Acute Exposure to Diesel Exhaust Increases IL-8 and GRO-α Production in Healthy Human Airways

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We have previously demonstrated that short-term exposure to diesel exhaust (DE) for 1 h induced a marked leukocytic infiltration in the airways of healthy human volunteers involving neutrophils, lymphocytes, and mast cells along with increases in several inflammatory mediators. We hypothesized that the leukocyte infiltration and the various inflammatory responses induced by DE were mediated by enhanced chemokine and cytokine production by resident cells of the airway tissue and lumen. To investigate this, 15 healthy human volunteers were exposed to diluted DE and air on two separate occasions for 1 h each in an exposure chamber. Fiberoptic bronchoscopy was performed 6 h after each exposure to obtain endobronchial biopsies and bronchial wash (BW) cells. Using reverse transcriptase/polymerase chain reaction enzyme-linked immunosorbent assay (RT-PCR ELISA), a novel and sensitive technique to quantify relative amounts of cytokine mRNA gene transcripts, and immunohistochemical staining with computer-assisted image analysis to quantify expression of cytokine protein in the bronchial tissue, we have demonstrated that DE enhanced gene transcription of interleukin-8 (IL-8) in the bronchial tissue and BW cells along with increases in IL-8 and growth-regulated oncogene-alpha (GRO-α) protein expression in the bronchial epithelium, and an accompanying trend toward an increase in IL-5 mRNA gene transcripts in the bronchial tissue. There were no significant changes in the gene transcript levels of interleukin-1B (IL-1β), tumor necrosis factor-alpha (TNF-α), interferon gamma (IFN-γ), and granulocyte macrophage colony–stimulating factor (GM-CSF) either in the bronchial tissue or BW cells after DE exposure at this time point. These observations suggest an underlying mechanism for DE-induced airway leukocyte infiltration and offer a possible explanation for the association observed between ambient levels of particulate matter and various respiratory health outcome indices noted in epidemiological studies. Salvi SS, Nordenhall C, Blomberg A, Rudell B, Pourazar J, Kelly FJ, Wilson S, Sandström T, Holgate ST, Frew AJ. Acute exposure to diesel exhaust increases IL-8 and GRO-α production in healthy human airways.

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Continuing expansion in the number of diesel engine powered vehicles has increased the concentration of diesel exhaust particles (DEP) in the ambient atmosphere of most urban areas worldwide, and DEP is now one of the major contributors to inhalable particulate matter pollution throughout the industrialized world. Although several epidemiological studies have reported associations between daily concentrations of ambient particulate matter and increased incidences of allergies, asthma, respiratory infections, increased hospitalization for respiratory diseases, decreased pulmonary function, and premature mortality among the general population (1), there are only few studies that have addressed the underlying biological mechanisms of particulate matter toxicity. We have previously demonstrated that short-term exposure to diesel exhaust (DE) for 1 h induced an acute inflammatory response in the airways of healthy human volunteers. Along with an increase in the concentrations of histamine and fibronectin in the bronchoalveolar lavage fluid (BALF), DE elicited a marked leukocytic infiltration in the airways involving neutrophils, lymphocytes, and mast cells (2).

Recruitment of inflammatory cells from the circulation into the airway mucosa during an inflammatory response is a highly complex process involving a series of coordinated events. These include movement of leukocytes from the blood to the luminal surface of postcapillary venules, adhesion to endothelial cells via the induced upregulation of adhesion molecules, transendothelial migration, and finally movement along a gradient of chemotactic stimuli toward the source of the inflammatory fo-

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cus. We have already shown that exposure to DE upregulates the expression of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on capillary endothelial cells of the bronchial submucosa and induces a concomitant increase in the number of leukocyte function–associated antigen 1–positive (LFA-1+) leukocytes in the airway tissue of healthy human subjects (2). Chemokines are low-molecular-weight signaling proteins which direct the migration of leukocytes from the blood vessel toward the inflammatory focus (3). The effects of DE on chemokine production have been studied in rats (4) and in epithelial and macrophage cell cultures exposed to DEP *in vitro* (5–8), and these have demonstrated increased production of interleukin-8 (IL-8) and macrophage inflammatory protein-1α (MIP-1α).

Cytokines are low-molecular-weight proteins which play an important role in the modulation of inflammatory responses, specifically, cellular activation, proliferation, differentiation, adhesion, migration, and synthesis of acute phase proteins. Exposure to DE has been shown to increase the production of several cytokines both *in vivo* in animal exposure studies and *in vitro*, including interleukin-1 (IL-1), IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, tumor necrosis factor-alpha (TNF-α), granulocyte macrophage colony–stimulating factor (GM-CSF), and MIP-1α (4–14). Although these studies have clearly demonstrated that DEP can induce the production of several proinflammatory cytokines, the concentrations of DEP used in these studies have been extremely high and over prolonged periods of time, levels that are unlikely to be experienced in ambient settings. Also, the wide variation in the proinflammatory responses of different animal species to various air pollutants makes it difficult to extrapolate these findings to humans. Similarly, the *in vitro* cell culture studies do not take into account the various defense mechanisms present *in vivo*, such as the protective epithelial lining fluid containing various antioxidants and anti-inflammatory mediators.

There have been no controlled exposure studies which have addressed the *in vivo* effects of DE on chemokine and cytokine production in the lower airways of healthy human subjects. We hypothesized that the leukocyte infiltration and the various inflammatory responses induced by DE exposure in healthy human airways were mediated by enhanced chemokine and cytokine production by resident cells of the airway tissue and lumen. The aim of this study was therefore to investigate the effects of acute exposure of DE on changes in cytokine gene transcription and protein production in the proximal bronchial tissue and in cells obtained from bronchial wash (BW) of healthy human volunteers.

METHODS

Subject Recruitment and Exposure Protocol

Fifteen healthy nonsmoking volunteers (11 male, 4 female), mean age 24 (range 21 to 28) yr, with no history of asthma, respiratory or other illnesses, normal lung function, and negative skin prick tests to common airborne allergens were recruited. The study was approved by the local ethics committee, and each subject gave his or her written informed consent. Each subject was exposed to air or DE for 1 h in a specially built diesel exposure chamber according to a previously described standard protocol (2, 15) on two different occasions in a randomized sequence, at least 3 wk apart. The DE was generated from an idling Volvo diesel engine and the exposures were standardized by keeping the concentration of particulates with a mass median diameter of less than 10 μm (PM_{10}) at 300 $\mu g/m^3$ which was associated with concentrations of NO_2 of 1.6 ppm, NO of 4.5 ppm, CO of 7.5 ppm, total hydrocarbons of 4.3 ppm, formaldehyde of 0.26 mg/m^3, and 4.3×10^6 suspended particles/cm^3. The exposure protocol has been described in detail previously (2, 3). The subjects were unaware of the actual exposure, and during each exposure they alternated moderate exercise (minute ventilation [V_E] = 20 L/min/m^2) on a bicycle ergometer with rest at 15-min intervals.

Bronchoscopies

Six hours after the end of each exposure, fiberoptic bronchoscopy was performed to obtain endobronchial biopsies and bronchoalveolar lavage (BAL) samples. The bronchoscopy procedure has been described in detail previously (2). A proximal BW with 2 × 20 ml sterile phosphate-buffered saline (PBS) (pH 7.3, 37° C) was obtained with the tip of the bronchoscope carefully wedged into the lingula or middle lobe bronchus.

Processing of Samples

Mucosal biopsies obtained during bronchoscopy were placed in ice-cooled acetone containing protease inhibitors and processed into glycolmethacrylate (GMA) resin for immunohistochemical staining as previously described (16). Biopsies obtained for reverse transcriptase/polymerase chain reaction enzyme-linked immunosorbent assay (RT-PCR ELISA) analysis were immediately suspended in liquid nitrogen, until further analysis was carried out.

The BW samples were collected into a siliconized container placed in iced water, filtered through a nylon filter (pore diameter 100 μm; Syntab AB, Malmö, Sweden) and centrifuged at 400 *g* for 15 min. Cell pellets were resuspended in PBS at 10^6 cells/ml and differential counts were measured on cytocentrifuge slides stained with May-Grunwald Giemsa, counting 400 cells per slide.

Immunohistochemistry

The biopsy specimens were stained according to Britten and coworkers (16). Briefly, the GMA sections were cut at 2 μm in thickness and floated onto ammonia water (1:500), picked onto 0.01% poly-L-lysine glass slides, and allowed to dry at room temperature for 1 h. The sections were treated with 0.1% sodium azide and 0.3% hydrogen peroxide in distilled water to block endogenous peroxidase. Nonspecific antibody binding was blocked with undiluted culture supernatant for 30 min followed by the primary monoclonal antibody (mAb) (Table 1) which was applied and incubated at room temperature overnight. After rinsing in TRIS-buffered saline (TBS), biotinylated rabbit anti-mouse IgG Fab (Dako Ltd, High Wycombe, UK) was applied for 2 h, followed by the streptavidin–biotin horseradish peroxide complex (Dako Ltd) for another 2 h. After rinsing in TBS, sections for submucosal analysis were developed with aminoethyl carbamazole (AEC) in acetate buffer (pH 5.2) and hydrogen peroxide to develop a peroxide-dependent red color reaction, whereas sections for epithelial measurements were developed as a brown color with 3,3-diaminobenzidine (DAB). All sections were counterstained with Mayer's hematoxylin.

The biopsies were stained with mAbs directed against specific cytokines, including IL-4, IL-5, IL-6, IL-8, TNF-α, GM-CSF, growth-regulated oncogene-alpha (GRO-α), and epithelial neutrophil activating peptide-78 (ENA-78), which were all used for submucosal analysis. Epithelial analysis was performed on sections stained with mAbs directed against IL-8, GM-CSF, GRO-α, and ENA-78. TBS and the mAbs IgG_1 and IgG_{2b} were used as negative controls.

TABLE 1

CYTOKINE MONOCLONAL ANTIBODIES USED FOR IMMUNOHISTOCHEMISTRY

Antibody	Clone	Catalog No.	Company
GRO-α	20,326.1	MAB 275	R&D Systems
IL-8	NADII	BMS 136	Bender Medical System
IL-5	14,611.3	MAB 205	R&D Systems
IL-4	4D9	211-44-134A	Nordic Biosite AB
IL-4	3H4	211-44-134B	Nordic Biosite AB
IL-6	—	1618-01	Genzyme Diagnostics
GM-CSF	—	ZM-213	Genzyme Diagnostics
ENA-78	33,160.111	MAB 254	R&D Systems
TNF-α	18,255.121	MAB 210	R&D Systems

Quantification of Immunostaining

Quantification of stained cytokine immunoreactivity was performed separately in the epithelium and in the submucosa according to a previously described protocol (17). Briefly, epithelial analysis was performed on sections developed with DAB as the substrate, whereas submucosal analysis was performed on sections developed with AEC. The epithelial expression of each cytokine was quantified with the assistance of computer-aided image analysis (QWin, Leica Q500IW; Leica, Cambridge, UK) and expressed as the percentage of epithelial area with positive immunostaining as compared with the total epithelial area. In the submucosa, the total number of positive cells were counted using a light microscope and expressed as cells/mm^2. The submucosal area was assessed by computer image analysis excluding all areas with smooth muscle, glands, large blood vessels, and torn or folded tissue.

RT-PCR ELISA

RT-PCR ELISA was used to quantify relative changes in cytokine messenger RNA (mRNA) gene transcripts as previously described (18). Briefly, total RNA was extracted from bronchial biopsies and BW cells, precipitated with isopropanol and washed in 80% ethanol. Using equal amounts of total RNA from paired samples, poly(A) mRNA was reverse transcribed to produce complementary DNA (cDNA). PCR amplification of the cDNA was carried out using primer pairs specific for the constitutively expressed gene adenine phosphoribosyl transferase (APRT) and the cytokines IL-1β, IL-4, IL-5, IL-8, TNF-α, interferon gamma (IFN-γ), and GM-CSF (for primer nucleotide sequences; Reference 18). A volume of 2.5 μl cDNA (1/10th of the reverse transcription product) was amplified using 1 U Taq DNA polymerase (Promega, UK) in the presence of 15 pmol of both primers, 2.5 μl PCR digoxigenin (DIG) labeling mix (0.2 mM deoxyadenosine triphosphate [dATP], deoxycytidine triphosphate [dCTP], deoxyguanosine triphosphate [dGTP]; 0.19 mM deoxythymidine triphosphate [dTTP]; 0.01 mM digoxigenin-11–deoxyuridine triphosphate [DIG-11-dUTP]) (Boehringer Mannheim, Mannheim, Germany), magnesium-free PCR buffer (50 mM KCl, 10 mM TRIS-HCl, pH 9.0, 0.1% TritonX-100) (Promega, Southampton, UK), and 1 mM $MgCl_2$. Target cDNA was amplified using a three-temperature PCR, with primer annealing temperatures optimized for each cytokine (56° C for APRT; 50° C for IL-1β, IL-4, IL-5, and IL-8; 54° C for TNF-α and IFN-γ; and 60° C for GM-CSF).

Ten microliters of the PCR product was then denatured with 40 μl denaturation solution from the PCR ELISA kit (Boehringer Mannheim) for 15 min. Biotinylated labeled "capture probes" (18) specifically designed to hybridize with each cytokine PCR product were then hybridized to the complementary DIG-labeled PCR product and immobilized on duplicate wells of streptavidin-coated microtiter plates at 37° C for 3 h. After thorough washing to remove free antibody, bound PCR products were detected by incubation for 30 min at 37° C with an anti-DIG antibody conjugated to horseradish peroxidase, followed by reaction with the substrate ABTS (2, 2′-azino-di-[3-ethyl benzthiazoline sulfonate]). During green color development the absorbance (at 405 nm) was measured with an ELISA plate reader. Absorbance readings were taken after 10 min and at 5-min intervals thereafter, up to 30 min. The upper limit of the colorimetric detection system used in this PCR ELISA was reached before the full 30-min color development had elapsed; therefore a time point of 20 min, when the absorbance values were still within the dynamic range of the ELISA, was used throughout. Mean absorbance values (at 20 min) were calculated for the duplicate samples. Before comparing between treatments (exposure to air and exposure to DE), the levels of cytokine transcripts were normalized to APRT and expressed as a percentage (level of cytokine products/level of APRT product × 100).

Optimizing the PCR for semiquantitative analysis. In preliminary experiments cDNA samples were subjected to 20, 25, 30, 35, and 40 PCR cycles in the presence of DIG labeling mix and levels of PCR products measured using the RT-PCR ELISA. Absorbance readings were plotted against PCR cycle number to identify the range over which PCR products were accumulating exponentially. For the housekeeping gene APRT, and the cytokines listed above, PCR products were still accumulating exponentially after 30 PCR cycles (data not shown). Measurement of levels of PCR products for APRT, IL-1β, IL-5, IL-8, TNF-α, IFN-γ, and GM-CSF in bronchial biopsies were therefore made after 30 PCR cycles.

Statistical Analyses

The subjects acted as their own controls. Immunohistochemical and cytokine PCR variables were analyzed using Wilcoxon's paired rank test (SPSS for Windows Version 8). Values of $p < 0.05$ were considered significant.

RESULTS

Constitutive Expressions of Cytokine mRNA in the Healthy Bronchial Tissue and Cells Obtained from BW

There was constitutive gene expression for IL-1β, IL-5, IL-8, TNF-α, and IFN-γ in the proximal bronchial tissue and in the cells obtained from BW, and GM-CSF mRNA expression in the bronchial tissue of healthy human subjects at baseline values. IL-8 and IFN-γ gene transcripts were expressed at relatively higher levels in the bronchial tissue when compared with cells from BW, whereas IL-1β and TNF-α mRNA levels were expressed at relatively higher levels in BW cells. The healthy bronchial tissue expressed IL-5 mRNA transcripts constitutively, whereas these were expressed minimally in BW fluid cells. IL-4 mRNA was expressed at very low levels in the bronchial tissue and was undetectable in BW cells (Table 2).

TABLE 2

RELATIVE BASELINE EXPRESSIONS FOR CYTOKINE mRNA TRANSCRIPTS IN THE BRONCHIAL TISSUE AND BW CELLS*

Cytokine mRNA	Bronchial Tissue	BALF Cells
IL-1β	30.3% (9.5–75.8)	60.4% (39.9–70.1)
IL-4	0.3% (0.2–0.4)	0.0% (0.0–0.2)
IL-5	10.3% (7.6–26.7)	1.1% (0.4–3.3)
IL-8	54.6% (50.7–61.0)	16.4% (9.8–28.6)
TNF-α	21.5% (9.6–43.2)	73.1% (44.9–105.3)
IFN-γ	45.7% (17.8–53.7)	10.6% (1.7–35.1)
GM-CSF	8.5% (3.2–13.1)	Not detected

* Values expressed as %APRT; median (interquartile range).

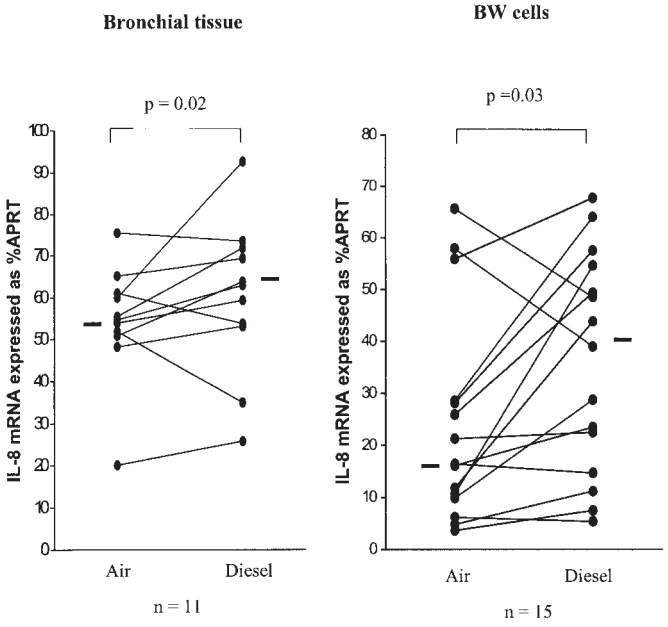

Figure 1. Increased expressions of IL-8 mRNA levels in the bronchial tissue and BW cells after acute exposure to DE.

TABLE 3

CHANGES IN CYTOKINE mRNA IN THE BRONCHIAL TISSUE 6 h AFTER ACUTE EXPOSURE TO DIESEL EXHAUST*

Cytokine mRNA	Air	Diesel	p Value
IL-1β	30.3 (9.5–75.8)	50.7 (16.15–70.5)	NS
IL-4	0.3 (0.2–0.4)	0.2 (0.1–0.3)	NS
IL-5	10.3 (7.6–26.7)	22.9 (13.7–29.0)	0.09
IL-8	54.6 (50.7–61.0)	63.8 (53.7–73.4)	0.02
TNF-α	21.5 (9.6–43.2)	19.1 (14.8–35.2)	NS
IFN-γ	45.7 (17.8–53.7)	33.7 (22.8–50.0)	NS
GM-CSF	8.5 (3.2–13.1)	8.6 (2.2–12.9)	NS

Definition of abbreviation: NS = not significant
* Values expressed as %APRT; median (interquartile range).

Upregulation of IL-8 mRNA in the Bronchial Tissue and BW Fluid Cells 6 h after Acute Exposure to DE

Six hours after acute exposure to DE there was a 17% median relative increase in the expression of IL-8 mRNA gene transcripts in the bronchial tissue ($p = 0.02$) and a 240% median relative increase in IL-8 mRNA gene transcripts in BW cells ($p = 0.03$) (Figure 1, and Tables 3 and 4).

Increased IL-8 and GRO-α Protein in the Bronchial Epithelium after Exposure to DE

Immunohistochemical analysis of the neutrophil attractant factors demonstrated constitutive baseline expressions for IL-8 and GRO-α in the bronchial epithelium. DE induced a 198% median relative increase in area staining for IL-8 [Air:Diesel—2.3 (0.7 to 2.9) : 4.5 (1.7 to 7.1); $p = 0.04$, values expressed as percentage of epithelial area with positive immunostaining, median (interquartile range)] and a 229% median relative increase in GRO-α staining [Air:Diesel—0.9 (0.6 to 1.3) : 2.0 (1.4 to 6.2); $p = 0.01$, values expressed as percentage of epithelial area with positive immunostaining, median (interquartile range)] in the bronchial epithelium 6 h postexposure (Figures 2–6). There were no differences in GM-CSF or ENA-78 immunostaining in the epithelium. Immunohistochemical analysis in the submucosa revealed only occasional stained cells without any difference between exposures.

Increased Expression of IL-5 mRNA in the Bronchial Tissue after Exposure to DE

After exposure to DE, nine of 11 subjects demonstrated a relative increase in the expression of IL-5 mRNA gene transcripts in the bronchial tissue (median increase 230%), but this did not reach statistical significance ($p = 0.09$). There was no change in the expression of IL-5 mRNA gene transcripts in the BW cells (Figure 7, Tables 3 and 4).

TABLE 4

CHANGES IN CYTOKINE mRNA IN THE BW CELLS 6 h AFTER ACUTE EXPOSURE TO DIESEL EXHAUST*

Cytokine mRNA	Air	Diesel	p Value
IL-1β	60.4 (39.9–70.1)	57.9 (31.8–81.0)	NS
IL-4	0.0 (0.0–0.2)	0.0 (0.0–0.3)	NS
IL-5	1.1 (0.4–3.3)	0.6 (0.4–2.0)	NS
IL-8	16.4 (9.8–28.6)	38.9 (14.7–54.6)	0.03
TNF-α	73.1 (44.9–105.3)	85.9 (71.7–94.7)	NS
IFN-γ	10.6 (1.7–35.1)	13.2 (5.8–21.6)	NS

* Values expressed as %APRT; median (interquartile range).

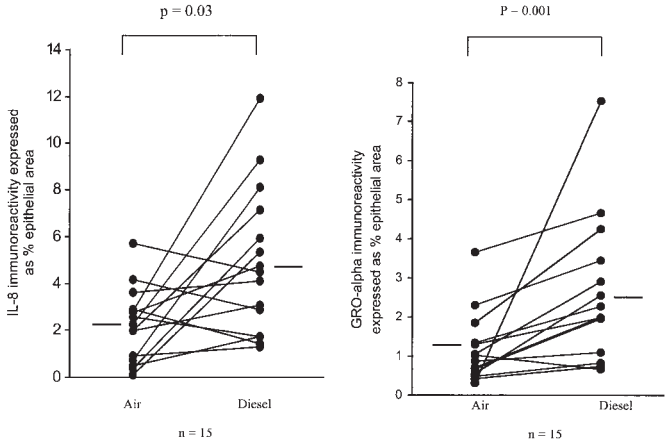

Figure 2. Increased IL-8 and GRO-α immunoreactivity in the bronchial epithelium after acute exposure to DE.

Changes in the Expression of Other Cytokines

There were no changes in the mRNA gene transcript levels for IL-1β, IL-4, TNF-α, IFN-γ, and GM-CSF after exposure to DE compared with air, either in the bronchial tissue or in BW cells (Tables 3 and 4), and no differences in immunoreactivity toward IL-4, IL-5, IL-6, IL-8, TNF-α, GM-CSF, GRO-α, and ENA-78 in the bronchial submucosa (data not shown).

DISCUSSION

Using RT-PCR ELISA, a novel and sensitive technique for quantifying relative amounts of cytokine mRNA gene transcripts in the bronchial tissue and airway lavage cells, and immunohistochemical staining with computer-assisted image analysis to quantify expression of cytokine protein in the bronchial tissue, we have demonstrated that DE enhances gene transcription of IL-8 in the bronchial tissue and airway cells of healthy human subjects, along with increases in IL-8 and GRO-α protein expression in the bronchial epithelium. DE also tended to increase the levels of IL-5 mRNA gene transcripts in the bronchial tissue, whereas there were no significant changes in the gene transcript levels of IL-1β, TNF-α, IFN-γ, and GM-CSF either in the bronchial tissue or BW cells

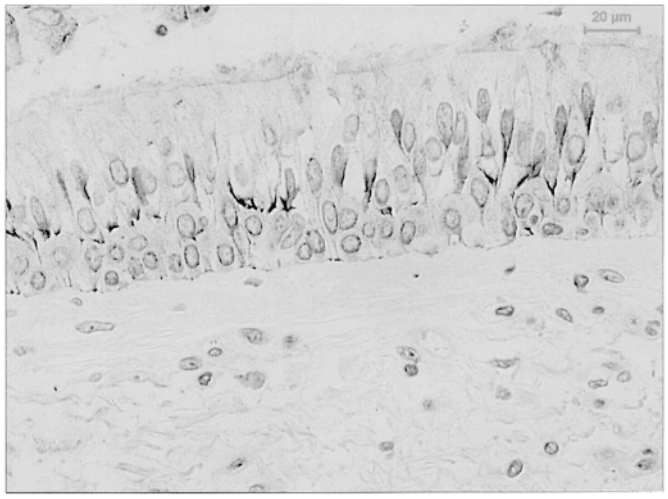

Figure 3. Immunohistochemical staining for IL-8 in the bronchial epithelium after air exposure.

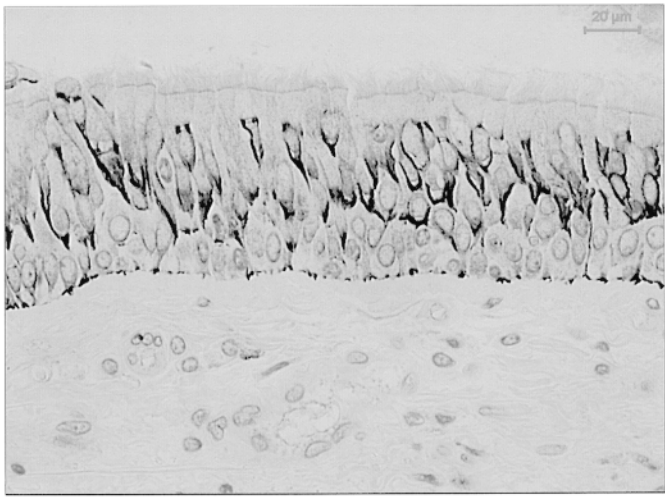

Figure 4. Immunohistochemical staining for IL-8 in the bronchial epithelium after diesel exposure.

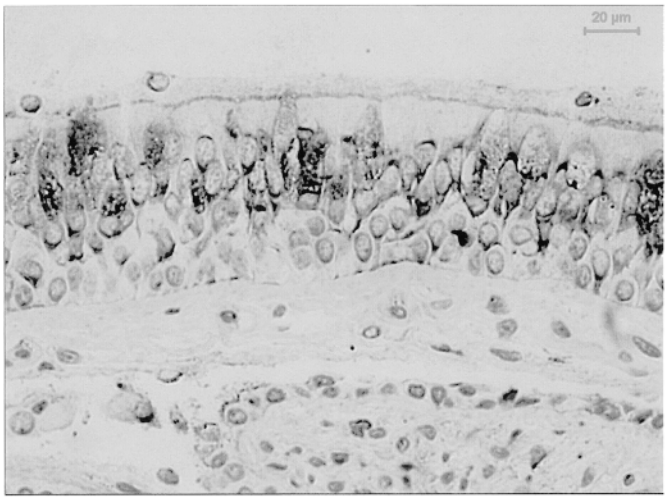

Figure 6. Immunohistochemical staining for GRO-α in the bronchial epithelium after diesel exposure.

at this time point. To our knowledge this is the first human *in vivo* study demonstrating an effect of exposure to DE on chemokine and cytokine production in the lower airways of healthy human subjects.

IL-8 and GRO-α are CXC chemokines which possess a number of biological effects on various cells, with a predominant effect on neutrophils. The major role of these chemokines is to attract and activate leukocytes. IL-8 induces neutrophil chemotaxis by activating the contractile cytoskeleton; increases adherence to unstimulated endothelial cells, fibrinogen, and matrix proteins; upregulates cell surface expression of Mac-1 (CD11/CD18), β_2 integrin subunits, and the complement receptor type 1; and induces L-selectin shedding from leukocytes. In addition, IL-8 and GRO-α induce transendothelial migration of leukocytes, stimulate degranulation and release of proteolytic enzymes from neutrophil intracellular storage granules, enhance production of leukotriene B_4 (LTB_4) and 5-hydroxyeicosatetraenoic acid (5 HETE) from exogenous arachidonate, and induce a respiratory burst with the formation of O_2^- and H_2O_2 (19–21). T lymphocytes and eosinophils have also been reported to migrate in response to IL-8 (20, 22, 23). IL-8 induces release of eosinophil peroxidase (24), and is a potent eosinophil activator when associated with secretory IgA (23). IL-8 possesses chemotactic activity for human basophils and induces the release of histamine (25) and peptidoleukotrienes (26) when basophils are pretreated with IL-3, IL-5, or GM-CSF (27). It is noteworthy that IL-8 is an extremely stable protein which maintains its biological activity even in the presence of significant changes in pH and proteolytic enzymes suggesting that once produced it may exert a prolonged biological activity (19).

In this study, baseline expression of IL-8 mRNA was relatively higher in the bronchial tissue as compared with BALF cells (54.6% versus 16.4%), which suggests that IL-8 mRNA is mainly expressed by resident cells of the airway tissue. Potential

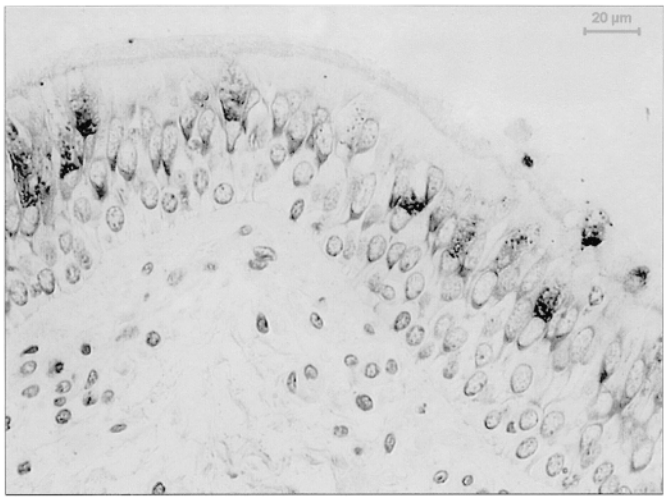

Figure 5. Immunohistochemical staining for GRO-α in the bronchial epithelium after air exposure.

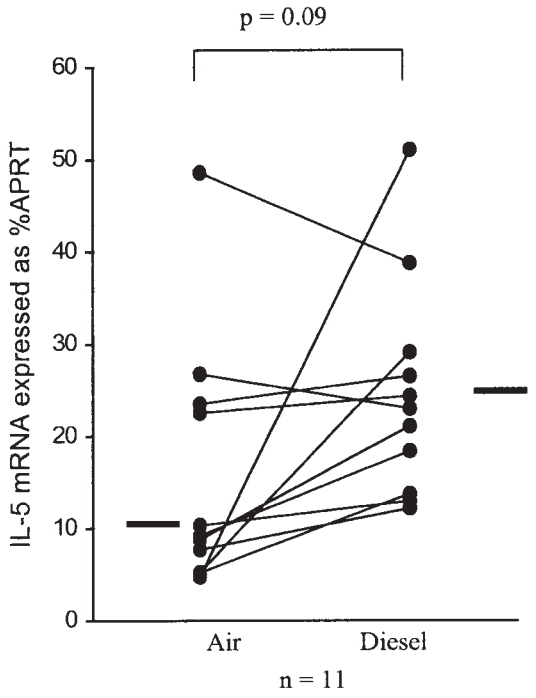

Figure 7. Increased expression of IL-5 mRNA in the bronchial tissue after acute exposure to DE.

cellular sources for IL-8 in the lung include monocytes, macrophages, neutrophils, T lymphocytes, B lymphocytes, eosinophils, mast cells, epithelial cells, endothelial cells, and fibroblasts (19, 28–30). Although monocytes, macrophages, and neutrophils are considered to be the major cellular sources for IL-8 in the lung, especially since they produce large amounts of IL-8 after stimulation with particles, bacteria, and antigens (31), these cells express only low levels of IL-8 mRNA at baseline. In contrast, ciliated human bronchial epithelial cells have been demonstrated to express large amounts of IL-8 mRNA gene transcripts in culture, at levels which are comparable to those produced by lipopolysaccharide (LPS)-activated monocyte–macrophage cells (30, 32). The increased baseline expression of IL-8 mRNA in the bronchial tissue compared with BW cells is therefore likely to represent bronchial epithelial cell expression.

DE induced a 17% relative median increase in IL-8 mRNA levels in bronchial tissue and a 240% relative increase in the BW cells 6 h after exposure. It is difficult to pinpoint the cellular source for increased IL-8 mRNA levels following DE exposures in these two compartments, because total RNA was extracted from the full bronchial tissue and from the total BW cell pellet. The airway epithelium is one of the first cell types to come in contact with inhaled particles and oxidant gases present in DE. *In vitro* exposure of human bronchial epithelial cells to NO_2 has been shown to increase the production of IL-8, GM-CSF, and TNF-α (30). Moreover, primary human bronchial epithelial cell cultures exposed to DEP for 24 h produce significantly increased amounts of IL-8 (5), suggesting that epithelial cells are a likely source of the increased IL-8 mRNA gene transcripts observed in the bronchial epithelium in this study. DEP can upregulate H_1 histamine receptors on airway epithelial cells and enhance IL-8 production in the presence of histamine (33). We have previously demonstrated that DE exposure increases histamine release in the airways (2), and this may be an additional mechanism for the increased IL-8 gene transcripts observed in this study. Neutrophils and macrophages express low levels of IL-8 mRNA at baseline. Following particle phagocytosis these cells can become activated to produce large amounts of IL-8 (12, 31). We have previously demonstrated increased numbers of neutrophils in both bronchial tissue (fourfold increase in the epithelium and threefold increase in the submucosa) and airway lavage fluid (threefold increase) after DE exposure (2), which could account for the increased IL-8 mRNA levels observed in BW cells after DE exposure in this study.

Oxidant gases such as NO_2 and various transition metals present in the DE are capable of generating reactive oxygen intermediate species (ROI), which can activate the redox-sensitive transcription factors, nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1). The promoter sequences of the IL-8 and GRO-α genes both have putative binding sites for NF-κB and AP-1 (34), and activation of these transcription factors by ROI present in DE could explain increased IL-8 gene transcription. Low levels of ROI are also produced by neutrophils and macrophages as a normal part of their cellular metabolism (35), and after particle phagocytosis they produce increased levels of ROI which could further enhance IL-8 gene transcription.

Increased gene transcription of IL-8 does not necessarily mean that the protein is produced and released. Using immunohistochemical staining with computer-assisted image analysis we have demonstrated increased IL-8 and GRO-α immunoreactivity in the bronchial epithelium, thereby suggesting that increased IL-8 mRNA gene transcription is associated with, or followed by, increased production of IL-8 protein. Increased IL-8 protein expression in the bronchial epithelium after DE exposure could be due to activation of epithelial cells or increased numbers of IL-8-producing leukocytes in the epithelium. Although it is difficult to ascribe a cellular source, the pattern of IL-8 staining suggests that epithelial cells are the more likely source. We have previously shown that IL-8 protein levels measured by ELISA in BW fluid increase 50% after DE exposure, although this is not a significant increase (2). It is likely that IL-8 protein produced by 6 h was held in complexed forms associated with cell surface or extracellular matrix heparin sulfate or glycosaminoglycans in the bronchial tissue (36). Another possible explanation is that release of IL-8 protein occurs at a later time point, as has been demonstrated *in vitro* in human epithelial cell cultures 24 to 48 h after DE.

The cytokine IL-5 is implicated in the recruitment, differentiation, activation, and survival of eosinophils in the airways, and in addition promotes the differentiation of basophils and mediates histamine release (18). In this study, constitutive expression of IL-5 mRNA was higher in the bronchial tissue compared with BALF cells at baseline values (10.3% versus 1.1%), suggesting that resident airway cells are the major cellular sources for IL-5 mRNA. We have recently demonstrated that human bronchial epithelial cells constitutively express IL-5 gene transcripts (18), which would account for the higher amount of IL-5 gene transcripts observed in the bronchial tissue at baseline. DE induced a 220% median increase in IL-5 mRNA levels in the bronchial tissue in nine of 11 subjects analyzed, which was however not statistically significant. In contrast, DE did not alter IL-5 mRNA levels in BW cells. Several *in vivo* animal exposure studies have recently demonstrated increased IL-5 production in the lung tissue after inhalation or intratracheal instillation of DEP with antigen (9–11). However, this is the first report to demonstrate that DE affects IL-5 gene transcription in human bronchial tissue. Apart from airway epithelial cells, there are several other potential cellular sources for IL-5 mRNA in the bronchial tissue, viz, T helper type 2 (Th2) and T cytotoxic type 2 (Tc2) type CD4+ and CD8+ T cells, mast cells, eosinophils, and basophils. Although it is difficult to ascribe a cellular source for the increased IL-5 mRNA levels observed in the bronchial tissue after DE exposure, we believe it is likely that epithelial cells are the primary source. Despite the trend toward an increase in IL-5 mRNA levels in the bronchial tissue after DE exposure, there was no difference in the bronchial IL-5 immunoreactivity at this time point, suggesting that increased IL-5 gene transcripts had not been translated into protein at this time point.

High ambient levels of particulate matter have been associated with increased rates of asthma exacerbations, increased need for asthma medications, and reductions in lung function. Although most studies have emphasized the importance of eosinophils and lymphocytes in the allergic airway inflammation, recent studies have suggested that chemokines and neutrophilic inflammation could also contribute to the pathophysiology of asthma and allergic rhinitis. Several studies have demonstrated increased production of IL-8 in the airways and nasal secretions of asthmatic and rhinitic subjects (29–32) and as described previously, IL-8 has a variety of proinflammatory biological effects relevant to asthma. Recently, IL-8 has also been demonstrated to play a significant role in IgE-mediated lung inflammation (37). In this study DE exposure induced an increase in IL-8 production in the human airways associated with neutrophilic and lymphocytic infiltration of the airways mucosa. These findings together suggest a plausible mechanism for the association observed between increased ambient particulate matter concentrations and asthma exacerbations.

Various *in vivo* and *in vitro* DE exposure studies have demonstrated increased production of several cytokines including

IL-1, IL-2, IL-4, IL-6, IL-10, TNF-α, GM-CSF, and MIP-1α and decreased production of IFN-γ (4–14). In the present study we did not find any differences in the mRNA levels of IL-1β, IL-4, TNF-α, IFN-γ, or GM-CSF. There are several possible explanations for this apparent discrepancy. The concentrations of diesel exhaust used in the various animal *in vivo* studies have been extremely high and the duration of exposure has been prolonged or repeated. In most animal exposure studies DEP was administered intratracheally, often in combination with allergens or antigens. Moreover, some of the cytokine changes seen in the animal studies may reflect the concurrent use of allergen or aspects of the challenge procedure that are not adequately controlled. The inflammatory response after inhalation of toxic substances in the lung is a highly dynamic process. Changes in cytokine gene transcripts in the bronchial tissue and airway lavage cells in this study were assessed at a single time point of 6 h after DE exposure, whereas most of the cytokine changes observed in previous animal exposure studies were found 24 to 48 h postexposure. It seems possible that the other cytokine genes may be transcribed later than 6 h, and it would be interesting to repeat our study using later time points.

Nevertheless, we have demonstrated for the first time that short-term exposure to DE at high ambient concentration, levels which have been recorded in several cities of the world (38), induced increased IL-8 gene transcription in the airways of healthy human subjects. This was associated with increased IL-8 and GRO-α protein expression in the bronchial epithelium and an accompanying trend toward an increase in IL-5 mRNA gene transcripts in the bronchial tissue. These observations suggest a plausible mechanism for DE-induced airway leukocyte infiltration, and hence a possible explanation for the association observed between ambient levels of particulate matter and various respiratory health outcome indices noted in epidemiological studies.

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