Acute Inflammatory Responses in the Airways and Peripheral Blood After Short-Term Exposure to Diesel Exhaust in Healthy Human Volunteers

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Several epidemiologic studies have demonstrated a consistent association between levels of particulate matter (PM) in the ambient air with increases in cardiovascular and respiratory mortality and morbidity. Diesel exhaust (DE), in addition to generating other pollutants, is a major contributor to PM pollution in most places in the world. Although the epidemiologic evidence is strong, there are as yet no established biological mechanisms to explain the toxicity of PM in humans. To determine the impact of DE on human airways, we exposed 15 healthy human volunteers to air and diluted DE under controlled conditions for 1 h with intermittent exercise. Lung functions were measured before and after each exposure. Blood sampling and bronchoscopy were performed 6 h after each exposure to obtain airway lavages and endobronchial biopsies. While standard lung function measures did not change following DE exposure, there was a significant increase in neutrophils and B lymphocytes in airway lavage, along with increases in histamine and fibronectin. The bronchial biopsies obtained 6 h after DE exposure showed a significant increase in neutrophils, mast cells, CD4+ and CD8+ T lymphocytes along with upregulation of the endothelial adhesion molecules ICAM-1 and VCAM-1, with increases in the numbers of LFA-1+ cells in the bronchial tissue. Significant increases in neutrophils and platelets were observed in peripheral blood following DE exposure. This study demonstrates that at high ambient concentrations, acute short-term DE exposure produces a well-defined and marked systemic and pulmonary inflammatory response in healthy human volunteers, which is underestimated by standard lung function measurements. Salvi S, Blomberg A, Rudell B, Kelly F, Sandtröm T, Holgate ST, Frew A. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers.

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Air pollution, especially from vehicular exhaust, has become an almost inescapable part of urban life throughout the world. Recent health concerns have emerged over pollutants generated from this source—particulate matter (PM) in particular, which, according to World Health Organization (WHO) estimates, is linked with half a million excess premature deaths each year. There is now extensive evidence to suggest that exposure to increased levels of inhalable particulate pollutants is associated with increases in mortality and morbidity from cardiovascular and respiratory causes (1–5).

Diesel engines have recently gained wider usage because of their efficiency, robustness, and low running costs, and until recently were thought to be more environmentally friendly on account of considerably lower emission levels of carbon monoxide, carbon dioxide (CO_2) and hydrocarbons (6). However, diesel engines, in addition to generating significant amounts of nitrogen oxides, also generate up to 100 times more particles than similar-sized petrol engines (7, 8), and form a major contributor to atmospheric PM pollution in most places over the world. Particles generated from diesel exhaust (DE) are extremely small and are present in the nuclei or accumulation modes, with diameters of 0.02 μm and 0.2 μm, respectively (9). These submicronic particles, by virtue of their greater surface-area-to-mass ratio, can carry a much larger fraction of toxic compounds, such as hydrocarbons and metals, on their surface (6, 10). Importantly, they can remain airborne for long periods of time and deposit in greater numbers and deeper into the lungs than larger-sized particles (11, 12).

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Area sampling measurements used routinely to monitor respirable PM (i.e., particles with an aerodynamic diameter < 10 μm [PM_{10}]) may underestimate the levels inhaled by people, since studies have shown personal exposure levels several times higher than the corresponding area measurements (13, 14). Also, in keeping with concerns over the effects of smaller particles ($PM_{2.5}$) (15), the particle number may be more important than the mass concentration in producing a biological response (16, 17), and close to a busy road, particle numbers are increased by a factor far greater than their mass (12).

The WHO and the United Nations Environment Programme have stated that particulate matter pollution is the most serious global air pollution problem today. Among the world's 20 megacities (cities with population $\geqslant 10$ million), 12 of them have serious levels of PM pollution that are persistently above the WHO guidelines both for long-term averages and peak concentrations. The annual average levels of suspended PM across these cities are in the range of 200–600 μg/m^3, and peak concentrations are frequently above 1,000 μg/m^3. Five of the remaining cities have moderate to severe levels exceeding the WHO guidelines by a factor of up to two, while only three of the world's megacities have PM levels within the WHO guidelines (18). Importantly, recent epidemiologic studies have found associations of PM pollution with increased mortality and morbidity at levels even below current WHO guidelines (19).

Although there is sufficient epidemiologic evidence linking PM pollution with several indices of health outcomes, as yet there are no established biological mechanisms to explain their underlying toxicity in humans. We hypothesized that exposure to DE might induce inflammatory and mediator responses in the airways and peripheral blood. To investigate this, we exposed healthy human volunteers to diluted DE for 1 h in a specially built exposure chamber at high ambient concentrations that are commonly encountered in most busy cities of the developing world, several occupational settings, at bus stops, and bus garages, and during peak pollution episodes in the developed world. Fiberoptic bronchoscopy with mucosal biopsy and lavages were used to investigate the pulmonary inflammatory response.

METHODS

Subjects

Fifteen healthy nonsmoking volunteers (11 males, 4 females) mean age, 24 (range, 21–28) yr, were recruited. None had a history of asthma, respiratory, or other illnesses or were taking any medication. They had normal lung function and negative skin prick tests to common airborne allergens and had no respiratory tract infections for at least 6 wk prior to or during the study period. The study was approved by the local ethics committee and each subject gave their written informed consent.

Study Design

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 (20) on two different occasions in a randomized sequence, at least 3 wk apart. The subjects were unaware of the actual exposure, and during each exposure they performed moderate exercise (minute ventilation [$\dot{V}_E$] = 20 L/min/m^2) on a bicycle ergometer, which was alternated with rest at 15-minute intervals.

Exposure Protocol

The DE was generated from an idling Volvo diesel engine (Volvo TD45, 4.5 L, 4 cylinders, 1991, 680 rpm; Diesel fuel used was: cetane number 51; aromatics, 25% vol; polycyclic aromatic hydrocarbons [PAHs], 0.5% vol; sulphur 0.06% weight; carbon, 86.4% weight; hydrogen, 13.5% weight; nitrogen, less than 0.02% weight; and oxygen, less than 0.1% weight. The 10% vol boiling point is 200 °C, 50% vol boiling point is 282 °C, and 95% vol boiling point is 355 °C). Over 90% of the exhaust was shunted away, and the remaining part was diluted with air and fed into the exposure chamber at a steady-state concentration. During the exposures, air was sampled in the breathing zone of the subjects and was continuously monitored for nitrogen oxides (NO, NO_2), CO, particles (number/cm^3), and total hydrocarbons (measured as propane). Formaldehyde and particulate matter were collected on different filters and analyzed separately. The exposures were standardized by keeping the PM_{10} concentration at 300 μg/m^3, which was associated with concentrations of NO_2, 1.6 parts per million (ppm); NO, 4.5 ppm; CO, 7.5 ppm; total hydrocarbons, 4.3 ppm; formaldehyde, 0.26 mg/m^3 and 4.3×10^6 suspended particles/cm^3. The temperature and humidity in the chamber were controlled at 20° C and 50%, respectively.

Lung Function Tests

Standard lung function tests (peak expiratory flow rate [PEFR], FVC, FEV_1, $FEF_{25–75\%}$) were measured immediately before and after each exposure using a computerized pneumotach spirometer (Vitalograph R, Buckingham, UK).

Bronchoscopy

Six hours after the end of each exposure, fiberoptic bronchoscopy was performed to obtain endobronchial biopsy and lavage samples using a standardized procedure as described before (21). Biopsies were obtained from one side and lavage carried out on the contralateral side. This was reversed during the second bronchoscopy to avoid biopsy artifacts. Three biopsies were taken from the anterior portion of main carina and the subcarinae of the third- and fourth-generation airways on the right side or from the posterior part of the main carina and the corresponding subcarinae on the left side. A proximal bronchial wash (BW) with 2×20 ml and bronchoalveolar lavage (BAL) with 3×60 ml sterile phosphate-buffered saline (PBS) (pH 7.3, 37° C) were obtained after the tip of the bronchoscope was carefully wedged into the lingula or middle lobe bronchus.

Processing of Samples

Mucosal biopsies were placed in ice-cooled acetone containing protease inhibitors and processed into glycolmethacrylate (GMA) resin as previously described (22). The GMA blocks were stored in air tight containers at −20° C until used for immunostaining.

The BW and BAL samples were collected into a siliconized container placed in iced water. The samples were then filtered through a nylon filter (pore diameter, 100 μm; Syntab AB, Malmö, Sweden) and centrifuged at 400 *g* for 15 min. The supernatants were separated from the cell pellet and analyzed for albumin, protein, and LDH, the remainder being stored at −70° C for further analyses. Cell pellets were resuspended in PBS at 10^6 cells/ml. Differential counts were measured on cytocentrifuge slides stained with May-Grünwald Giemsa, counting 400 cells per slide. Lymphocyte subsets were determined by flow cytometry (FACS scan; Becton Dickinson, Oxon, UK). Albumin, total protein, and LDH were assayed by autoanalyzer using commercial kits from Boehringer Mannheim (Mannheim, Germany). Interleukin (IL)-8 and soluble intercellular adhesion molecule-1 (ICAM-1) were measured with commercial ELISA kits (R & D Systems, Inc., MN). Methylhistamine and fibronectin were determined with commercial RIA kits from Pharmacia (Uppsala, Sweden) and Dako Ltd. (High Wycombe, UK), respectively. The complement products C3a and C5a were quantified using RIA kits (Amersham, Buckinghamshire, UK).

Immunohistochemistry

The GMA sections were cut at 2 μm 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 to block endogenous peroxide. Nonspecific antibody binding was blocked with undiluted culture supernatant for 30 min, followed by the primary monoclonal antibody (mAb), which was applied and incubated at room temperature overnight. After rinsing in TRIS-buffered saline (TBS), biotinylated rabbit anti-mouse immunoglobin G (IgG) Fab (Dako Ltd.) was applied for 2 h, followed by the Streptavidin-biotin horseradish peroxide complex (Dako Ltd.) for another 2 h. After rins-

TABLE 1

CHANGES IN LUNG FUNCTION PARAMETERS IMMEDIATELY FOLLOWING EXPOSURES

	Air (*Mean* ± *SEM*)		Diesel Exhaust (*Mean* ± *SEM*)	
Parameter	Before	After	Before	After
PEFR, L	662 ± 44.5	656 ± 43.0	633 ± 39.7	609 ± 57.2
FEV_1, L/min	4.53 ± 0.26	4.52 ± 0.26	4.47 ± 0.25	4.48 ± 0.26
FVC, L	5.92 ± 0.32	5.93 ± 0.31	5.83 ± 0.30	5.92 ± 0.30
$FEF_{25-75\%}$, L	4.36 ± 0.37	4.38 ± 0.39	4.35 ± 0.37	4.21 ± 0.36

ing in TBS, amino ethyl carbamazole (AEC) in acetate buffer (pH 5.2) and hydrogen peroxide were used as substrate to develop a peroxide-dependent red color reaction. The sections were then counterstained with Mayer's hematoxylin.

The biopsies were stained with mAbs directed against specific cellular markers, which included neutrophils, lymphocytes (CD3+, CD4+, CD8+ cells), mast cells, macrophages, and eosinophils, and for the adhesion molecules ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), and their respective ligands leukocyte function-associated antigen-1 (LFA-1) and very late antigen-4 (VLA-4).

Quantification of Immunohistochemistry

Stained inflammatory cells were counted separately in the epithelium and in the submucosa, excluding mucosal glands, blood vessels, and muscle. The counts were expressed as cells/mm in the epithelium and cells/mm^2 in the submucosa. The length of the epithelium and the area of the submucosa were calculated using a computer-assisted image analysis program (Color Vision Software; Improvision System, Birmingham, UK). In the case of endothelial adhesion molecules, the number of blood vessels stained with a specific mAb was compared with the total complement of the microvessels identified by staining with the endothelial marker EN4, as described before (23). The number of vessels staining positive for specific adhesion molecules in consecutive 2-μm sections were expressed as a percentage of the total vessel population. Assessment of immunostaining was carried out by an observer blinded to the exposure protocol.

Blood Samples

Peripheral blood was collected 6 h after each exposure and was analyzed for total cells, differential count, and platelet counts using an autoanalyzer.

Statistical Analyses

The subjects acted as their own controls. Since the lung function data were normally distributed, Student's *t* test was used to compare variables before and after exposure. Blood, BAL, and immunohistochemistry indices were analyzed using Wilcoxon's paired rank test. p Values < 0.05 were considered significant.

RESULTS

There were no changes noted in the standard lung function parameters measured (PEFR, FVC, FEV_1, $FEF_{25-75\%}$) immediately following exposure to air or DE (Table 1).

In the BW obtained 6 h after DE exposure, there was an increase in the number of neutrophils compared with the control day (median, 1.86 vs 0.57×10^4 cells/ml; $p = 0.009$), while the macrophage numbers (median, 9.57 vs 7.36×104 cells/ml; $p = 0.08$) and lactic dehydrogenase activity (median, 77 vs 57 microkatals/L; $p = 0.08$) showed a tendency to increase.

In the BAL 6 h after DE exposure, flow cytometry showed an increase in B lymphocytes ($p < 0.05$) but no significant changes in HLA-DR+, CD3+, CD4+, CD8+, or CD25+ cells. DE exposure also induced an increase in BAL concentrations of methyl histamine (median, 0.06 versus 0.04 μg/L; $p < 0.05$) and fibronectin (median, 800 versus 760 μg/L; p =

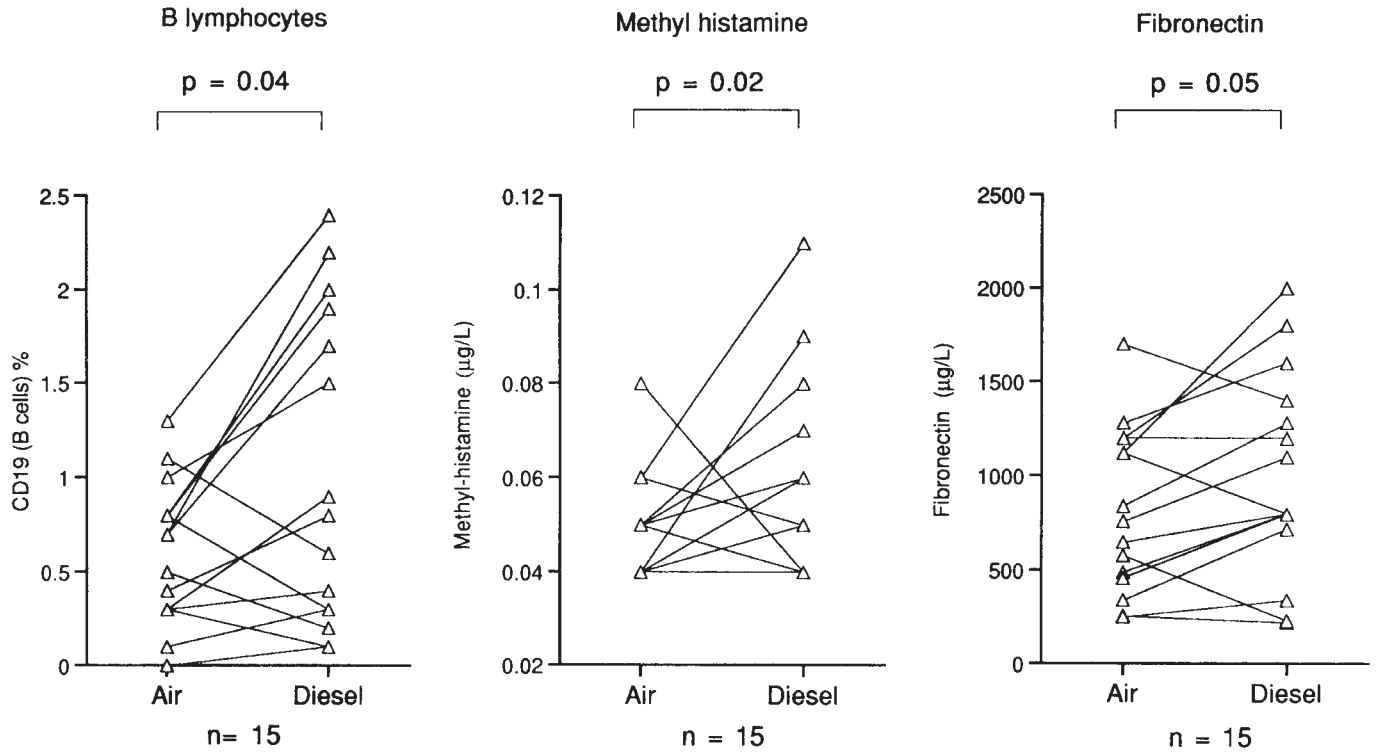

Figure 1. Changes in BAL after exposure to air and diesel exhaust.

TABLE 2

INFLAMMATORY CELLS IN THE BRONCHIAL TISSUE FOLLOWING EXPOSURE TO AIR OR DIESEL EXHAUST

Parameter	Air (*Median, IQR*)	DE (*Median, IQR*)	p Value
Neutrophils - Epi	0.83 (0.0–2.5)	3.69 (1.8–6.7)	0.01
Neutrophils - SM	21.8 (13.7–35.3)	59.9 (32.4–90.9)	0.003
Mast cells - Epi	0.0 (0.0–0.4)	0.0 (0.0–0.0)	NS
Mast cells - SM	8.8 (7.7–14.9)	32.7 (14.7–46.2)	0.002
CD3+ cells - Epi	3.8 (0.0–13.7)	23.5 (9.9–31.9)	0.01
CD3+ cells - SM	5.9 (1.2–18.5)	24.9 (9.3–59.9)	0.02
CD4+ cells - Epi	0.36 (0.0–0.79)	2.97 (1.4–6.09)	0.002
CD4+ cells - SM	3.28 (0.76–10.6)	13.1 (5.1–35.3)	0.04
CD8+ cells - Epi	2.47 (0.0–5.5)	7.2 (2.9–20.4)	0.04
CD8+ cells - SM	2.36 (0.27–15.5)	17.4 (2.9–37.1)	0.09

Definition of abbreviations: DE = diesel exhaust; Epi = epithelium; IQR = interquartile range; SM = submucosa, expressed as cells/mm in Epi and cells/mm^2 in SM.

0.05) (Figure 1) but no changes in the levels of total protein, albumin, IL-8, C3a, C5a, or soluble ICAM-1.

After DE exposure the bronchial biopsies immunostained for inflammatory cells exhibited an increase in the number of neutrophils in the submucosa and epithelium (Table 2) (Figure 2). Mast cell numbers were also increased in the submucosa. Total T lymphocytes were elevated both in the submucosa and in the epithelium, comprising an increase in the numbers of CD4+ cells both in the submucosa and epithelium, and CD8+ cells in the epithelium (Table 2). There were no submucosal or epithelial changes in the number of activated T lymphocytes (CD25+ cells), macrophages, eosinophils, or B lymphocytes.

In the bronchial submucosa there was constitutive basal expression of endothelial ICAM-1 (median, 49% vessels), E-selectin (median, 25% vessels), and P-selectin (25% vessels). Six hours after DE exposure, there occurred a marked increase in immunostaining for ICAM-1 and VCAM-1 (Table 3) (Figure 3), but no detectable changes in E-selectin and P-selectin expression (Table 3). Similarly, cells expressing the ligand for ICAM-1, LFA-1 (CD11a/CD18) were increased both in the submucosa and epithelium, while cells expressing the ligand for VCAM-1, VLA-4 (CD29/CD45a) showed a trend toward an increase in the submucosa (Table 3).

In the peripheral blood sampled 6 h after DE exposure, there was a significant increase in neutrophils ($p < 0.05$) and platelets ($p < 0.05$), while HLA-DR+ lymphocytes were significantly reduced when compared with the control day ($p < 0.05$) (Figure 4).

DISCUSSION

In this study carefully controlled exposure of healthy human volunteers for 1 h to diluted DE produced a marked cellular inflammatory response in the airways involving neutrophils, mast cells, and lymphocytes and accompanied by increased expression of endothelial adhesion molecules and their complementary ligands on inflammatory cells. These changes were associated with an increase in a number of inflammatory mediators in the airway lining fluid and a systemic response in the form of neutrophilia, thrombocytosis, and selective lymphope-

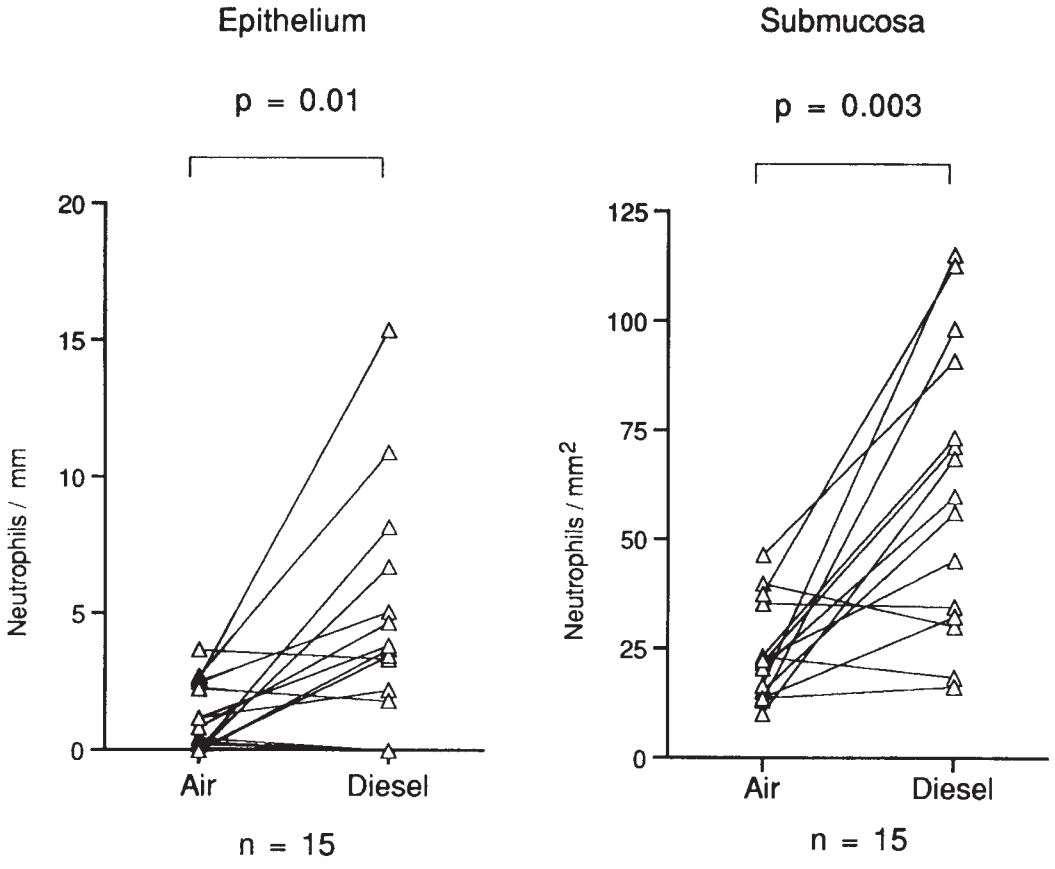

Figure 2. Neutrophils in the bronchial tissue after exposure to air and diesel exhaust.

TABLE 3

EXPRESSION OF ENDOTHELIAL AND LEUKOCYTE ADHESION MOLECULES IN THE BRONCHIAL TISSUE FOLLOWING EXPOSURE TO AIR AND DIESEL EXHAUST IN HEALTHY VOLUNTEERS

Parameter	Air (*Median, IQR*)	DE (*Median, IQR*)	p Value
ICAM-1 %	48.9 (27.6–58.6)	93.2 (83.8–95.8)	0.0007
VCAM-1 %	0.67 (0.0–3.5)	2.21 (0.8–5.6)	0.03
E-selectin %	24.5 (16.0–33.3)	23.5 (10.6–35.0)	NS
P-selectin %	25 (17.2–35.7)	30.7 (19.3–40.2)	NS
LFA-1+ cells - Epi	0.09 (0.0–2.7)	5.7 (3.0–22.4)	0.001
LFA-1+ cells - SM	1.4 (0.0–16.6)	43.9 (10.3–76.9)	0.01
VLA-4+ cells - Epi	0.0 (0.0–0.0)	0.0 (0.0–5.0)	NS
VLA-4+ cells - SM	0.69 (0.0–5.9)	5.77 (0.0–16.6)	0.08

For definition of abbreviations, *see* Table 2.

nia in the peripheral blood. Studies in experimental animals have shown that exposure to very high concentrations of DE is capable of producing an acute inflammatory response (24), but to our knowledge this is the first study describing such effects in healthy humans following exposure to concentrations of DE encountered in the environment.

The exposure system used in this study has been carefully developed and validated (20). The running conditions of the diesel engine generated DE with a constant composition, and concentrations of the key constituents were continuously monitored to ensure consistent exposure of all subjects throughout the study. The concentration of PM used in the DE exposure selected here falls within the high ambient range and is comparable to the PM_{10} concentrations encountered in enclosed spaces such as ferry decks, garages, bus stations, railway stations, tunnels, and mines, as well as in the congested streets of most major cities in the developing world (18, 25).

By combining BAL with bronchial biopsy, we have been able not only to demonstrate the neutrophilic response and mediator release but also to display some of the subjacent mechanisms, including mast cell activation and upregulation of adhesion molecules. Recognizing that particle deposition occurs throughout the lung but particles of different sizes will deposit differentially in the central and peripheral airways, we considered it important to analyze the proximal (BW) and distal airways (BAL) separately. In this study, the neutrophil response was most marked in the BW, while the lymphocyte and histamine responses were found in the distal (BAL) samples. This may partly reflect the different resident cell populations at the two sites, but is also consistent with the view that particles of different sizes may have distinct effects on the airways.

Concern has been expressed about the choice of an appropriate level of statistical significance in studies, such as the present work, which assess a range of inflammatory parameters simultaneously. The key to successful analysis is to ensure that the parameters being studied are appropriate and are de-

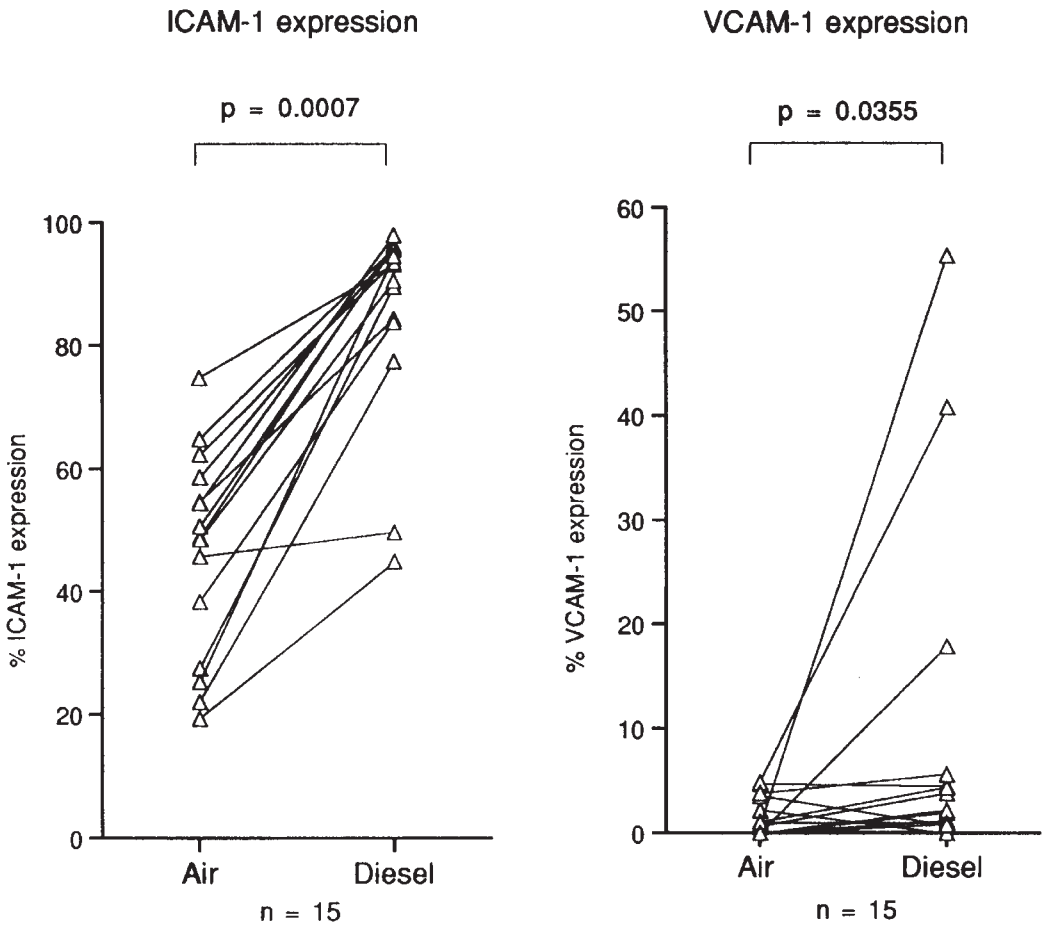

Figure 3. Expression of endothelial adhesion molecules after exposure to air and diesel exhaust.

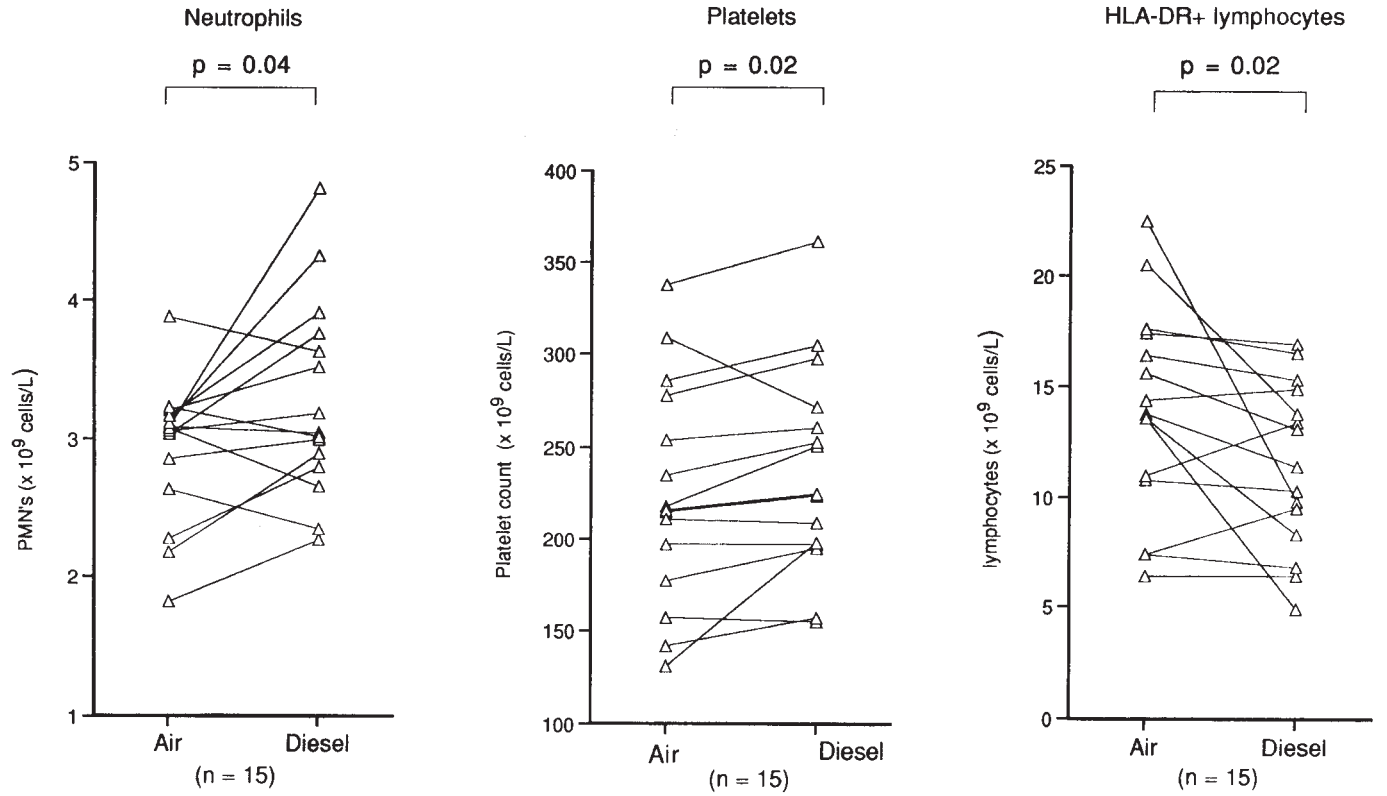

Figure 4. Changes in blood parameters after exposure to air and diesel exhaust.

cided before the study is performed. Differences that achieve a p value of 0.05 could arise by chance 1 in 20 times. Several of the changes observed here showed p values less than 0.003 (i.e., less than 3 chances in 1,000 that this has occurred by chance) and are therefore genuine. Some other parameters showed changes that are consistent with an inflammatory response to diesel exhaust, but either because of a small magnitude response or a wide scatter the p values were between 0.01 and 0.05, and these should be regarded with more caution.

Exposure to DE for only 1 h produced a marked increase in the number of neutrophils in the peripheral blood. There was a fourfold increase in their numbers in the bronchial epithelium and a threefold increase in the submucosa, which was associated with increased numbers in the bronchial wash, thereby suggesting that DE offers a potent stimulus for release of neutrophils from the bone marrow and their transit from blood to the airway tissues.

Acute exposure to DE was also associated with thrombocytosis. Thrombocytosis has been described as an acute-phase reactant response and has been shown to be associated with a range of inflammatory pulmonary disorders (26). Peters and colleagues (27) have recently demonstrated increased plasma viscocity during increased levels of PM in a large sample of the population. An increase in platelet numbers during a PM pollution episode, especially in elderly people compromised with cardiovascular functions, may increase their risk of developing strokes and coronary vessel thrombosis, thereby increasing cardiovascular mortality and morbidity.

DE exposure was associated with an increase in the number of mast cells in the submucosa and elevated BAL histamine concentrations. In addition to producing bronchoconstriction and vasodilatation, histamine upregulates P-selectin on endothelial cells (28), which through interacting with sialyl-Lewis^x on leukocytes is responsible for the initial rolling of inflammatory cells on their surface before passing through the endothelium into the perivascular space (29).

It is well established that a small number of T lymphocytes reside in the bronchial tissue of normal subjects. In the present study CD4+ cells predominated in the submucosa and CD8+ cells in the epithelium. Six hours following DE exposure, a marked increase in the number of T lymphocytes infiltrating the bronchial submucosa and epithelium occurred, with CD4+ cells predominating. Following exposure to DE, the B lymphocytes in the bronchial tissue did not change, but their numbers increased in BAL with a corresponding decrease in the blood, suggesting trafficking of circulating B cells into the bronchial lumen.

The transit of neutrophils and other inflammatory cells from the blood into tissue occurs in a highly regulated manner involving sequential upregulation of several adhesion molecules on the endothelial cells and their respective ligands on the leukocytes, along with release of chemoattractants from the epithelial and inflammatory cells. In this study and in previous work, we have shown basal constitutive expression of the endothelial adhesion molecules ICAM-1, E-selectin, and P-selectin on the bronchial capillary endothelial vessels of normal and asthmatic subjects. Following acute exposure to DE, we found a significant increase in endothelial ICAM-1 and VCAM-1 expression. This change was an order of magnitude greater than the effects of allergen challenge in atopic asthmatics on adhesion molecule expression assessed by identical methodology in our previous study (23). Upregulation of endothelial ICAM-1 is dependent upon protein synthesis and normally peaks at about 6–8 h following endothelial cell acti-

vation, while P-selectin is preformed and can be translocated to the cell surface within minutes of activation by stimuli such as histamine, thrombin, leukotriene C_4, or free radicals (29). However, P-selectin expression is usually followed by rapid cleavage such that one would not expect to see P-selectin persisting 6 h after the initial insult. The numbers of LFA-1+ cells were significantly increased in the bronchial submucosa and epithelium following DE exposure. The integrin LFA-1 is a ligand for ICAM-1 and is expressed on most leukocytes following activation by a variety of cytokines and inflammatory mediators (30). We also detected upregulation of VCAM-1 on the endothelial cells, along with a trend toward an increase in the number of VLA-4+ cells in the submucosa. These adhesion molecules are specifically implicated in the recruitment of eosinophils, basophils, and T lymphocytes. Thus, although practical and ethical considerations have limited the present study to a single time point, it is clear that acute exposure to DE induces upregulation of endothelial and leukocyte adhesion molecules, thereby providing a cellular basis for the rapid influx of neutrophils and other inflammatory cells into the airways.

DE consists of a combination of several pollutants, including the gaseous and particulate components. The gaseous phase consists of oxides of nitrogen, carbon, and sulphur. The levels of NO_2 from DE used in this study (1.6 ppm) are encountered during peak pollution episodes, certain occupational settings, and in some homes with gas stoves (31). However, the inflammatory response seen in our study cannot be attributed to NO_2 since our previous human exposure studies with NO_2 alone, used at higher concentration for a longer duration (2 ppm NO_2 for 4 h) using a similar study protocol, did not show any cellular inflammatory response in the airway tissue sampled at the same time point (32). It therefore seems more likely that the particulate component of DE is responsible. Previous *in vitro* studies have shown that ultrafine particles elicit a greater inflammatory response in the alveolar space when compared with larger-sized particles, probably due to an interaction between the larger surface area of the small particles with alveolar macrophages and interstitial cells (29). PM_{10} collected by environmental sampling has recently been shown to generate hydroxyl radicals in aqueous solution by an iron-dependent process (33). These free radicals are known to cause activation of redox-sensitive transcription factors, such as nuclear factor kappa B (NFκB) and AP-1 (34). NFκB is important in the transcription of many cytokine and chemokine genes, inducible enzymes (iNOS, COX-2, c-PLA_2), and vascular and epithelial adhesion molecules (35). Oxidative stress induced by these free radicals is also known to increase the permeability of epithelial cells, which would further favor the transfer of particles into the interstitium (36). The proximity of the interstitial inflammatory cells to the endothelium and the blood spaces means that signals such as cytokines could be released into the blood, causing systemic effects. A combination of several pollutants present in DE may be more potent in producing a biological response than each pollutant in isolation. At the same time we recognize that chronic low-dose exposure to PM with intermittent acute high-dose exposures may elicit a response that is different from the acute response seen in the present study. The subjects used in this study group lived in areas that have low levels of PM (average $PM_{10} < 25\ \mu g/m^3$). An acute exposure to high levels of PM in these subjects during the study may have produced a burden of excess PM deposition in their airways to produce an acute inflammatory response. People who are routinely exposed to such high ambient levels may, however, produce a different inflammatory response. Nevertheless, this study clearly suggests the potential of PM in mounting an acute cellular inflammatory response.

No significant changes were observed in the standard lung function tests measured after DE exposure. We have previously shown an increase in airway resistance in healthy volunteers following exposure to DE, which is a more sensitive marker for airflow obstruction than forced expiratory variables (37). The present study, however, suggests that standard lung function parameters used routinely to assess acute health effects of particulate pollution in epidemiologic studies are likely to underestimate the tissue insult.

In conclusion, the findings in this study reinforce the view that diesel emissions are capable of causing a marked pulmonary and systemic inflammatory response involving a variety of cell types. It is now clearly important to determine whether there is a threshold for these effects, whether similar or augmented responses are found in those with respiratory diseases such as asthma, and what happens in those who are chronically exposed to diesel emissions in the course of their daily activities.

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