air and O₃, respectively.

cytokine response to O₃ exposure. The asthmatic group displayed a higher degree of expression of Th2-related cytokines at baseline and a greater O₃-induced increase in IL-5 and GM-CSF compared to the healthy group. Furthermore, neutrophil chemoattractants were increased in the asthmatics, but not in healthy control subjects, after O₃ exposure. It is therefore suggested that the bronchial epithelium contributes to the airway inflammatory response to O₃ exposure.

Airway epithelial cells are positioned at the interface with the external environment and are therefore, together with alveolar macrophages, the first cells exposed to inhaled irritants, allergens and air pollutants. It is now well established that the airway epithelium not only acts as a physical barrier but is also a metabolically active physiochemical layer, capable of synthesizing and releasing a large number of inflammatory mediators and of expressing a range of cytokines, such as IL-5, IL-6, IL-8, IL-10, TNF- α , GRO- α and GM-CSF [19–23]. All of these cytokines could contribute to the development of an airway inflammatory response to irritants, including O₃.

At baseline, the asthmatic group showed a greater degree of expression of IL-4 and IL-5 in the bronchial epithelium compared to the healthy group. Asthma is associated with a Th2-related airway response, and IL-4 and IL-5 are both important Th2-related cytokines. IL-4 acts on B cells to stimulate production of antigen-specific IgE, whereas IL-5 promotes the recruitment, differentiation, activation and survival of airway eosinophils, cells that are related to a chronic asthmatic response [23]. IL-4 also has the ability to induce production of the Th2-related cytokines IL-4 and IL-5 from CD4⁺ T lymphocytes and to inhibit production of Th1-cytokines such as IFN- γ [24, 25]. The O₃-induced increase in IL-5, and also in GM-CSF, which affects the growth, activation and survival of eosinophils, may indicate an effect of O₃ on the Th2-related airway response and on airway eosinophilia. Increased numbers of eosinophils have earlier been detected in airway lavage 18 h after exposure to 0.16 ppm of O₃ [26]. The O₃-induced Th2-related cytokine responses that were found within the asthmatic group may predict a subsequent worsening of their asthmatic airway inflammation and thus suggest a link to the epidemiological data indicating O₃-associated increases in bronchial reactivity and hospital admissions.

ENA-78 and IL-8 are C-X-C chemokines, with a number of biological effects, predominantly on neutrophils. The major role of these chemokines is to attract and activate neutrophils. Given that asthmatics often have airways neutrophilia, it is noteworthy that expression of GRO- α , ENA-78 and IL-8 was lower at baseline in the asthmatic group compared with the healthy subjects. O₃ exposure, however, increased the expression of these cytokines in the asthmatic group, whereas no significant change was noted within the healthy group. When comparing the O₃-induced effects between the two groups, there was evidence of a dissociated response with a pronounced neutrophilic mucosal inflammation in the healthy group at this time point, but not in the asthmatics, despite the increased presence of neutrophil chemoattractants in the latter group [10]. In the asthmatics, it may be assumed that a neutrophilic infiltration would have occurred during the hours after the endobronchial mucosal biopsy sampling took place. Consequently, a difference in time-kinetics of the O₃-induced airway inflammation between the two groups is suggested, with the asthmatic subjects responding more slowly than the healthy controls. The reason

behind this delayed response in the asthmatics is not clear but may be due to endogenous anti-inflammatory or immunomodulatory mechanisms that may operate in the inflamed asthmatic airway and that can dampen or delay the acute inflammatory response to the O₃ challenge. Another possible explanation could be that the epithelial cells are 'exhausted' due to the ongoing chronic inflammation within the asthmatic airway mucosa and thus respond more slowly to an exogenous irritant such as O₃.

IL-10 is an immunoregulatory cytokine with a number of properties relevant to airway inflammation and bronchial reactivity. Reduced levels of IL-10 have been detected in sputum of subjects with asthma when compared with healthy controls [27]. Here, as in another recent investigation, expression of IL-10 at baseline was significantly lower in the asthmatic group than in the healthy controls [28]. In contrast to our recent study in which exposure to diesel exhaust significantly increased the epithelial expression of IL-10 in mild asthmatics, no such effects were detected after O₃. In healthy subjects, a trend towards a decrease was found after O₃, but the O₃-induced responses did not differ significantly between the two groups.

Fractalkine has been proposed as a potent chemoattractant for monocytes and T lymphocytes, and may play an important role in inflammatory responses by recruiting mononuclear leucocytes to the bronchial epithelium [29]. Mast cells in the skin have been found to migrate, but not to degranulate, in response to fractalkine [30]. The response of airways mast cells remains to be elucidated. In the present study, fractalkine was expressed in both groups with no baseline differences and with no O₃-induced responses in either group. Previously, mast cells were found to increase in the bronchial mucosa of healthy subjects after O₃ exposure, whereas a decrease in epithelial mast cells was detected in mild asthmatics [10, 15]. The decrease of epithelial mast cells in the asthmatic group may have been due to degranulation, but the findings in healthy subjects are more difficult to interpret. Nevertheless, fractalkine does not seem to be of great importance in the airway response to O₃.

Increased levels of IL-6, but not of TNF- α , have previously been detected in airway lavage following exposure to O₃ in healthy and asthmatic subjects [7, 31, 32]. In contrast, bronchial epithelial cells have been shown to release both IL-6 [33] and TNF- α [34] after O₃ exposure *in vitro*. Except for a small difference in the baseline levels of TNF- α , no O₃-induced effects were detected in the epithelial expression of the proinflammatory cytokines TNF- α and IL-6 in the present study.

In conclusion, the present study demonstrates a baseline difference in epithelial cytokine pattern between healthy and mild atopic asthmatic subjects, as well as a differential epithelial cytokine response to O₃. We imply that the airway epithelium plays a key role in driving the airway inflammatory response to O₃ exposure. O₃-induced upregulation of Th2-related cytokines and neutrophil chemoattractants in asthmatics may result in a subsequent worsening of the airway inflammation, and hence go some way to explain the differential sensitivity of asthmatics to O₃.

Acknowledgements

The authors wish to thank Ann-Britt Lundström, Lena Skedebant, Maj-Cari Ledin, Gerd Lindén and Helen Bertilsson for

their expert technical assistance. This study was supported by Swedish Asthma and Allergy Association and The Swedish Heart and Lung Foundation.

References

- Bascom R, Bromberg PA, Costa DA et al. Health effects of outdoor air pollution. *Am J Respir Crit Care Med* 1996; 153:3–50.
- Schelegle ES, Siefkin AD, McDonald RJ. Time course of ozone-induced neutrophilia in normal humans. *Am Rev Respir Dis* 1991; 143:1353–8.
- Koren HS, Devlin RB, Graham DE et al. Ozone-induced inflammation in the lower airways of human subjects. *Am Rev Respir Dis* 1989; 139:407–15.
- Devlin RB, McDonnell WF, Mann R et al. Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. *Am J Respir Cell Mol Biol* 1991; 4:72–81.
- Aris RM, Christian D, Hearne PQ, Kerr K, Finkbeiner WE, Balmes JR. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. *Am Rev Respir Dis* 1993; 148:1363–72.
- Bayram H, Sapsford RJ, Abdelaziz MM, Khair OA. Effect of ozone and nitrogen dioxide on the release of proinflammatory mediators from bronchial epithelial cells of non-atopic non-asthmatic subjects and atopic asthmatic patients *in vitro*. *J Allergy Clin Immunol* 2001; 107:287–94.
- Basha MA, Gross KB, Gwizdala CJ, Haidar AH, Popovich J Jr. Bronchoalveolar lavage neutrophilia in asthmatic and healthy volunteers after controlled exposure to ozone and filtered purified air. *Chest* 1994; 106:1757–65.
- Scannell C, Chen L, Aris RM et al. Greater ozone-induced inflammatory responses in subjects with asthma. *Am J Respir Crit Care Med* 1996; 154:24–9.
- Balmes JR, Aris RM, Chen LL et al. Effects of ozone on normal and potentially sensitive human subjects. Part I. Airway inflammation and responsiveness to ozone in normal and asthmatic subjects. *Res Rep Health Eff Inst* 1997; 78:1–37.
- Stenfors N, Pourazar J, Blomberg A et al. Effect of ozone on bronchial mucosal inflammation in asthmatic and healthy subjects. *Respir Med* 2002; 96:352–8.
- Kelly FJ, Mudway I, Krishna MT, Holgate ST. The free radical basis of air pollution: focus on ozone. *Respir Med* 1995; 89:647–56.
- Mudway IS, Kelly FJ. Ozone and the lung: a sensitive issue. *Mol Aspects Med* 2000; 21:1–48.
- Thompson AB, Robbins RA, Romberger DJ et al. Immunological functions of the pulmonary epithelium. *Eur Respir J* 1995; 8:127–49.
- National Institute of Health. Global Strategy for Asthma Management and Prevention. Geneva, NHLBI/WHO, 1995.
- Blomberg A, Mudway IS, Nordenhäll C et al. Ozone-induced lung function decrements do not correlate with early airway inflammatory or antioxidant responses. *Eur Respir J* 1999; 13:1418–28.
- Blomberg A, Krishna MT, Bocchino V et al. The inflammatory effects of 2 ppm NO₂ on the airways of healthy subjects. *Am J Respir Crit Care Med* 1997; 156:418–24.
- Britten KM, Howarth PH, Roche WR. Immunohistochemistry on resin sections: a comparison of resin embedding techniques for small mucosal biopsies. *Biotech Histochem* 1993; 68:271–80.
- Wilson SJ, Leone BA, Anderson D, Manning A, Holgate ST. Immunohistochemical analysis of the activation of NF-kappaB and expression of associated cytokines and adhesion molecules in human models of allergic inflammation. *J Pathol* 1999; 189:265–72.
- Stadnyk AW. Cytokine production by epithelial cells. *FASEB J* 1994; 8:1041–7.
- Levine SJ. Bronchial epithelial cell–cytokine interactions in airway inflammation. *J Invest Med* 1995; 43:241–9.
- Bonfield TL, Konstan MW, Burfeind P, Panuska JR, Hilliard JB, Berger M. Normal bronchial epithelial cells constitutively produce the anti-inflammatory cytokine interleukin-10, which is downregulated in cystic fibrosis. *Am J Respir Cell Mol Biol* 1995; 13:257–61.
- Calderon MA, Devalia JL, Prior AJ, Sapsford RJ, Davies RJ. A comparison of cytokine release from epithelial cells cultured from nasal biopsy specimens of atopic patients with and without rhinitis and nonatopic subjects without rhinitis. *J Allergy Clin Immunol* 1997; 99:65–76.
- Salvi S, Semper A, Blomberg A et al. Interleukin-5 production by human airway epithelial cells. *Am J Respir Cell Mol Biol* 1999; 20:984–91.
- Maggi E, Parronchi P, Manetti R et al. Reciprocal regulatory effects of IFN- γ and IL-4 on the *in vitro* development of human Th1 and Th2 clones. *J Immunol* 1992; 148:2142–7.
- Parronchi P, De Carli M, Manetti R et al. IL-4 and IFN (alpha and gamma) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones. *J Immunol* 1992; 149:2977–83.
- Peden DB, Boehlecke B, Horstman D, Devlin R. Prolonged acute exposure to 0.16 ppm ozone induces eosinophilic airway inflammation in asthmatic subjects with allergies. *J Allergy Clin Immunol* 1997; 100:802–8.
- Takanashi S, Hasegawa Y, Kanehira Y et al. Interleukin-10 level in sputum is reduced in bronchial asthma, COPD and in smokers. *Eur Respir J* 1999; 14:309–14.
- Holgate ST, Sandström T, Frew AJ et al. The health effects of acute exposure to diesel exhaust and concentrated ambient particles. Part 1: Exposure of normal and asthmatic subjects to fresh diesel exhaust. *Res Rep Health Eff Inst*, 2002; 112:1–30.
- Bazan JF, Bacon KB, Hardiman G et al. A new class of membrane-bound chemokine with a CX3C motif. *Nature* 1997; 385:640–4.
- Papadopoulos EJ, Fitzhugh DJ, Tkaczyk C et al. Mast cells migrate, but do not degranulate, in response to fractalkine, a membrane-bound chemokine expressed constitutively in diverse cells of the skin. *Eur J Immunol* 2000; 30:2355–61.
- Devlin RB, McDonnell WF, Becker S et al. Time-dependent changes of inflammatory mediators in the lungs of humans exposed to 0.4 ppm ozone for 2 h: a comparison of mediators found in bronchoalveolar lavage fluid 1 and 18 h after exposure. *Toxicol Appl Pharmacol* 1996; 138:176–85.
- Torres A, Utell MJ, Morow PE et al. Airway inflammation in smokers and nonsmokers with varying responsiveness to ozone. *Am J Respir Crit Care Med* 1997; 156:728–36.
- Devlin RB, McKinnon KP, Noah T, Becker S, Koren HS. Ozone-induced release of cytokines and fibronectin by alveolar macrophages and airway epithelial cells. *Am J Physiol* 1994; 266: L612–9.
- Rusznak C, Devalia JL, Sapsford RJ, Davies RJ. Ozone-induced mediator release from human bronchial epithelial cells *in vitro* and the influence of nedocromil sodium. *Eur Respir J* 1996; 9:2298–305.