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Effects of 0.2 ppm ozone on biomarkers of inflammation in bronchoalveolar lavage fluid and bronchial mucosa of healthy subjects

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ABSTRACT: Short-term exposure to ozone at peak ambient levels induces neutrophil influx and impairs lung function in healthy humans.

In order to investigate the mechanisms contributing to neutrophil recruitment and to examine the role of T-cells in the acute inflammatory response, we exposed 12 healthy humans to 0.2 parts per million (ppm) of ozone and filtered air on two separate occasions for 2 h with intermittent periods of rest and exercise (minute ventilation=30 L·min^{-1}). Fibreoptic bronchoscopy was performed 6 h after the end of exposures. Total protein, tryptase, histamine, myeloperoxidase, interleukin (IL)-8 and growth-related oncogene-α (Gro-α) were measured and total and differential cell counts were performed in bronchoalveolar lavage (BAL) fluid. Flow cytometry was performed on BAL cells to study total T-cells, T-cell receptors (αβ and γδ), T-cell subsets (CD4+ and CD8+ cells) and activated T-cell subsets (CD25+). Using immunohistochemistry, neutrophils, mast cells, total T-cell numbers, T-cell subsets, CD25+ T-cells and leukocyte endothelial adhesion molecules including P-selectin, E-selectin, intercellular adhesion molecule (ICAM)-1 and vascular adhesion molecule (VCAM)-1 were quantified in the bronchial biopsies.

Paired samples were available from nine subjects. Following ozone exposure there was a threefold increase in the proportion of polymorphonuclear neutrophils (PMNs) (p=0.07) and epithelial cells (p=0.05) in BAL fluid. This was accompanied by increased concentrations of IL-8 (p=0.01), Gro-α (p=0.05) and total protein (p=0.058). A significant positive correlation was demonstrated between the two chemokines and proportion of PMNs in BAL fluid. After ozone exposure there was a significant decrease in the CD4/CD8 ratio (p=0.05) and the proportion of activated CD4+ (p=0.01) and CD8+ T-cells (p=0.04). However, no significant changes were demonstrable in any of the inflammatory markers studied in the biopsies.

Short-term exposure of healthy humans to 0.2 ppm ozone induced a neutrophil influx in peripheral airways at 6 h post exposure, but no apparent inflammatory response in proximal airways. This response seems to be mediated at least in part by interleukin-8 and growth-related oncogene-α.

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Several studies evaluating the effects of ozone on healthy as well as asthmatic subjects have shown that short-term exposure to 0.08–0.40 parts per million (ppm) ozone impairs lung function and induces an acute inflammatory response [1–3]. These studies have reported an influx of polymorphonuclear neutrophils (PMNs) and secretion of interleukins (IL)-6 and 8, granulocyte macrophage colony stimulating factor (GM-CSF), total protein, albumin, fibronectin and complement C3a [1–3].

Endothelial adhesion molecules are considered to play a key role in leukocyte recruitment in an inflammatory response. The first step in that recruitment process involves the upregulation of P- and E-selectins (CD62 P and E), which mediate the early adhesion and "rolling" of leukocytes on the vessel wall [4]. Depending on the stimulus, there then occurs an upregulation of a second set of adhesion molecules belonging to the immunoglobulin superfamily including intercellular adhesion molecule-1 (ICAM-1, CD54) and vascular cell adhesion molecule-1 (VCAM-1, CD106) on the vessel wall, which interact with their counterligands on the leukocyte surface: leukocyte functional antigen (LFA-1, CD11a/CD18) and very late antigen (VLA-4, CD49d/CD29), respectively. This leads to firm adhesion, leukocyte priming, and transmigration of the leukocyte from the intravascular compartment to the extravascular space [4].

It has been shown previously that short-term exposure to 0.12 ppm ozone induces rapid upregulation of P-selectin in the microvascular endothelium of healthy bronchial submucosa and that this represents one of the earliest

events in the inflammatory response to ozone [5]. The present study was carried out to examine the inflammatory effects of short-term exposure to 0.2 ppm ozone on healthy human airways at a later point (6 h after the end of exposure). Based on the above model of leukocyte recruitment, it is likely that some of the key steps involved in neutrophil influx take place at this time point, including the release of chemoattractants such as IL-8 and growth-related oncogene-α (Gro-α) and the upregulation of endothelial adhesion molecules. E-selectin and ICAM-1, in particular have been linked to neutrophil recruitment under other circumstances [6]. In addition, a recent study [7] has suggested that T-cells could play a regulatory role in the acute inflammatory response to ozone and the present study examines this hypothesis.

Subjects and methods

Details of the experimental design, subjects, ozone exposure and fibreoptic bronchoscopy have been described previously [8]. In brief, 12 healthy, nonsmoking young adults (10 males and two females, age 27.6±6.2 yrs (mean ±SD)) were recruited into the study. A written informed consent was obtained from all subjects and this study received the approval of Southampton University and Hospitals Joint Ethics Committee. A detailed medical history was obtained from all subjects and a physical examination performed at screening. None of the subjects had any history of allergies or asthma, or any history of respiratory tract infection for at least 6 weeks before the study days. Exposures were carried out under controlled conditions with a temperature of 25°C and relative humidity of 40–60%. All subjects were exposed to 0.2 ppm ozone and filtered air for 2 h in a randomized, double-blind, cross-over control fashion. During the exposures the volunteers were subjected to intermittent 15 min alternating cycles of rest and exercise (minute ventilation=30 $L \cdot min^{-1}$). The average ozone concentration during the exposures was 0.199±0.009 ppm (mean±SD). None of the subjects had received any form of anti-inflammatory drugs, antihistamines or dietary antioxidant supplementation. Fibreoptic bronchoscopy was performed 6 h after the end of exposures. Bronchoalveolar lavage (BAL) was performed using 160 mL prewarmed (37°C) saline in the right upper lobe and left upper lobes on days 1 and 2, respectively, and bronchial mucosal biopsies were obtained from proximal airways as described previously [8]. Paired samples were obtained from only nine subjects, because one subject had a 39% drop in the forced expiratory volume in one second (FEV_1) following ozone exposure and was considered unsuitable for bronchoscopy, one subject was unable to tolerate bronchoscopy at all (ozone day) while another underwent bronchoscopy after filtered air exposure, became dist-ressed 3 h after bronchoscopy, was observed overnight in hospital and was subsequently withdrawn from the study.

Analysis of mediators in bronchoalveolar fluid

BAL fluid samples were filtered through a 100 μm filter (Becton-Dickinson, Oxford, UK), centrifuged at 300×g for 10 min and cells washed once in sterile phosphate-buf-fered saline (PBS). Cells were counted using trypan blue exclusion of dead cells on a Neubauer haemocytometer (BDH, Dagenham, UK), and an aliquot was separated for cytospin preparation and fluorescence-activated cell scan (FACS) analysis. Cytospin preparations were prepared using a Shandon cytospin device (Shandon Southern Instruments, Runcorn, UK). The slides were air dried and differential cells counts were performed after staining with a rapid Giemsa stain (HemaGurr, BDH, Poole, UK) with at least 400 cells being counted. BAL fluid supernatant was aliquoted and stored at -70°C for mediator analysis.

Mediators were measured in BAL fluid supernatant using the following methods. The total protein assay was carried out by modification of the LOWRY method [9], proposed by SMITH *et al.* [10], which measures protein by spectrophotometery at 562 nm.

Myeloperoxidase, histamine and tryptase were measured by radioimmunoassay (RIA) using commercially available kits (Pharmacia, Uppsala, Sweden). The sensitivities of the assays were 8 $\mu g \cdot mL^{-1}$, 0.1 $ng \cdot mL^{-1}$ and 0.5 $ng \cdot mL^{-1}$, respectively.

Total IL-8 was measured by enzyme-linked immunosorbent assay (ELISA) (PeliKine Compact, human IL-8 ELISA kit; CLB, Amsterdam, The Netherlands) with a sensitivity of 1 $pg \cdot mL^{-1}$.

Gro-α was measured using a commercially available ELISA kit (R&D systems, Abingdon, UK) with a sensitivity of 15 $pg \cdot mL^{-1}$.

Immunohistochemistry. Two biopsies were obtained from proximal airways, fixed overnight in acetone containing protease inhibitors, and processed into glycol methacrylate on the following day as described previously [6, 11]. Sections (2 μm thick) were cut using a microtome (Supercut 2065, Leica, Germany), floated on to ammonia water (1:500), then picked on to glass slides coated with 0.01% poly-L-lysine and allowed to dry at room temperature for 1–4 h. Sequential 2 μm sections were taken for adhesion molecules and pan-vessel marker endothelium clone (EN)-4 and for CD4, CD8 and CD25. Immunostaining by the avidin biotinylated complex (ABC) method was performed using the standard protocol in our laboratory, as des-

Table 1. – Monoclonal antibodies used for immunohistochemistry

Name	Marker	Cells	Source
CD3	CD3	T-cells	DAKO* Becton Dickinson+
CD4	CD4	T-cells	DAKO*
CD8	CD8	T-cells	A. Walls#
AA1	Tryptase	Mast cells	DAKO*
NE	Elastase	Neutrophils	DAKO*
CD25	T-lymphocyte	Activated T-lymphocyte	Sanbio‡
EN4	Endothelium	Pan-endothelial marker	Serotec§
ICAM-1 (RR1)	ICAM-1	Microvasculature	Serotec§ Immunotec†
E-selectin	E-selectin	Microvasculature	Sanbio‡
P-selectin	P-selectin	Microvasculature	
VCAM	VCAM	Microvasculature	

ICAM: intracellular adhesion molecule; VCAM: vascular adhesion molecule. *: High Wycombe, Bucks, UK; +: Oxford, UK; #: University of Southampton, UK; ‡: Loughborough, UK; §: Kidlington, UK; †: Marseille, France.

cribed previously [11]. Details of monoclonal antibodies used for immunostaining are given in table 1.

Quantification of inflammatory markers in sections was performed using the standard protocol as described by MONTEFORT *et al.* [6]. Immunostained cells were counted separately in the epithelium and submucosa. Areas including smooth muscle, glands, large blood vessels, torn and folded tissue were not included. The length of the epithelium and area of submucosa were determined using "Color Vision Software" (Improvision, UK) computer-assisted image analysis and results of inflammatory cell numbers were expressed as cells·mm^{-1} and cells·mm^{-2} in the epi-thelium and submucosa, respectively. Quantification of adhesion molecules in the submucosa was undertaken by expressing the number of vessels staining with specific antiadhesion antibody in the section as a percentage of the total number of blood vessels staining with pan-vessel marker EN4 in the adjacent section.

Flow cytometry. Flow cytometry was performed using a standard protocol described previously [12]. BAL cells were resuspended at 1×10^6 cells·mL^{-1}, and 100 μL of this suspension was added to each FACS tube containing peridin chlorophyll-protein conjugate (CD3PerCP) and CD4 or CD8 fluoroscein isothiocyanate (FITC), together with CD25 PE (phycoerythrin); 100 μL was also added to a tube containing T-cell receptor (TCR) αβ FITC and TCRγδ PE. Cells were incubated for 30 min at room temperature in the dark. BAL cells were washed once in PBS. Three-colour analysis was performed on a Becton Dickinson FACS scan using lysis II software. The results were recorded as total CD3, T-cell αβ and γδ cells per 10,000 events. CD4 and CD8 were expressed as the percentage of total CD3 T-cells. CD25 were expressed as the percentage of CD4+ and CD8+ T-cells, respectively. Monoclonal antibodies against T-cell markers CD3, CD4, CD8, CD25 were purchased from Becton Dickinson while TCRαβ and γδ monoclonal antibodies were purchased from T-cell Sciences (Woburn, MA, USA).

Statistical analyses. As the data were not normally distributed, Wilcoxon's paired sign rank test was used to compare the inflammatory indices in the BAL fluid and biopsies between the 2 days. Spearman rank correlations were performed to study the association between the percentage of PMNs and IL-8 in BAL fluid, and the percentage of PMNs and Gro-α in BAL fluid.

Results

Bronchoalveolar lavage fluid

Total and differential cell counts. As reported previously [8], there was no change in the total number of leukocytes between the two exposures. After ozone exposure there was a threefold increase in the proportion of neutrophils and epithelial cells with reciprocal changes in macrophages (table 2).

Soluble mediators. At 6 h after exposures no significant changes were detectable in myeloperoxidase, histamine and tryptase. However, there was a 1.8-fold increase in Gro-α (median (interquartile range (IQR)): 369 (165–679) *vs* 653 (273–1057) pg·mL^{-1}, p=0.05), a fourfold increase in total IL-8 (median (IQR): 7.5 (6–16.1) *vs* 27.8 (7.3–37.4) pg·mL^{-1}, p=0.01) and a trend towards an increase in total protein (median (IQR): 0.15 (0.08–0.27) *vs* 0.24 (0.2–0.37), p=0.058) following ozone exposure (table 3, fig. 1a and b). Significant correlations were demonstrable between levels of PMNs and Gro-α (r=0.80, p=0.015) and between PMN and total IL-8 (r=0.71, p=0.047) in BAL fluid following exposure to ozone (fig. 2a and b).

Bronchial biopsies. Ring staining was observed with anti-CD3, -CD4, -CD8 and -CD25 antibodies demonstrating the surface expression of receptors. Mast cells were stained on account of the high concentration of immunoreactive tryptase within their cytoplasmic granules. Neutrophils also showed cytoplasmic staining for elastase. The mono-

Table 2. – Analysis of total and differential cell count in bronchoalveolar lavage fluid

	Total lymphocytes $\times10^6$ cells·mL^{-1}	Macrophages %	Neutrophils %	Eosinophils %	Lymphocytes %	Epithelial cells %
Air	0.21 (0.1–0.3)	90.6 (88.1–93.1)	3.5 (2–5.3)	0 (0–0.2)	3.6 (1.9–5.7)	1.6 (1.3–3.4)
Ozone	0.12 (0.09–0.19)	80.3 (74.9–87.6)	9.8 (4.2–16.3)	0 (0–0.4)	3.1 (1.5–4.2)	5 (2.2–9.8)
p-value* (n=8)	NS	<0.05	0.07	NS	NS	0.05

All air and ozone values represent median with interquartile range in parentheses. *: Wilcoxon's matched paired rank test. NS: nonsignificant. (See also Ref. [8]).

Table 3. – Concentrations of soluble mediators in bronchoalveolar lavage fluid

Mediator	n	Air	Ozone	p-value*
Total protein mg·mL^{-1}	8	0.15 (0.08–0.27)	0.24 (0.2–0.37)	0.058
IL-8 pg·mL^{-1}	9	7.5 (6–16.1)	27.8 (7.3–37.4)	0.01
Gro-α pg·mL^{-1}	9	369 (165–379)	653 (273–1057)	0.05
Histamine nM·L^{-1}	9	1 (0.15–5.6)	2.7 (0–6.4)	0.36
Myeloperoxidase μg·L^{-1}	9	3.3 (1.75–8)	4.1 (2.05–28.05)	0.20
Tryptase U·L^{-1}	9	0.48 (0.23–0.78)	0.55 (0.2–0.78)	0.67

All values represent median with interquartile range in parentheses. *: Wilcoxon's matched paired sign rank test. IL-8: interleukin-8; Gro-α: growth-related oncogene-α.

clonal antibodie EN4 clearly picked out the endothelium of the microvasculature. The monoclonal antibodies directed against P-selectin, E-selectin, ICAM-1 and VCAM-1 easily identified mucosal vasculature but between the antibodies there was a considerable difference in the proportion of vessels stained (table 4). Anti-ICAM-1 occasionally produced scant staining of the basal layer of the bronchial epithelium and also occasional mucosal leukocytes.

No significant changes were seen in neutrophils, mast cells, total T-cells (CD3+), T-cell subsets (CD4+, CD8+), the CD4+/CD8+ ratio and activation marker CD25+ in the bronchial submucosa. Similarly, no changes were seen in the expression of vascular endothelial adhesion molecules, including P-selectin, E-selectin, ICAM-1 and VCAM-1 (table 4).

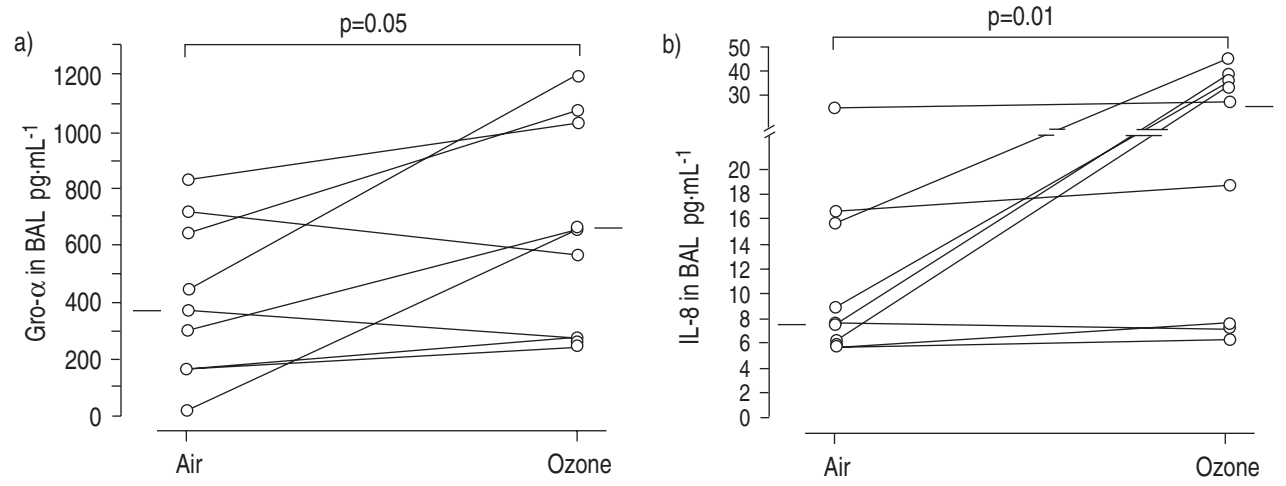

Fig. 1. – Concentration of: a) growth-related oncogene-α (Gro-α); and b) interleukin (IL)-8 in bronchoalveolar lavage (BAL) fluid following exposure to filtered air or ozone. Horizontal lines represent medians. Statistical assessment by Wilcoxon test. Significant differences between groups: a) p=0.05 (n=9); b) p=0.01 (n=9).

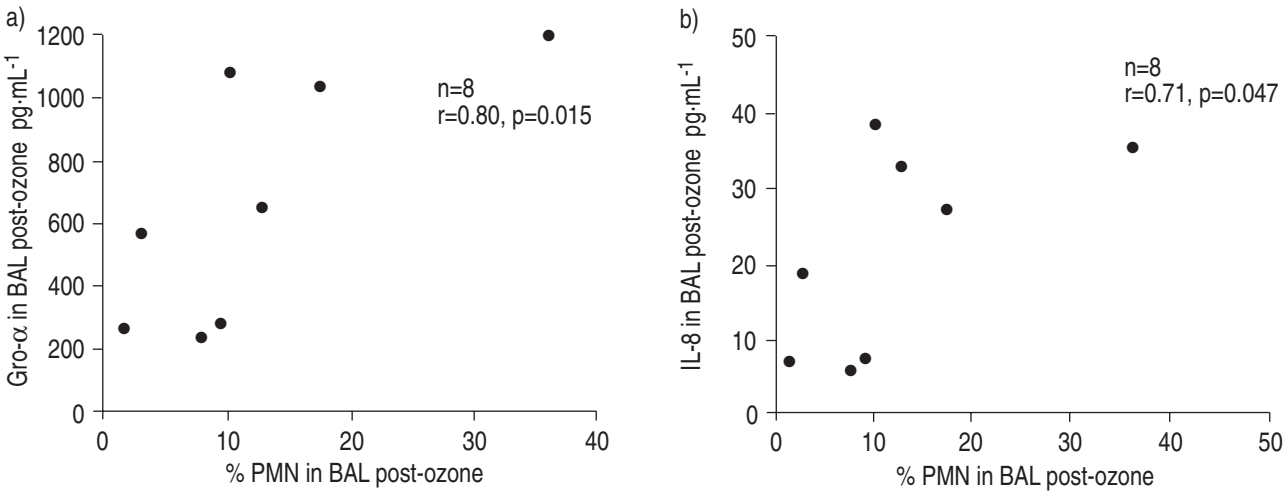

Fig. 2. – Correlations between: a) growth-related oncogene-α (Gro-α) and per cent polymorphonuclear neutrophils (PMN) and b) interleukin (IL)-8 and per cent PMN. BAL: bronchoalveolar lavage. Statistical assessment by Spearman rank correlation. Significant correlations between groups: a) r=0.80, p=0.015 (n=8); b) r=0.71, p=0.047 (n=8).

Table 4. – Inflammatory indices in bronchial submucosa

mAB	n	Air	Ozone	p-value*
Neutrophil elastase cells·mm^{-2}	8	75.9 (23.5–268.7)	80.3 (62.6–160)	0.78
CD3 cells·mm^{-2}	8	86.8 (23–179)	122.33 (12.6–198.6)	0.58
CD4 cells·mm^{-2}	8	30.4 (14–104.8)	53.4 (4.2–93.1)	0.73
CD8 cells·mm^{-2}	8	22 (9.2–57)	40.4 (2.8–105.9)	0.67
CD25 cells·mm^{-2}	8	0 (0–0.3)	0 (0–0.6)	0.46
Mast cells cells·mm^{-2}	7	35.6 (14.6–39.8)	32.9 (12.3–56.5)	0.13
ICAM-1 %	9	86 (71–100)	80 (66.5–98.5)	0.55
P-selectin %	9	30 (10–67.5)	22 (5.5–47)	0.44
E-selectin %	9	25 (12–67.5)	23 (8.5–53)	0.77
VCAM %	9	1 (0–10.5)	4 (0–10)	0.86
CD4/CD8 ratio	8	1.5 (0.61–2)	0.86 (0.67–3.1)	0.75

All values represent median with interquartile range in parentheses. *: Wilcoxon's matched paired sign rank test. mAB: monoclonal antibody; ICAM: intracellular adhesion molecule; VCAM: vascular cell adhesion molecule.

Table 5. – Analysis of T-lymphocytes in bronchoalveolar lavage (BAL) fluid by flow cytometry

mAB	n	Air	Ozone	p-value*
CD3·10,000 total BAL cells^{-1}	9	513 (344–761)	143 (73–566)	0.09
TCRαβ·10,000 total BAL cells^{-1}	9	501 (325–775)	189 (155–522)	0.07
TCRγδ·10,000 total BAL cells^{-1}	9	11 (0–58)	20 (14–27)	0.87
CD4 % of CD3	9	54 (49–64)	53 (39–61)	0.21
CD8 % of CD3	9	34 (28–42)	45 (31–48)	0.17
Activated CD4+ T-cells %	9	26 (18–32)	14 (7–22)	0.01
Activated CD8+ T-cells %	8	7 (4–13)	2 (0.4–7)	0.04
CD4/CD8 ratio	9	1.6 (1–2)	1 (0.8–1.6)	0.05

All values represent median with interquartile range in parentheses. *: Wilcoxon's matched paired sign rank test. mAB: monoclonal antibody; TCR: T-cell receptor.

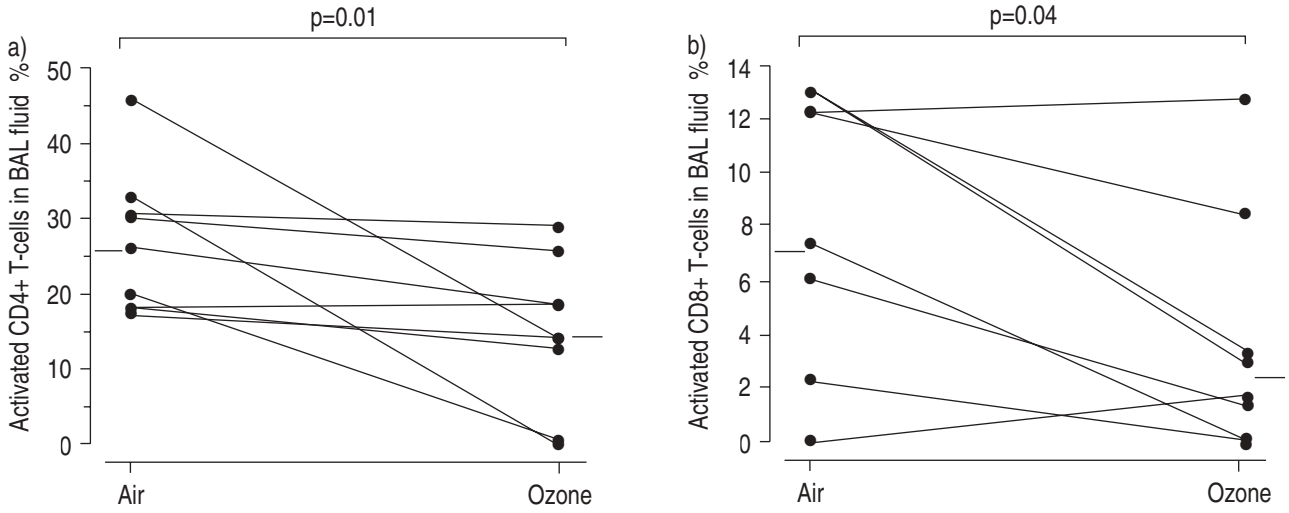

Fig. 3. – Percentage of bronchoalveolar lavage (BAL) a) CD4+ and b) CD8+ T-cells expressing CD25 following exposure to filtered air or ozone. Horizontal lines represent medians. Statistical assessment by Wilcoxon test. Significant differences between groups: a) p=0.01 (n=9); b) p=0.04 (n=8).

Flow cytometry. After ozone exposure there was a significant decrease in the proportion of activated (CD25+) T-cells in BAL fluid. This decrease was apparent in both the CD4+ and CD8+ T-cell subsets (table 5, fig. 3a and b). The CD4/CD8 ratio altered in favour of CD8 cells (median values of 1 after ozone *vs* 1.6 after air) and there was a trend towards a decreased proportion of CD3+ cells in BAL fluid.

Discussion

The present data show that short-term exposure to 0.2 ppm ozone induces epithelial shedding, neutrophil recruitment, an increase in the C-X-C chemokines Gro-α and IL-8 in BAL fluid, and decrements in T-cell numbers, activation markers and the CD4+/CD8+ ratio in BAL fluid. However, no changes were seen in any of the inflammatory cells or adhesion molecules examined in the submucosa of biopsies following exposure to ozone.

The exposure system used in this study has been carefully standardized and validated. The concentration of 0.2 ppm represents peak ozone levels encountered during summertime air pollution episodes. A minute ventilation rate of 30 L·min^{-1} was chosen in order to simulate mild outdoor activity and the sampling time of 6 h was chosen because inflammatory response to ozone has been reported to peak between 6–10 h after short-term exposures [13].

Previous studies have shown that ozone induces a significant airway inflammatory response in both proximal and distal airways of healthy subjects [2, 3]. Aris *et al.* [3] reported neutrophilia in biopsies taken from proximal airways of healthy subjects 18 h after exposure to 0.2 ppm ozone for 4 h and, more recently, Aris *et al.* [14] have shown increased expression of ICAM-1 in the microvascular endothelium of the bronchial submucosa of healthy human volunteers 18 h after short-term exposure to ozone, thus highlighting the role of ICAM-1 in PMN recruitment. In addition, increased expression of P-selectin was reported in the vascular endothelium of the bronchial submucosa of healthy Swedish subjects 1.5 h after exposure to a lower concentration of ozone (0.12 ppm for 2 h) [5]. Data from *in vitro* studies suggest that the expression of E-selectin and ICAM-1 peaks 3–4 and 12–18 h, respectively following stimulation with agents such as histamine, thrombin, oxygen radicals and tumour necrosis factor (TNF)-α [15–19]. However, in the present study neither tissue neutrophilia nor upregulation of E-selectin and ICAM-1 was found in the proximal airway biopsies.

The absence of PMN influx in the bronchial submucosa in the present study could be due to the relatively lower dose of ozone and early sampling time employed in this study. Given that the proximal alveolar region is the major site for the deposition of ozone [20, 21], the trend towards neutrophilia and chemokine responses seen in BAL fluid probably represents a peripheral airway response. Since approximately 40–45% of inhaled ozone is deposited in

the proximal airways (at the level of bronchus intermedius) it is plausible that the effective dose of ozone might have been too low to elicit an inflammatory response in the proximal airways in the present study. In addition, it is likely that, owing to differential deposition of ozone in the respiratory tract, the kinetics of the inflammatory response are different between proximal and distal airways and the time point of 6 h may thus have been too early to detect PMN influx in the proximal airways.

In the context of the neutrophil response, an interesting observation seen in this study is an increase in Gro-α, a C-X-C chemokine known to exhibit a significant chemotactic property for PMNs and also to induce granule exocytosis, weak respiratory bursts and transient elevations in cytosolic calcium [22, 23]. VILLARD *et al.* [24] recently showed that Gro-α is probably as important as IL-8 in the pathogenesis of acute lung injury in patients with adult respiratory distress syndrome, bacterial pneumonia or *Pneumocystis carinii* pneumonia. In that study, significant correlations were found between levels of Gro-α and absolute numbers of PMN in BAL fluid. In the present study there was a significant positive correlation between levels of Gro-α and PMN in BAL fluid, suggesting that this chemokine could play a key role in the acute inflammatory response to ozone.

Similarly, a significant increase was seen in total IL-8 (immunoreactive) in all subjects in BAL fluid following exposure to ozone in this study. Elevated concentrations of this C-X-C chemokine have been reported in healthy and asthmatic airways in both bronchial wash and BAL fluid after ozone exposure [25, 26]. IL-8 is the most prominent member of the C-X-C family and is reported to cause the release of lysosomal enzymes from neutrophils, to increase the adherence of neutrophils to unstimulated endothelial cells, fibrinogen and matrix proteins, to increase granulocyte surface expression of Mac-1 (CD11/CD18), to induce L-selectin shedding from leukocytes and to upregulate the β_2-integrin subunit [22]. Although a number of cell types produces IL-8, including monocytes, alveolar macrophages, fibroblasts, lymphocytes, epithelial cells, endothelial cells and neutrophils, it is most likely that epithelial cells are the major source in response to ozone since they are adjacent to the airway lumen [22]. Significant positive correlations between IL-8 and Gro-α and the percentage of PMN in BAL fluid suggest that these C-X-C chemokines are important in the early inflammatory response to ozone.

The present data on the effects of ozone on T-lymphocytes are novel. Effects were observed that were mainly confined to the BAL compartment, since no corresponding changes were found in the total T-cells, their subsets or CD25 expression in the biopsies. In contrast to the original hypothesis, there was a significant decrease in the proportion of activated CD4+ (T-helper) and activated CD8+ (T-cytotoxic/suppressor) cells. In addition, the CD4/CD8 ratio decreased after exposure to ozone, probably through a relative increase in CD8+ cells. The absence of any corresponding change in T-cell numbers in the bronchial biopsies following ozone exposure suggests that these changes take place more in the peripheral airways, once again reiterating the concept that the kinetics of airway inflammation are probably different in proximal and distal airways.

BLEAVINS *et al.* [7] have also suggested that T-cells may play a regulatory response in ozone induced airway inflammation. In their study, when CD-1 mice were exposed repeatedly to ozone in the presence of cyclosporin A, the number of T-lymphocytes had halved by day 14 of the exposure, as opposed to the placebo group (ozone plus vehicle). In addition, the cyclosporin group developed a significantly greater inflammatory response than the control group as gauged by the number of PMNs and "lesion volume". These observations suggest that T-lymphocytes could play a protective role in the airway inflammatory response to ozone, particularly following repeated exposures.

In conclusion, short-term exposure of healthy humans to 0.2 ppm ozone with intermittent moderate exercise induces neutrophil influx in the peripheral airways at 6 h but no apparent inflammatory response in the proximal airways. This response seems to be mediated, at least in part, by chemokines, including interleukin-8 and growth-related oncogene-α. In addition, such an exposure has an effect on T-cell subsets and activation, the significance of which is unclear at the present time. These findings confirm the ability of a moderate to high concentration of ozone to induce airway inflammation, and indicate a pattern that is both localized and distinct from the inflammatory responses that have been described in asthma and other related airway diseases.

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