

Comparison of ciliary activity and inflammatory mediator release from bronchial epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients and the effect of diesel exhaust particles in vitro

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Background: Recent studies have suggested that asthmatic patients may be more susceptible to the adverse effects of air pollutants, including diesel exhaust particles (DEP). The underlying mechanisms, however, are not clear.

Methods: We cultured bronchial epithelial cells from bronchial biopsy specimens of well-characterized groups of nonatopic, nonasthmatic individuals and atopic patients with mild asthma and compared the ciliary beat frequency (CBF) and release of IL-8, GM-CSF, regulated on activation, normal T-cell expressed and secreted (RANTES), and soluble intercellular adhesion molecule-1 (sICAM-1) from these cells both before and after exposure for 24 hours to 10 to 100 $\mu\text{g/mL}$ DEP in vitro.

Results: The baseline CBF was not found to be significantly different in the bronchial epithelial cell cultures of nonasthmatic and asthmatic individuals. Incubation with DEP significantly attenuated the CBF of both the nonasthmatic and asthmatic bronchial epithelial cell cultures at all concentrations of DEP investigated and were maximal (55.5% and 45.2%, respectively) after incubation with 100 $\mu\text{g/mL}$ DEP. The bronchial epithelial cell cultures of asthmatic patients constitutively released significantly greater amounts of IL-8, GM-CSF, and sICAM-1 than bronchial epithelial cell cultures of nonasthmatic subjects. The cultures of only asthmatic patients additionally released RANTES. Incubation of the asthmatic cultures with 10 $\mu\text{g/mL}$ DEP significantly increased the release of IL-8 (from 102.0 to 167.8 $\text{pg}/\mu\text{g}$ cellular protein; $P < .01$), GM-CSF (from 0.43 to 1.87 $\text{pg}/\mu\text{g}$ cellular protein; $P < .01$), and sICAM-1 (from 14.7 to 38.1 $\text{pg}/\mu\text{g}$ cellular protein; $P < .02$) after 24 hours. Incuba-

tion with 50 to 100 $\mu\text{g/mL}$ DEP, however, significantly decreased the release of IL-8 and RANTES from these cultures. In contrast, only the higher concentrations of 50 to 100 $\mu\text{g/mL}$ DEP significantly increased release of IL-8 (from 37.9 to 71.5 $\text{pg}/\mu\text{g}$ cellular protein; $P < .05$) and GM-CSF (from 0.06 to 0.34 $\text{pg}/\mu\text{g}$ cellular protein; $P < .05$) from the bronchial epithelial cells of nonasthmatic individuals.

Conclusions: These results suggest that bronchial epithelial cells of asthmatic subjects are different from bronchial epithelial cells of nonasthmatic subjects with regard to the amounts and types of proinflammatory mediators they can release and that the increased sensitivity of bronchial epithelial cells of asthmatic subjects to DEP may possibly result in exacerbation of their disease symptoms. (*J Allergy Clin Immunol* 1998;102:771-82.)

Key words: Atopic asthma, diesel exhaust particles, bronchial epithelial cells, ciliary beat frequency, cytokines, sICAM-1

The infiltration of bronchial mucosa by inflammatory cells, such as eosinophils, neutrophils, lymphocytes, mast cells, and macrophages is a characteristic feature of asthma.¹⁻⁴ Although these cells have been shown to synthesize and release different types of proinflammatory mediators, which could directly contribute to the pathogenesis of allergic airway diseases, the mechanisms underlying the recruitment of these cells into the airways are not clear. Recent evidence suggests that airway epithelial cells may contribute markedly to airway inflammation because they are capable of synthesizing and releasing inflammatory mediators that influence the activity of the inflammatory cells.⁵⁻⁹ Indeed, some recent studies have suggested that the airway epithelial cells of atopic and nonatopic individuals may be different in their capacity to synthesize and release specific proinflammatory mediators.^{6,10-13}

Epidemiologic studies have suggested that there may be an association between an increase in the incidence of allergic disease and air pollution resulting from increased use of liquid petroleum and natural gas.^{14,15} Studies of particulate air pollution (PM_{10}) have reported that there is an association between an increase in the levels of this pollutant and increases in cardiopulmonary mortality¹⁶ and respiratory symptoms in asthmatic subjects.¹⁷⁻¹⁹

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Abbreviations used

DEP:	Diesel exhaust particles
CBF:	Ciliary beat frequency
RANTES:	Regulated on activation, normal T-cell expressed and secreted
sICAM-1:	Soluble intercellular adhesion molecule-1
PM ₁₀ :	Respirable particulate matter
SF-1 medium:	Serum-free supplemented medium

Mechanistic studies of individuals undergoing nasal allergen challenge with diesel exhaust particles (DEP) have reported that DEP induces local IgE and cytokine synthesis in the nasal mucosa of these individuals.²⁰ More recently, we have demonstrated that exposure of bronchial epithelial cells, cultured from tissue of individuals undergoing surgery for lung cancer, to DEP leads to a significant decrease in the ciliary beat frequency (CBF) and increase in the release of inflammatory mediators from these cells.²¹ To date, however, there is no information on the effect of DEP on bronchial epithelial cells of asthmatic patients, who are generally thought to be at a greater risk from the adverse respiratory effects of this pollutant.

In this study, we cultured bronchial epithelial cells from biopsy specimens of well-characterized groups of nonatopic nonasthmatic subjects and atopic mildly asthmatic patients and investigated the effect of DEP on CBF of these cells in vitro. Additionally, we have investigated both constitutive and DEP-induced release of specific proinflammatory mediators, including IL-8, GM-CSF, regulated on activation, normal T-cell expressed and secreted (RANTES), and soluble intercellular adhesion molecule-1 (sICAM-1) from these cells.

METHODS**Nonatopic nonasthmatic subjects and atopic asthmatic patients**

Twenty-eight volunteers (12 women and 16 men) with a mean age of 29 years (range, 19 to 45 years) took part in the study. Of these, 13 were nonatopic nonasthmatic subjects (control subjects) and 15 were atopic mildly asthmatic patients. The characteristics of these individuals are shown in Table I and demonstrated that there were no significant differences in the age and sex ratios. Atopy was confirmed by positive skin prick reactions (wheals with a mean diameter of >3 mm with associated flare and itching, in absence of any reaction to the negative control) to house dust mite (*Dermatophagoides pteronyssinus*) and/or to other allergens, such as grass pollen, *Aspergillus fumigatus*, dog, or cat (ALK, Allergolast Laboratorium, Horsholm, Denmark). All asthmatic patients had an FEV₁ level of greater than 70% predicted for their age and height and a provocative concentration of less than 2 mg/mL histamine, leading to a 20% fall in FEV₁ (PC₂₀FEV₁). Before entry into the study, the patients were clinically stable and had not required systemic antihistamine, nonsteroid antiinflammatory, β -blocker, topical steroid, long-acting β_2 -agonist, or oral bronchodilator medication for 6 weeks or inhaled steroid, nedocromil sodium, or sodium cromoglycate for 6 months. During the study, only inhaled salbutamol or terbutaline was permitted as required. None of the volunteers entering into the

study were smokers or exsmokers and had not had upper or lower respiratory tract infections within 1 month of the study. The study was approved by the ethics committee of St Bartholomew's Hospital, and all volunteers provided written informed consent on entry.

Reagents

All reagents were of tissue culture grade and purchased from Sigma Chemical Company (Poole, Dorset, UK) unless stated otherwise.

Bronchial tissue

Bronchial biopsy specimens were obtained from the right segmental and subsegmental carinae of the nonasthmatic subjects and asthmatic patients at bronchoscopy, performed under light sedation with midazolam and local anaesthesia with lignocaine, according to the American Thoracic Society guidelines.²² A maximum of 6 specimens, of approximately 1 to 2 mm³ in size, were obtained from each individual with a rotatable Olympus FB-19KR cup-biopsy forceps (KeyMed Ltd, Southend-on-Sea, UK) and immediately placed in ice-cold medium 199 before further processing for culture of bronchial epithelial cells.

Culture of bronchial epithelial cells

Bronchial epithelial cells were cultured by modification of an explant cell culture technique developed in our laboratory.²³ Each specimen was observed under a dissecting microscope, and the epithelium was dissected from the underlying tissue. The epithelium was then cut further into smaller sections, approximately 0.5 mm³ in size, and the sections sterilized by gently washing 3 times in prewarmed and pregassed medium 199 containing 1% (vol/vol) antibiotic/antimycotic solution (Sigma, Poole, UK). Two to 3 sections were explanted into untreated 6-cm-diameter Falcon Primaria plastic culture dishes (Becton Dickinson Ltd, Oxford, England) and incubated at 37°C in 5% CO₂ in air atmosphere, in 2 mL of freshly prepared and micropore filter-sterilized culture medium containing 2.5 mL FBS (Flow Laboratories, Scotland), 250 μ g bovine pancreatic insulin, 250 μ g of human transferrin, 36 μ g of hydrocortisone, 1 mg L-glutamine and 1 mL of antibiotic/antimycotic solution, in 100 mL medium 199. The medium in each culture dish was replaced with fresh culture medium containing 2.5% Nu-serum IV (Universal Biologicals Ltd, Gloucestershire, UK) after 3 days and then every 48 hours for 2 to 3 weeks until the outgrowing cells had grown to confluence.

The purity and identity of the cells were checked in randomly selected cultures and confirmed by (1) light microscopy and (2) indirect immunoperoxidase staining techniques, using specific monoclonal antibodies directed toward cytokeratin and specific ciliated epithelial cell antigens, as we have described before.²³ Similarly, randomly selected cultures were also analyzed for the presence of contaminating cell types by staining with specific monoclonal antibodies. T and B lymphocytes and endothelial cells were analyzed by staining with mAbs CD3, CD37, and M616, respectively (Serotec Ltd, Oxford, UK). Eosinophils were analyzed by staining with mAb EG1 (Pharmacia Ltd, Milton Keynes, UK). Similarly, the other contaminating cells, including fibroblasts, mast cells, neutrophils, and macrophages were analyzed by staining with mAbs 5B5, AA1, NP57, and Ber-MAC3, respectively (Dako Ltd, Bucks, UK).

Preparation of DEP suspensions

DEP (mass median aerodynamic diameter = 0.4 μ m, Q1 = 0.195 μ m, and Q3 = 1.42 μ m) were purified and characterized as we have described previously.^{21,24} Briefly, exhaust generated from a diesel engine was introduced into a stainless steel dilution tunnel and the

TABLE I. Characteristics of study volunteers

Group	Subject	Age (y)	Sex	Atopy (SPT)					PC ₂₀ FEV ₁ (mg/mL)
				HDM	GP	AF	Dog	Cat	
Nonatopic nonasthmatic subjects									
	1	25	F	—	—	—	—	—	>4
	2	32	M	—	—	—	—	—	>4
	3	24	F	—	—	—	—	—	>4
	4	30	M	—	—	—	—	—	>4
	5	30	M	—	—	—	—	—	>4
	6	29	F	—	—	—	—	—	>4
	7	19	M	—	—	—	—	—	>4
	8	30	M	—	—	—	—	—	>4
	9	25	M	—	—	—	—	—	>4
	10	38	F	—	—	—	—	—	>4
	11	28	F	—	—	—	—	—	>4
	12	23	F	—	—	—	—	—	>4
	13	33	M	—	—	—	—	—	>4
Atopic asthmatic patients									
	1	23	M	+	+	—	—	+	0.29
	2	43	F	+	+	—	—	+	0.63
	3	22	M	+	—	—	—	—	0.11
	4	32	F	+	—	—	+	+	0.64
	5	29	M	+	—	—	—	—	0.66
	6	25	F	+	+	—	+	+	0.03
	7	45	F	+	—	—	—	—	1.3
	8	40	M	+	+	—	—	+	0.25
	9	26	M	+	+	—	+	+	0.06
	10	24	M	+	—	—	—	+	0.07
	11	32	F	+	+	—	—	—	0.15
	12	37	M	+	+	—	—	+	1.35
	13	21	M	+	+	—	—	+	0.92
	14	22	F	+	—	—	+	—	2
	PL	23	M	+	+	—	—	—	2

SPT, Skin prick test; HDM, house dust mite; GP, grass pollen; AF, *Aspergillus fumigatus*; PC₂₀FEV₁, dose of bronchoconstrictor that leads to a 20% fall in FEV₁.

DEP were collected on a glass fiber filter situated in a constant-volume sampler placed at the end of the tunnel. The diameter of the particles was measured with the use of a low-pressure Andersen Air Sampler and then investigated further by scanning electron microscopy to determine the general shapes of the particles collected.

The purified DEP were suspended in SF-1 serum-free supplemented medium (SF-1 medium) at concentrations of 10 to 100 µg/mL before use in further investigations. The SF-1 medium was prepared by mixing 0.5 mL SF-1 serum-free supplement (Northumbria Biologicals Ltd, Cramlington, UK) and 1.0 mL antibiotic/antimycotic solution in 100 mL of medium 199.

Effect of DEP on CBF of bronchial epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients

Cultures 1 to 2 weeks old were used for these investigations, and CBF was measured by a modification of the analogue contrast enhancement technique, as we have described before.²³ Before experimentation, the bronchial explants were removed and all the cultures were equilibrated by incubation for 24 hours in SF-1 medium. After equilibration, the cultures were gently washed 3 times with fresh, prewarmed and pregassed SF-1 medium and sets of at least 6 different nonasthmatic and asthmatic bronchial epithelial cell

cultures, each from a different nonasthmatic or asthmatic individual, were investigated further. Before treatment, each culture was equilibrated for 2 minutes at room temperature and monitored for the baseline CBF in at least 6 randomly chosen areas of ciliated cells to account for any variance in the distribution and CBF of the ciliated cells in the culture. The positions of each of these 6 areas were noted accurately by means of a horizontal and a vertical Vernier scale on the microscope stage, and the culture was then incubated in 2 mL of culture medium containing a known concentration of DEP ranging from 0 to 100 µg/mL. At 2, 4, 6, and 24 hours of incubation, the culture was equilibrated for 2 minutes at room temperature and monitored for CBF in the same areas as at the beginning of the experiment. The CBF in each culture was calculated as the mean of 6 areas within the culture at baseline and at each time point during incubation; the effect of treatment was expressed as percentage change from the baseline.

Effect of DEP on release of IL-8, GM-CSF, RANTES, and sICAM-1 from bronchial epithelial cells of nonasthmatic subjects and asthmatic patients

Confluent bronchial epithelial cell cultures 2 to 3 weeks old from nonasthmatic and asthmatic individuals were used in these investi-

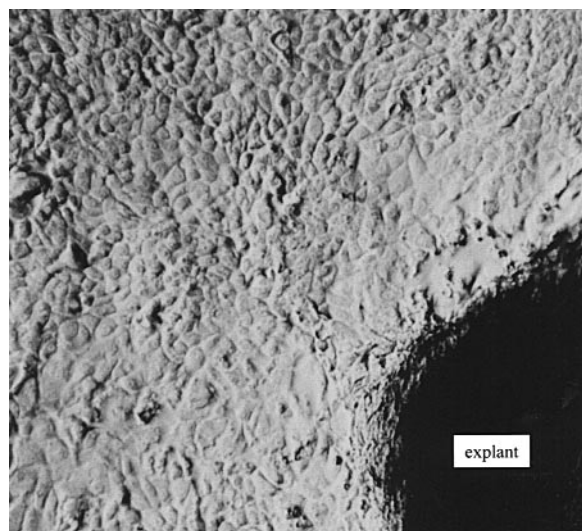


FIG 1. Typical confluent human bronchial epithelial cell culture after 2 to 3 weeks in culture.

gations. Before experimentation, the bronchial explants were removed and all the cultures were equilibrated and washed with pre-warmed, pregassed SF-1 medium, as described.

In the case of bronchial epithelial cell cultures of nonasthmatic subjects, 4 sets of 8 different cultures each were incubated in 2.0 mL of fresh SF-1 medium containing either 0, 10, 50, or 100 $\mu\text{g/mL}$ DEP for 24 hours at 37°C in 5% CO_2 in air atmosphere. At the end of incubation, the culture medium was collected from each culture and the cells were gently washed with 1 mL of SF-1 medium. The culture medium and the wash were pooled, and all samples were stored at -70°C until analysis for IL-8, GM-CSF, RANTES, and sICAM-1, with the use of commercially available ELISA kits (R&D Systems, Abingdon, England). The cells were scraped off into 1.0 mL 1 mol/L NaOH solution and stored at -20°C until analysis for cellular protein, according to the method of Lowry et al.²⁵

In the case of asthmatic bronchial epithelial cell cultures, the experimental protocol was modified slightly because of difficulties in obtaining suitable numbers of cultures required for these studies. Three sets of at least 10 separate cultures, each devoid of their bronchial explants, were equilibrated and washed as described. The cultures were then incubated in 2.0 mL of fresh SF-1 medium for 24 hours at 37°C in 5% CO_2 in air atmosphere, and at the end of incubation, the medium was collected and stored as described. After this incubation, the cultures were incubated in 2.0 mL complete culture medium for 6 to 8 hours and then for a further 24 hours in SF-1 medium. At the end of this incubation, the cultures were washed with SF-1 medium and then each set of cultures finally incubated in 2.0 mL of SF-1 medium containing either 10, 50, or 100 $\mu\text{g/mL}$ DEP for 24 hours at 37°C in 5% CO_2 in air atmosphere. After the final incubation, all culture medium and cellular samples were collected and treated as described.

Statistical analysis

All statistical analyses were carried out in collaboration with a resident statistician. Data were tested for skewness and then analyzed further with nonparametric statistical tests. The Mann-Whitney U test was applied to test for differences in the age of nonatopic nonasthmatic subjects and atopic asthmatic patients. Before further statistical evaluation, all results for CBF were calculated as per-

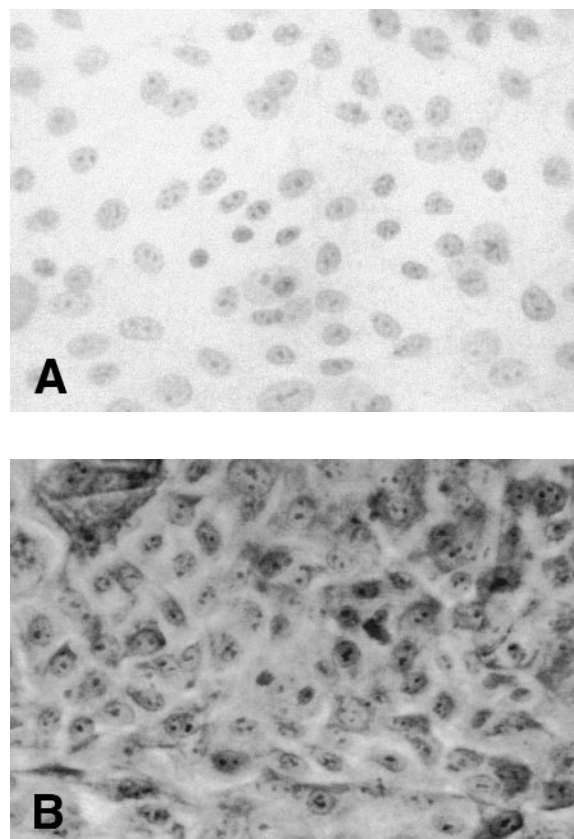


FIG 2. Typical human bronchial epithelial cell culture: **A**, negative control; **B**, stained for cytokeratin.

centage changes from baseline to account for variance in the CBF within and between cultures. In the case of all data resulting from nonasthmatic bronchial epithelial cell cultures and CBF data resulting from asthmatic bronchial epithelial cell cultures, the Kruskal-Wallis 1-way analysis of variance by ranks test was applied to determine the significance of any differences between all the sets of cultures investigated. This was followed by further analysis with the Mann-Whitney U test to assess the significance of any differences between the individual treatment groups. Bonferroni's correction was used for multiple comparisons. In the case of data obtained for mediator release from asthmatic bronchial epithelial cell cultures, Wilcoxon's matched pair, signed rank test was applied to test for significance in differences before and after exposure to DEP. The significance of any differences observed in CBF and release of mediators from nonasthmatic and asthmatic bronchial epithelial cell cultures was analyzed with the Mann-Whitney U test. All statistical tests were performed with a standard computer package (Minitab Data Analysis Software Release 6.1.1, 1987; Minitab Inc, State College, Pa), and results were expressed as median and interquartile range (Q1 to Q3) on the recommendation of the statistician. All *P* values less than .05 were considered to be significant.

RESULTS

These studies have demonstrated that epithelial cells can be grown as explant cultures from bronchial biopsy specimens of well-characterized nonatopic nonasthmatic subjects and atopic asthmatic patients (Fig 1). The cells

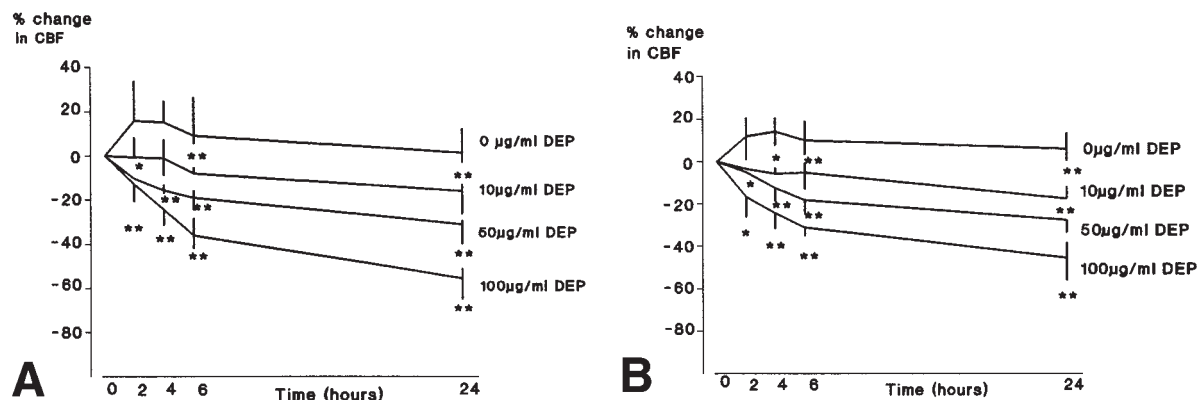


FIG 3. Effect of DEP on the CBF of **A**, nonasthmatic ($n = 7$) and **B**, asthmatic ($n = 10$) bronchial epithelial cell cultures after incubation for 2 to 24 hours. Results are calculated as percent change from baseline in each culture and expressed as median and interquartile ranges (* $P < .05$ and ** $P < .01$ vs 0 µg/mL DEP at all time points).

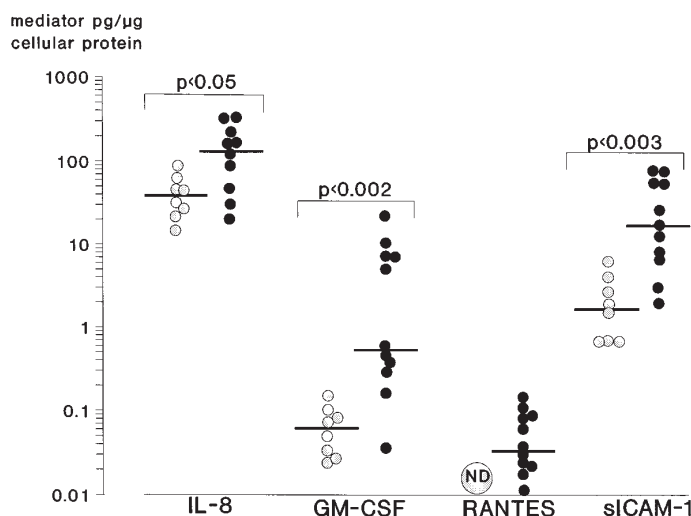


FIG 4. Constitutive release of IL-8, GM-CSF, RANTES, and sICAM-1 from bronchial epithelial cell cultures of nonasthmatic subjects (shaded circles) and asthmatic patients (filled circles) after incubation for 24 hours. Results are expressed as median and individual values. ND, Not detected.

reached confluence after 2 to 3 weeks in culture and demonstrated typical polygonal morphology characteristic of epithelial cells. The epithelial nature of the cells was confirmed by positive staining of the cells for cytokeratin (Fig 2, B) when compared with the negative control (Fig 2, A). Staining with specific monoclonal antibodies against contaminating cell types including fibroblasts, muscle cells, endothelial cells, macrophages, T and B lymphocytes, mast cells, eosinophils, and neutrophils did not show any indication of these cells. Despite the high specificity and sensitivity of these monoclonal antibody staining techniques, the possibility that the presence of a very small number of contaminating cells not being detected because of masking resulting

from the great excess of epithelial cells present in the culture, however, cannot be excluded completely.

Effect of DEP on CBF of bronchial epithelial cell cultures of nonasthmatic and asthmatic individuals

Light microscopic examination of bronchial epithelial cell cultures of both nonasthmatic and asthmatic subjects incubated in the presence of DEP for 24 hours revealed that this agent did not lead to any gross cellular damage or detachment of the cells at any concentration between 10 and 100 µg/mL DEP studied.

TABLE II. Constitutive and DEP-induced (10 to 100 µg/mL) mediator release from bronchial epithelial cells of asthmatic versus nonasthmatic subjects

	pg Mediator/µg cellular protein			
	IL-8	GM-CSF	RANTES	sICAM-1
Atopic asthmatic patients				
Constitutive	108.2* (37.2-189.8)	0.49† (0.31-7.08)	0.034† (0.02-0.09)	17.9† (6.0-58.4)
DEP 10 µg/mL	167.8* (31.8-202.4)	1.87† (0.44-8.41)	0.04† (0.018-0.071)	38.13† (10.47-57.95)
DEP 50 µg/mL	112.6 (50.7-145.6)	1.34† (0.43-7.11)	0.016† (0.009-0.028)	22.51* (9.05-45.99)
DEP 100 µg/mL	93.1 (15.0-144.1)	1.79† (0.85-19.07)	0.008† (0.005-0.021)	33.78* (8.10-56.81)
Nonatopic nonasthmatic subjects				
Constitutive	37.94 (24.02-49.03)	0.059 (0.029-0.091)	ND	1.68 (0.67-3.84)
DEP 10 µg/mL	25.39 (16.25-48.85)	0.052 (0.016-0.101)	ND	1.37 (0.71-1.46)
DEP 50 µg/mL	71.50 (47.5-98.5)	0.341 (0.162-0.873)	ND	4.78 (1.67-16.02)
DEP 100 µg/mL	42.59 (32.48-79.53)	0.175 (0.087-0.778)	ND	2.59 (1.84-15.41)

RANTES, Regulated on activation, normal T-cell expressed and secreted; DEP, diesel exhaust particles; ND, not detected.

Results are expressed as median and interquartile (Q1 and Q3) ranges.

* $P < .05$, † $P < .005$ for constitutive and DEP-induced release of mediator from bronchial epithelial cells of asthmatic vs bronchial epithelial cells of nonasthmatic individuals.

Incubation of bronchial epithelial cell cultures of nonasthmatic subjects with DEP progressively attenuated the CBF of the cultures in a time- and dose-dependent manner (Fig 3, A). Exposure to 10, 50, and 100 µg/mL DEP significantly attenuated the CBF by 15.9% ($Q1 = 12.9$ and $Q3 = 26.3\%$; $P < .05$), 31.0% ($Q1 = 29.1$ and $Q3 = 39.8\%$; $P < .01$), and 55.5% ($Q1 = 29.1$ and $Q3 = 39.8\%$; $P < .01$), respectively, from baseline after 24 hours. Compared with untreated control cultures, the changes observed in CBF of these cells were significant from 2 hours onward after incubation with 50 to 100 µg/mL DEP and from 6 hours onward after incubation with 10 µg/mL DEP (Fig 3, A).

Incubation for 24 hours with 10, 50, and 100 µg/mL DEP also significantly attenuated the CBF of bronchial epithelial cell cultures of asthmatic patients by 17.3% ($Q1 = 11.6$ and 28.7 ; $P < .01$), 27.5% ($Q1 = 22$ and $Q3 = 33.3\%$; $P < .01$), and 45.2% ($Q1 = 38.1$ and $Q3 = 56\%$; $P < .01$), respectively, from baseline (Fig 3, B). The changes in the CBF of these cells also were significant from 2 hours onward at all concentrations of DEP studied, compared with untreated control cultures (Fig 3, B).

There was no statistical difference between the CBF of asthmatic and nonasthmatic patients' cultures, either untreated or DEP-treated.

Constitutive release of inflammatory mediators from bronchial epithelial cell cultures of nonasthmatic and asthmatic individuals

Bronchial epithelial cell cultures of both nonasthmatic and asthmatic individuals released IL-8, GM-CSF, and

sICAM-1 constitutively. Release of these mediators from bronchial epithelial cells of asthmatic subjects was significantly greater than release from bronchial epithelial cell cultures of nonasthmatic subjects after incubation for 24 hours (Fig 4 and Table II). In marked contrast, constitutive release of RANTES was observed only from bronchial epithelial cell cultures of asthmatic individuals after 24 hours (Fig 4 and Table II).

Effect of DEP on release of inflammatory mediators from bronchial epithelial cell cultures of nonasthmatic and asthmatic individuals

IL-8. Incubation of bronchial epithelial cell cultures of nonasthmatic subjects for 24 hours with 50 µg/mL DEP significantly increased the release of IL-8 compared with the untreated control cultures ($P < .05$) (Fig 5, A, and Table II).

In contrast, release of IL-8 from bronchial epithelial cell cultures of asthmatic subjects was significantly increased after incubation for 24 hours with a much lower concentration of 10 µg/mL DEP ($P < .01$) when compared with constitutive release of IL-8 observed from these cells after incubation for 24 hours. However, the release of IL-8 from the asthmatic cells was significantly decreased by incubation for 24 hours with 50 µg/mL ($P < .05$) and 100 µg/mL DEP ($P < .01$) (Fig 5, B, and Table II).

A comparison of DEP-induced release of IL-8 from bronchial epithelial cell cultures of nonasthmatic and asthmatic individuals demonstrated that this was significantly greater in bronchial epithelial cells of asthmatic subjects after incubation with 10 µg/mL DEP for 24 hours ($P < .03$, Table II).

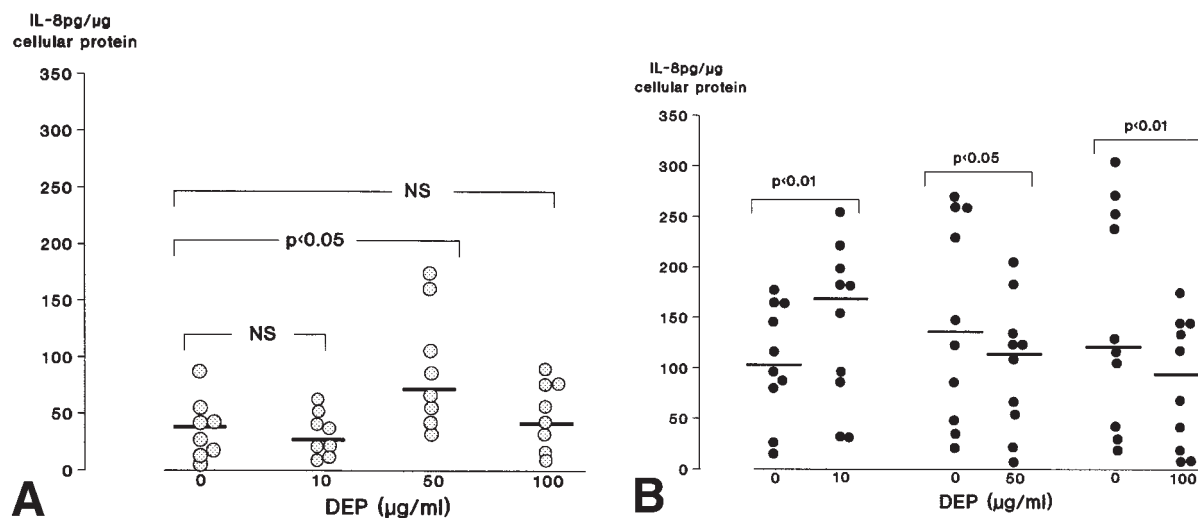


FIG 5. Effect of DEP on release of IL-8 from **A**, nonasthmatic, and **B**, asthmatic bronchial epithelial cell cultures after incubation for 24 hours. Results are expressed as median and individual values (all asthmatic bronchial epithelial cell cultures served as controls before incubation with DEP, as described in text).

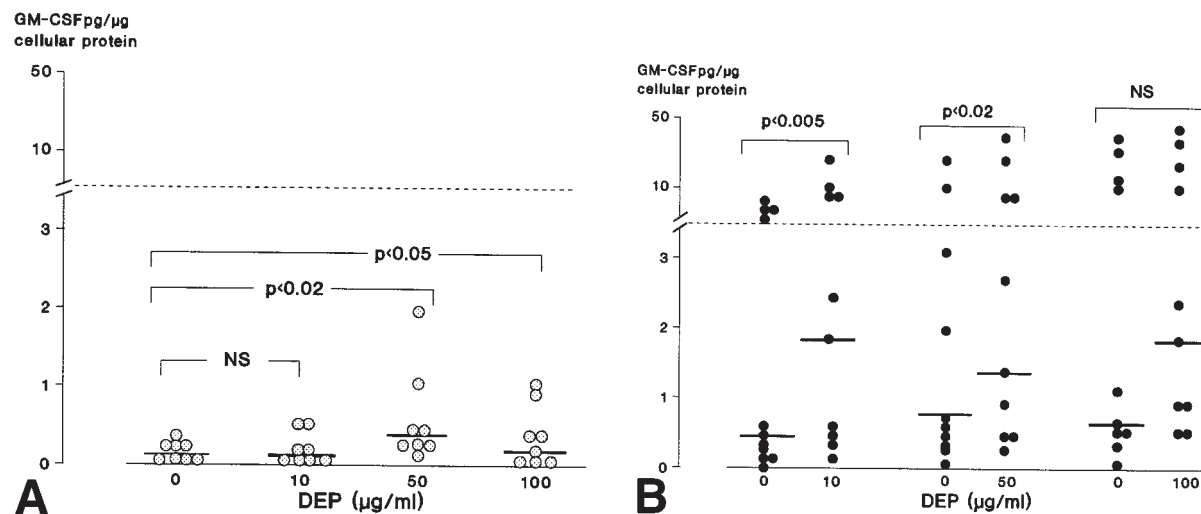


FIG 6. Effect of DEP on release of GM-CSF from **A**, nonasthmatic, and **B**, asthmatic bronchial epithelial cells after incubation for 24 hours. Results are expressed as median and individual values (all asthmatic bronchial epithelial cell cultures served as controls before incubation with DEP, as described in text).

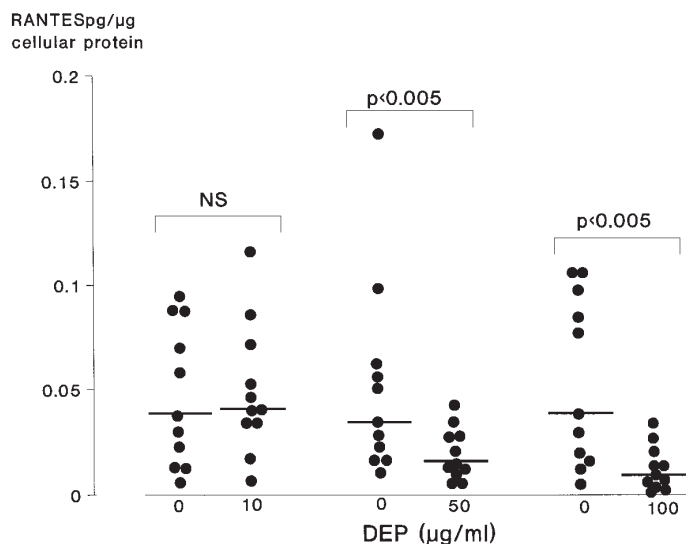


FIG 7. Effect of DEP on release of RANTES from asthmatic bronchial epithelial cell cultures after incubation for 24 hours. Results are expressed as median and individual values (bronchial epithelial cell cultures served as controls before incubation with DEP, as described in text).

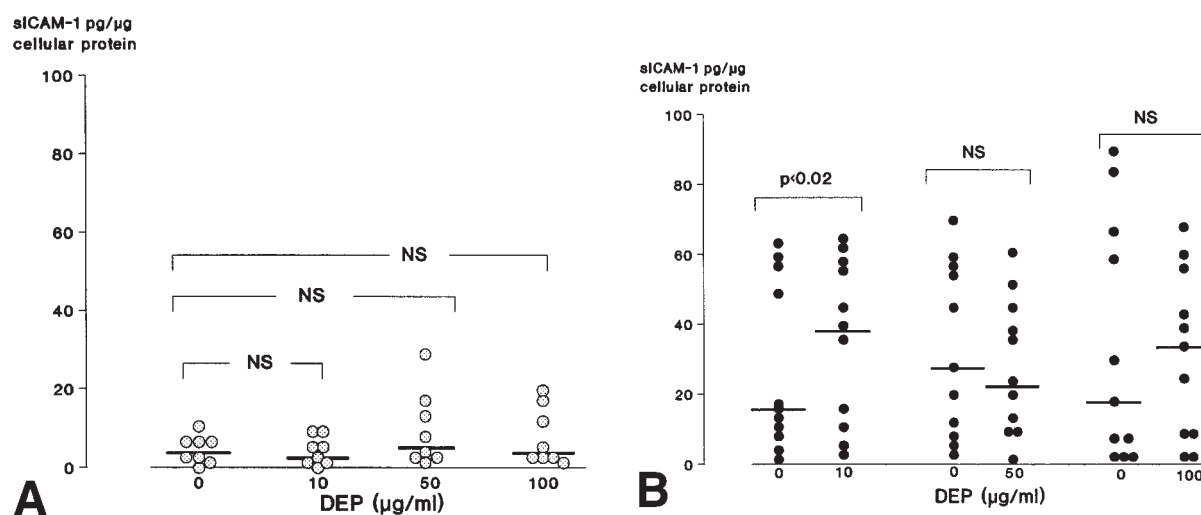


FIG 8. Effect of DEP on release of sICAM-1 from **A**, nonasthmatic, and **B**, asthmatic bronchial epithelial cell cultures after incubation for 24 hours. Results are expressed as median and individual values (all asthmatic bronchial epithelial cell cultures served as controls before incubation with DEP, as described in text).

GM-CSF. Incubation of bronchial epithelial cell cultures of nonasthmatic subjects with DEP for 24 hours at the higher concentrations of 50 $\mu\text{g/mL}$ DEP and 100 $\mu\text{g/mL}$ DEP also led to significantly increased release of GM-CSF from these cultures when compared with untreated control cultures (Fig 6, A, and Table II).

Similarly, analysis of GM-CSF released from bronchial epithelial cell cultures of asthmatic subjects

incubated with DEP demonstrated that this was significantly increased at the low concentration of 10 $\mu\text{g/mL}$ ($P < .005$) and of 50 $\mu\text{g/mL}$ DEP ($P < .02$) when compared with constitutive release of GM-CSF from these cells after incubation for 24 hours. In contrast to nonasthmatic cultures, incubation with 100 $\mu\text{g/mL}$ DEP did not significantly increase the release of GM-CSF from asthmatic bronchial epithelial cell cultures (Fig 6, B, and Table II).

A comparison between DEP-induced release of GM-CSF from bronchial epithelial cell cultures of nonasthmatic and asthmatic individuals demonstrated that this was significantly greater from the bronchial epithelial cell cultures of asthmatic subjects after incubation for 24 hours in the presence of 10 $\mu\text{g/mL}$ ($P < .001$), 50 $\mu\text{g/mL}$ ($P < .005$), and 100 $\mu\text{g/mL}$ DEP ($P < .003$) investigated (Table II).

RANTES. Release of RANTES was not detected from bronchial epithelial cell cultures of nonasthmatic individuals after incubation for 24 hours with 10 to 100 $\mu\text{g/mL}$ of DEP.

Although the release of RANTES from bronchial epithelial cell cultures of asthmatic patients was not significantly altered after 24 hours by incubation with 10 $\mu\text{g/mL}$ DEP when compared with constitutively released RANTES, this was, however, significantly decreased by incubation of the asthmatic cultures with 50 $\mu\text{g/mL}$ ($P < .005$) and 100 $\mu\text{g/mL}$ DEP ($P < .005$) (Fig 7 and Table II).

sICAM-1. Incubation with 10 to 100 $\mu\text{g/mL}$ DEP did not significantly alter the release of sICAM-1 from bronchial epithelial cell cultures of nonasthmatic subjects over a period of 24 hours (Fig 8, A, and Table II).

In contrast, incubation with 10 $\mu\text{g/mL}$ DEP ($P < .02$) significantly increased the release of sICAM-1 from bronchial epithelial cell cultures of asthmatic subjects compared with the constitutive release of sICAM-1 after incubation for 24 hours (Fig 8, B). Incubation with 50 and 100 $\mu\text{g/mL}$ DEP, however, did not significantly alter the release of sICAM-1 from asthmatic bronchial epithelial cell cultures (Fig 8, B, and Table II).

Analysis of DEP-induced release of sICAM-1 from the bronchial epithelial cell cultures of nonasthmatic subjects and asthmatic subjects demonstrated that this was significantly greater from the cells of asthmatic subjects after incubation for 24 hours with all 3 concentrations of 10 $\mu\text{g/mL}$ ($P < .003$), 50 $\mu\text{g/mL}$ ($P < .05$), and 100 $\mu\text{g/mL}$ DEP ($P < .03$) investigated (Table II).

DISCUSSION

These studies have demonstrated that bronchial epithelial cells can be grown as confluent explant cultures from biopsy specimens of well-characterized groups of both atopic asthmatic patients and nonatopic nonasthmatic subjects. Additionally, these studies have demonstrated that the cells of atopic asthmatic patients are different from the cells of nonatopic nonasthmatic subjects, with regard to the quantities and types of specific proinflammatory mediators that they can release constitutively, possibly reflecting a generally increased state of activation of these cells in asthma. Collectively, these findings suggest that the *in vitro* model used in these studies may provide a suitable means for investigating the role of bronchial epithelial cells in the mechanisms underlying the development of asthma.

The technique used for culturing bronchial epithelial cells from biopsy tissue in this study was based on the technique for culturing these cells from surgical tissue, developed and widely used in our laboratory.^{9,23} Although others have also documented investigations of

bronchial epithelial cells isolated or cultured from tissues of asthmatic and nonasthmatic individuals,¹⁰⁻¹² a major difference between our studies and those of others is that the bronchial epithelial cells used in the studies documented by the other authors have not been derived from tissues of strictly characterized groups of atopic asthmatic and nonatopic nonasthmatic individuals. In all these studies, the groups of asthmatic patients were composed of both atopic and nonatopic individuals. Indeed, the group of control nonasthmatic subjects investigated by Marini et al¹⁰ was composed of both atopic and nonatopic individuals, and, contrary to our findings, these authors were not able to demonstrate release of GM-CSF from cells grown from tissue of nonatopic individuals. Although Gormand et al¹² have demonstrated that bronchial epithelial cells from asthmatic subjects released significantly greater amounts of GM-CSF compared with nonasthmatic subjects, no significant changes were found between the release of IL-8 from the 2 groups. It is possible that the comparatively high levels of IL-8 released from bronchial epithelial cells of the control nonasthmatic group was a consequence of the atopic status, of at least some of these individuals, which was not determined. Indeed, studies by Calderon et al¹³ have demonstrated that nasal epithelial cells cultured from atopic subjects without rhinitis (patients with atopic eczema) release significantly greater amounts of GM-CSF, IL-8, and TNF- α compared with nonatopic non-rhinitic subjects. Another methodologic difference between our studies and those of others is that we have obtained bronchial epithelial cells as confluent explant cultures of high viability and purity compared with cells obtained after mechanical and proteolytic enzyme disaggregation, which could lead to detrimental morphologic and biochemical cellular changes²⁶ and possibly explain the discrepancies between our findings and those of others highlighted.

Despite successfully culturing bronchial epithelial cells as explant cultures from biopsy tissues of nonatopic nonasthmatic and atopic asthmatic individuals, a major problem encountered over the course of these studies was not being able to readily recruit suitable atopic mildly asthmatic patients and nonatopic nonasthmatic individuals willing to undergo bronchoscopy to provide the specimens required to undertake these studies. The lack of suitable biopsy tissue was further compounded by the observation that all explants did not produce suitable confluent cultures and occasionally the epithelial cell cultures of the atopic asthmatic patients appeared to grow at a slower rate than the epithelial cell cultures of nonatopic nonasthmatic subjects. On such occasions, these cultures were discarded.

Using this model, we have investigated the effect of exposure to DEP on the ciliary activity and release of proinflammatory mediators, which may play a prominent role in the development of allergic airway disease, from bronchial epithelial cells of well-characterized atopic mildly asthmatic patients and nonatopic nonasthmatic subjects. Our studies have demonstrated that although there are no marked differences between bronchial epithelial

cells of asthmatic subjects and nonasthmatic subjects, with respect to their ciliary activity, the two cell types do indeed differ significantly with respect to the amounts and types of inflammatory mediators that they can generate constitutively. Our studies have demonstrated that asthmatic bronchial epithelial cells constitutively release significantly greater concentrations of IL-8, GM-CSF, and sICAM-1 compared with nonasthmatic bronchial epithelial cells. A novel finding from our studies, however, has been the demonstration that release of RANTES, a potent chemokine affecting the activity of eosinophils,^{27,28} was detected only from the cells of asthmatic subjects. In view of these findings, it is tempting to speculate that the predisposition of atopic asthmatic individuals to greater airway inflammation and increased fragility of their airway epithelium may at least in part be a consequence of the increased release of proinflammatory mediators leading to increased interstitial trafficking and activation of inflammatory cells. Indeed, the ability of asthmatic bronchial epithelial cells to release specifically greater quantities of RANTES than bronchial epithelial cells of nonasthmatic subjects may explain why the asthmatic airways are predisposed to infiltration by eosinophils, the chief effector cell.^{1-4,29} In view of the wide array of cytotoxic compounds that could be generated by activated eosinophils,^{30,31} it is not too difficult to envision how these compounds may lead to the marked bronchial epithelial damage, which is thought to be a characteristic feature of asthma.

The findings of our investigations of the effects of DEP on bronchial epithelial cells of asthmatic subjects and nonasthmatic subjects have confirmed our previous findings with bronchial epithelial cells cultured from human surgical bronchial explants²¹ and demonstrated that exposure to 10 to 100 $\mu\text{g/mL}$ DEP results in attenuation of the CBF of these cells and an increase in release of IL-8, GM-CSF, and sICAM-1 from these cells. The precise mechanisms underlying DEP-induced effects on function and mediator release from bronchial epithelial cells of asthmatic subjects and nonasthmatic subjects, however, are not entirely clear. Our finding that DEP attenuated the ciliary activity of bronchial epithelial cells of both asthmatic subjects and nonasthmatic subjects, without any significant differences between the two cell types, suggests that DEP-mediated effects at the cell membrane level are likely to be nonspecific and lead to generalized cell damage, possibly as a consequence of formation of reactive oxygen species from the organic compounds adsorbed to the DEP. Although any significant differences in DEP-mediated changes in CBF were not observed between asthmatic and nonasthmatic bronchial epithelial cells, probably as a consequence of the "inhibitory" concentrations of DEP investigated, it is possible that such differences may indeed have been seen at concentrations lower than 10 $\mu\text{g/mL}$ DEP.

Although there is little or no information on concentrations at which DEP may demonstrate activating rather than "inhibitory" effects, preliminary studies in our laboratory have demonstrated that exposure for 6 hours to

400 ppb nitrogen dioxide, a better defined and investigated air pollutant, leads to significantly greater changes in the permeability of bronchial epithelial cell cultures of asthmatic subjects than those of nonasthmatic subjects.³² Similarly, studies with nasal epithelial cell cultures established from nasal biopsy specimens of atopic rhinitic patients and nonatopic nonrhinitic subjects have suggested that the cells of the atopic rhinitic patients are more susceptible to the detrimental effects of ozone, another well-defined and investigated air pollutant, than the cells of the nonatopic nonrhinitic subjects.³³

Certainly, our investigations of inflammatory mediator release from bronchial epithelial cells of asthmatic and nonasthmatic subjects suggest that asthmatic bronchial epithelial cells may be more sensitive to the effects of DEP than nonasthmatic bronchial epithelial cells. Our studies have demonstrated that significant release for all mediators investigated ensues from bronchial epithelial cells of asthmatic subjects at the lowest concentration of 10 $\mu\text{g/mL}$ DEP compared with release of IL-8 and GM-CSF from bronchial epithelial cells of nonasthmatic subjects at the higher concentrations of 50 to 100 $\mu\text{g/mL}$ DEP. Indeed, exposure to DEP does not affect the release from bronchial epithelial cells of nonasthmatic subjects of either RANTES or sICAM-1, both mediators that are thought to play a major role in the migration and activation of eosinophils.^{8,9,27,28} Although it is possible that the increased sensitivity of asthmatic bronchial epithelial cells, with respect to DEP-induced mediator release, may be a consequence of the slight difference in the experimental protocols used (namely that the asthmatic bronchial epithelial cell cultures were additionally incubated for 24 hours in SF-1 medium and 6 to 8 hours in complete culture medium), this short time span over which the asthmatic cells were treated differently is unlikely to have dramatically altered the physiology and the subsequent "responsivity" of the asthmatic cells to DEP. Also, because the constitutive release of mediators from bronchial epithelial cells of asthmatic subjects was shown to be greater than constitutive release from bronchial epithelial cells of nonasthmatic subjects and that incubation with 50 to 100 $\mu\text{g/mL}$ DEP led to decreased release of IL-8 and RANTES but increased release of GM-CSF from the cells of asthmatic subjects, our findings suggest that the DEP-induced release of specific mediators may be differential.

Irrespective of what the underlying mechanisms may be, the findings of our studies generally complement the findings of other studies. Several studies in animals have reported that exposure to DEP leads to significantly increased generation of superoxide and hydroxyl radicals and inhibition of the activities of superoxide dismutase, glutathione peroxidase, and catalase in epithelial lining fluid and have suggested that the increased oxygen radical load leads to increased lung injury and mortality.^{24,34-36} Ichinose et al³⁷ have investigated the effects of intratracheal instillation of DEP in mice and reported that this results in dose-dependent intra-alveolar hemorrhage, perivascular edema, and bronchiolar cell hypertrophy 18 to 24 hours after DEP

instillation. More recently, these authors have reported that intratracheal instillation of DEP, once per week for 16 weeks, resulted in marked infiltration of inflammatory cells, particularly eosinophils in the submucosa of the proximal bronchi and medium bronchioles, which was attenuated by pretreatment of the animals with polyethyleneglycol-conjugated superoxide dismutase.³⁵ Similarly, Ulfvarson et al³⁸ have investigated the effect of exposure to diluted diesel exhaust in rabbits and found that this leads to moderate inflammation in the larger and smaller bronchi, as indicated by increased numbers of eosinophils and mononuclear and polymorphonuclear cells in the submucosa and the epithelium. Studies by Rudell et al^{39,40} and Salvi et al⁴¹ have suggested that exposure to diesel exhaust may also lead to significant inflammatory changes in human beings. These authors have demonstrated that the numbers of neutrophils, CD19+ cells, and fibronectin are increased in bronchial wash and/or bronchoalveolar lavage fluid of healthy nonasthmatic volunteers 6 to 24 hours after exposure.

Similarly, our finding that DEP can induce release of proinflammatory mediators from both asthmatic and nonasthmatic bronchial epithelial cells is also in accordance with the findings of Diaz-Sanchez et al,²⁰ who have investigated the effect of DEP on the synthesis of cytokines in the nasal mucosa of subjects allergic to ragweed allergen. These authors have demonstrated that intranasal challenge with 0.3 mg purified DEP led to increased and readily detectable levels of mRNA for IL-2, IL-4, IL-5, IL-6, IL-10, and IL-13 and IFN- γ in the nasal mucosal cells of these individuals 18 hours after challenge. Additionally, these authors have demonstrated that although the levels of mRNAs for IL-4, IL-5, IL-6, IL-10, and IL-13 were increased even further when allergen and DEP challenge were applied simultaneously, the levels of IL-2 were unchanged and the levels of IFN- γ mRNA were decreased, suggesting that DEP upregulated specifically the activity of T_{H2}-like lymphocytes.

In conclusion, these results suggest that bronchial epithelial cells of asthmatic individuals may be different from the bronchial epithelial cells of nonasthmatic individuals with regard to their ability to release different types and quantities of specific inflammatory mediators and their reactivity to DEP. Whether the differences are a consequence of an intrinsic defect, an acquired property caused by the disease process itself, or a combination of the two, remains to be determined.

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