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Diesel exhaust exposure enhances the expression of IL-13 in the bronchial epithelium of healthy subjects

Jamshid Pourazar[a], Anthony J. Frew[b], Anders Blomberg[a],
Ragnberth Helleday[a], Frank J. Kelly[c], Susan Wilson[b], Thomas Sandström[a,*]

[a]*Department of Respiratory Medicine and Allergy, University Hospital, Umeå SE-901 85, Sweden*
[b]*Respiratory Cell and Molecular Biology, Division of Infection, Inflammation and Repair, Southampton General Hospital, Southampton, UK*
[c]*Lung Biology, School of Health and Life Sciences, King's College London, London, UK*

Summary Epidemiological studies have demonstrated adverse health effects of environmental pollution. Diesel exhaust (DE) is an important contributor to ambient particulate matter pollution. DE exposure has been shown to induce a pronounced inflammatory response in the airways, with an enhanced epithelial expression of IL-8, and Gro-α in healthy subjects. The present investigation was aimed to further characterise the epithelial response to DE in vivo, with particular reference to possible TH2 response, in non-atopic healthy subjects. To determine this response, 15 healthy, non-atopic non-smoking subjects with normal lung function were exposed to DE (PM10 $300\,\mu g/m^3$) and filtered air during 1 h on two separate randomised occasions. Bronchoscopy sampling of bronchial mucosal biopsies was performed 6 h after exposure. Immunohistochemical staining were performed using mAb for IL-10, IL-13 and IL-18 expression. DE exposure induced a significant increase in the expression of IL-13 in the bronchial epithelium cells, 2.1 (1.35–4.88) Md (Q1–Q3) vs. air 0.94 (0.53–1.23); $P = 0.009$. No significant changes were seen in IL-10 and IL-18 expression. This finding suggests an TH2-inflammatory response in the airways of non-atopic healthy individuals.
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Introduction

There is now extensive evidence showing that exposure to increased levels of ambient particulate matter (PM) pollution is associated with worsening of airway symptoms and lung function, increase in asthma medication use, emergency room visits and hospital admissions for respiratory diseases.[1–3]

Asthmatic subjects have been suggested to be particularly susceptible to the adverse health effects of PM pollution, but it is clear that even subjects without allergy and respiratory disease are affected and will experience mucosal and airway symptoms associated with elevation of PM pollution levels.[1,3] Diesel exhaust (DE) is a major contributor to PM air pollution and has been identified as a reactive pollutant, which contributes to adverse health effects.

The capacity of DE to produce adverse effects on human airways has been addressed in a series of experimental studies in healthy and asthmatic

*Corresponding author. Tel.: +46-90-7852516; fax: +46-90-141369.
E-mail address: thomas.sandstrom@lung.umu.se
(T. Sandström).

subjects using a validated system of controlled chamber exposure.[4–6]

DE exposure for 1 h at a PM concentration of 300 $\mu g/m^3$ has been shown to result in an inflammatory response in airways, with epithelial production of IL-8 and GRO-α causing a prominent recruitment of neutrophils into air spaces and bronchial mucosa. This was accompanied by a migration of CD4+ and CD8+ lymphocytes to the airways and mast cell activation leading to secretion of histamine into the air spaces, even in non-atopic healthy individuals.[7–9] DE has also been demonstrated to adversely affect the phagocytic function of alveolar macrophages in vitro.[6] In asthmatic subjects treated with inhaled corticosteroids (800–1200 $\mu g/d$). DE induced a doubling of bronchial responsiveness to methacholine 1 day after exposure.[10]

The relationship between DE and allergy has recently come in focus. Cell culture work and challenge studies in animals have demonstrated that sensitisation to allergens and development of allergic responses may be enhanced by a variety of air pollutants, but most prominently by DE particles.[11] Studies by Diaz-Sanchez and co-workers, with local DE particle installations in the nose, clearly suggest that DE may enhance allergen responsiveness as well as promoting sensitisation to neo-allergens in the nose.[12–15] The question was consequently raised whether DE could also cause a shift towards Th2 response in the lungs. If this does indeed occur, it would greatly affect how we regard the increasing use of diesel engines in our society.

Therefore, in this study, we examined the hypothesis that, in healthy non-atopic subjects, exposure to DE would induce a shift towards a TH2 cytokine response in the bronchial mucosa, and/or suppress a TH1 response.

Methods

The exposure protocol has been presented in detail previously.[7,8] Briefly, fifteen healthy non-atopic, non-smoking subjects (11 males, 4 females) mean age 24 years (range 21–28 yr), were recruited. Each subject was exposed to air or DE for 1 h in an exposure chamber, on two different occasions. Air and DE exposure were performed in a randomised sequence at least 3 weeks apart. Six hours after each exposure, bronchoscopy was performed to obtain endobronchial mucosal biopsies. To avoid biopsy artefacts, biopsies were taken from one side during the first bronchoscopy and from the contralateral side during the second bronchoscopy.

Table 1 Monoclonal antibodies used for immuno-histochemical staining of the bronchial epithelium.

Antibody	Clone	Source
IL-10	23738.111	R&D SYSTEM Abingdon, UK
IL-13	32116.11	R&D SYSTEM Abingdon, UK
IL-18	125-2H	R&D SYSTEM Abingdon, UK

Biopsies were fixed overnight in chilled acetone (−20°C) containing the protease inhibitors iodoacetamide and phenylmethyl sulfonyl fluoride. After fixation, biopsies were processed into glycolmethacrylate (GMA) resin and stored at −20°C until cutting and immunostaining.[16] Two micron thick sections were cut and placed on poly L-lysine treated glass slides. Endogenous peroxidases were inhibited using 0.1% sodium azide and 0.3% hydrogen peroxide. Non-specific antibody binding was blocked with undiluted culture medium containing bovine serum albumin and fetal calf serum, before the primary monoclonal antibodies (mAb) directed against IL-10, IL-13 and IL-18 (Table 1) were applied and incubated at room temperature overnight. After rinsing in Tris-buffered saline (TBS), biotinylated rabbit anti-mouse IgG F(ab′)$_2$ (Dako Glostrup Denmark) was applied for 2 h, followed by the streptavidin–biotin horseradish peroxidase complex (Dako) for another 2 h. Sections were developed with 3,3-diaminobenzidine and counterstained with Mayer's hematoxylin. TBS and mAb IgG were used as negative controls. Insufficient biopsy material was available to allow analysis of other cytokines.

All slides were coded and analysed by an observer blinded to the exposure regime. Positive staining was analysed based on red/green/blue colour composition, in areas of intact well orientated epithelium, with the assistance of computerised image analysis (Leica Q500IW, Leica, Cambridge, UK), as previously described.[8]

Statistic analyses

Each subject acted as their own control, and variables were analysed using Wilcoxon's paired rank test (SPSS for Windows version 10.1.4). P-values < 0.05 were considered significant.

Results

Following DE exposure, immunohistochemical analysis showed a significant increase in IL-13 in the

epithelium (2.05; 1.35–2.05, median and inter-quartile range, compared to biopsies obtained after filtered air (0.94; 0.53–1.23; $P = 0.009$) (Table 2, Fig. 1). No difference was detected in expression of IL-10 or IL-18 (Table 2).
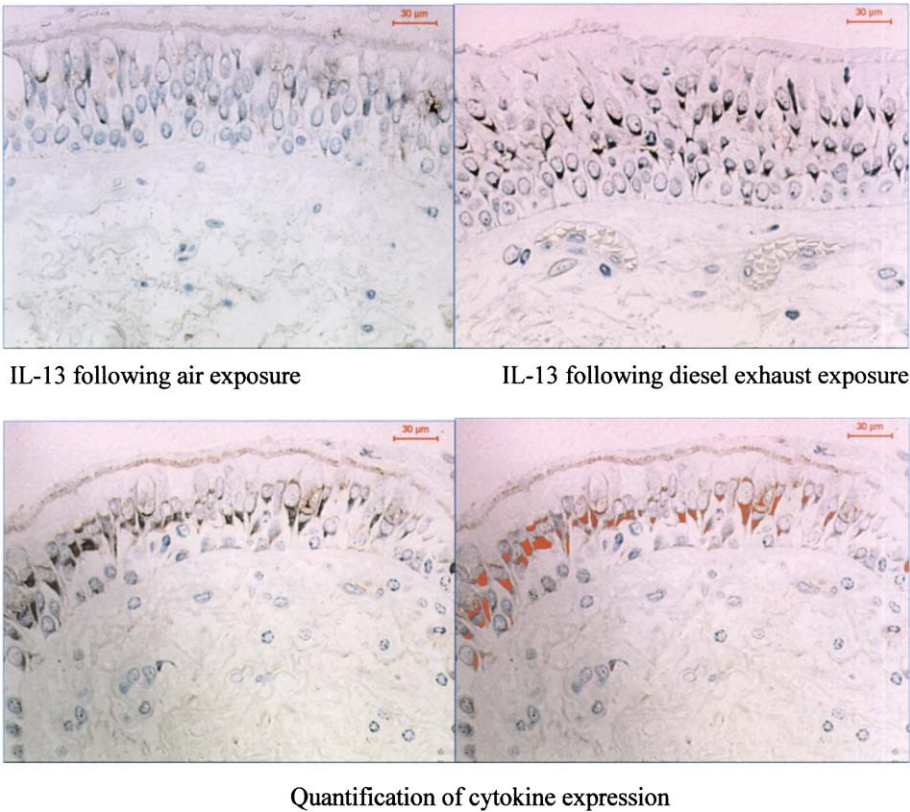
Discussion

The present study demonstrated that exposure of healthy human subjects to freshly generated DE significantly enhances expression of IL-13 protein in the bronchial epithelium, but does not significantly change the expression of either IL-10 or IL-18. To the best of our knowledge, this is the first study demonstrating a TH2 related cytokine response in the lungs of non-atopic individuals exposed to DE.

DE is a major component in ambient air pollution and has been shown to cause mucosal symptoms, increase BHR and have strong pro-inflammatory effects in the airways.[8–10] Since diesel particles have previously been demonstrated to cause Th2 responses in the nose, we hypothesised that this might also occur in the lower airways. To examine this, we determined the expression of Th1 and Th2

Table 2 Percentage area of bronchial epithelium expressing IL-10, IL-13 and IL-18 in biopsies obtained 6 h after exposure of healthy subjects to diesel exhaust vs. filtered air.

	IL-10	IL-13	IL-18
AIR	1.92 (1.52–2.61)	0.94 (0.53–1.23)	3.83 (3.02–6.41)
DE	2.31 (1.03–2.94)	2.05 (1.35–4.88)	6.1 (1.80–11.0)
	NS	$P = 0.009$	NS

All values are presented as percentage of epithelial area and given as medians with interquartile ranges. Statistical comparisons by Wilcoxon's paired rank test.

IL-13 following air exposure   IL-13 following diesel exhaust exposure

Quantification of cytokine expression

Figure 1 IL-13 expression in the bronchial epithelial mucosa after exposure to filtered air and diesel exhaust (upper panel). The black DAB colour is selected using an image analyser system and quantified within the epithelial area, as expressed by the red computer generated colour (lower panel). Bar represents 30 μm.

cytokines expression in the bronchial mucosa, which represents a major immunocompetent surface and barrier which interacts with the diesel particles and which is also a major source of cytokines in the airways. We were selective and limited in the number of Th1–Th2 cytokine investigated due to the limited availability of bronchial mucosal biopsy material.

IL-13 is an important Th2 cytokine and was selected as the main Th2 marker in this study. It is normally expressed in larger amounts in the airways than IL-4, with which it shares many properties. The similarities in effects of IL-4 and IL-13 are due to both cytokines using the IL-4 receptor-α chain, which is a common component of the IL-4 and IL-13 receptors.[17,18] Secretion of IL-13 following local allergen challenge in atopic asthma has been shown to strongly correlate with eosinophil numbers.[19] In mice IL-13 directly stimulates B cells, causing extended survival and enhanced antibody levels. As we have previously reported that DE results in a T-cell influx into the airways, the present findings may provide an additional mechanism for IL-13 to stimulate IgE antibody production by T-cell activated B cells.[7,20] Consequently, the increase of the Th2 cytokine IL-13 in the airway mucosa of non-atopic individuals may shift the immune response in these individuals towards an allergic pattern of response, as suggested by Diaz-Sanchez et al. in their human nasal provocation model with diesel particles.[8–15] This response could be of particular importance in subjects with allergy or asthma, as IL-13 has been shown to be most potent inducer of eotaxin in airway epithelial cells as well as being able to induce bronchial hyperresponsiveness (BHR) in animal models of allergic asthma. This is also consistent with our findings of a doubling of BHR in allergic asthmatics one day after DE exposure.[10] IL-13 also has a role in mucin secretion.

IL-10, was selected as a cytokine of interest for this study, because it contributes to Th2 responses and also has anti-inflammatory properties.[21] IL-10 enhances Th2 responses, potentiates IgE production by B cells that have become committed to IgE production.[22] The fact that IL-10 concentrations were relatively unchanged in the present study may reflect the early stage of the inflammatory process at which the biopsies were collected. It would be of interest to address this by studying biopsies at later timepoints after exposure.

IL-18 is a proinflammatory regulatory Th1 cytokine that plays an important role in NK cell activation. Together with IL-12, IL-18 has a synergistic effect on IFN-γ production from T cells.[23] Constitutive expression of IL-18 has been observed within epithelium and mononuclear cells in mice.[24] Mice challenged with LPS show a higher level of IL-18 expression in their lungs than control mice and conversely, ovalbumin-challenged mice show lower levels of IL-18 expression compared to control animals. In man, airways tissue from patients with sarcoidosis express a higher level of IL-18 than control individuals, while asthmatic airways show lower levels of IL-18 expression compared to controls.[24] In the current study, the expression of IL-18 varied widely between subjects, and although the median value was slightly increased, there was no statistically significant effect of DE exposure.

In this study, DE exposure induced an increase in IL-13 expression in the human bronchial epithelium of non-atopic subjects. This rise occurred at the same time as neutrophilic, lymphocytic and mast cell infiltration of the airways epithelium, upregulation of the adhesion molecules ICAM-1 and VCAM-1, and increased epithelial expression of the chemokines Gro-α and IL-8.[7,8] These data are consistent with the nasal provocation studies of Diaz-Sanchez et al. and suggest that DE may act as an immune modulator which enhances Th2 responses in the airways of exposed individuals. DE-enhanced IL-13 expression may increase the risk of allergic sensitisation in previously non-atopic individuals, while in allergic asthmatic subjects, this could be one of the mechanisms underpinning the clinical observation of enhanced susceptibility to particulate pollution.[10]

Acknowledgements

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