

Diesel exhaust particles directly induce activated mast cells to degranulate and increase histamine levels and symptom severity

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Background: The ability of combustion products, such as diesel exhaust particles (DEPs), to modulate the immune system has now been firmly established. DEPs can synergize with allergen at the human upper respiratory mucosa to enhance allergen-specific IgE production, initiate a T_H2 cytokine environment, and even promote primary allergic sensitization. Experiments suggest that these effects result from the initial activation of mast cells to produce IL-4.

Objective: We sought to demonstrate that in vivo mast cell activation by DEPs plus allergen will also affect the release of classic mast cell mediators and consequently enhance the immediate-phase response.

Methods: Dust mite-sensitive subjects were challenged intranasally with allergen, and symptom scores and histamine levels in nasal wash samples were compared after prechallenge with 0.3 mg of DEPs.

Results: If the subjects were first sprayed with DEPs, mean symptom scores rose from 3.7 to 9.9; additionally, only one fifth of the amount of intranasal dust mite allergen was required to induce clinical symptoms. DEPs alone had no effect. The changes in symptoms correlated with histamine levels measured in nasal lavage specimens from these subjects. Although challenge with DEPs alone did not induce histamine release, challenge with both DEPs and allergen resulted in 3-fold higher histamine concentrations than those seen with allergen alone. In contrast, carbon black particles (elemental carbon devoid of chemicals) had no effect. The role of chemicals was confirmed because degranulation of a murine mast cell line by FcεRI cross-linking was increased significantly (by 72%) by the soluble organic chemicals extracted from DEPs.

Conclusions: Overall, these results suggest that exposure to DEPs can enhance the severity of clinical symptoms to allergen

Abbreviations used

DEP: Diesel exhaust particles

DEP-SOC: Soluble organic chemical fraction of DEP

by enhancing mast cell degranulation. (*J Allergy Clin Immunol* 2000;106:1140-6.)

Key words: Allergy, pollution, mediators, clinical outcomes, hypersensitivity

The prevalence of allergic rhinitis, atopic eczema, urticaria, and asthma are all increasing worldwide,¹ probably as a result of many different factors linked to urbanization and Westernization. Although lifestyles, exposure to infection, and diet are all likely important factors, many studies have also shown a strong link between industrialization and atopy. In particular, the ability of combustion products, such as diesel exhaust particles (DEPs), to modulate the immune system has now been firmly established,² at least under experimental conditions. In cities such as Los Angeles, the major fraction of airborne particulate pollution emanates from diesel vehicle engines. This has become an area of increasing public health concern because diesel engines have come to dominate the trucking industry in recent decades; for example, 88% of all heavy-duty trucks (>26,000 lbs) now run on diesel fuel.³

Both human and animal models of allergic rhinitis and asthma have shown that DEPs can have both short- and long-term effects on the immediate hypersensitivity allergic response. Acute coexposure to DEPs plus an allergen in the human upper respiratory tract markedly increases allergen-specific IgE while skewing the cytokine pattern toward a T_H2-like profile.⁴ In addition, DEPs can interact with allergen to drive in vivo isotype switching to IgE in the nasal mucosa.⁵ Chronic exposure of mice to diesel exhaust after ovalbumin sensitization results in enhanced allergic airway inflammation, goblet cell hyperplasia, and airway hyperresponsiveness.⁶ In human subjects repeated exposure to DEPs in combination with a neoallergen will induce a de novo IgE response and may increase allergic sensitization.⁷

In this study we focus on a novel area of interaction between DEPs and allergen: the initial effector phase of the immediate hypersensitivity response. Previous studies have shown that the release of preformed mast cell

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mediators (eg, histamine and toluenesulfonylarginine methyl ester esterase) in response to nasal allergen challenge directly correlates with symptoms.⁸ Using in vivo and in vitro approaches, we demonstrate that DEPs and their constituent chemicals can augment degranulation from allergen or anti-IgE-activated mast cells and thereby increase symptoms in human subjects.

METHODS

Subjects

Eleven nonsmoking volunteers (6 men and 5 women), ranging in age from 21 to 55 years, were recruited in Los Angeles, California. Subjects all had a history of allergic upper airway disease (allergic rhinitis) and a positive skin prick test response to house dust mite (*Dermatophagoides pteronyssinus*). A wheal greater than 4 mm in diameter with surrounding erythema was considered positive because this was clearly discernable and distinguishable from the background controls. In addition, 5 subjects had a history of seasonal allergies but were asymptomatic and were outside their allergy season. None of the volunteers had previously had any known unusual or extraordinary exposure to air pollutants. Throughout the period of the study, there were no air pollution alerts in the Los Angeles area. None of the subjects had used topical or systemic steroids in the 3 months before the study or oral antihistamines for the prior week. None had ever received allergy immunotherapy. All studies were approved by the Human Subject Protection Committee of the University of California at Los Angeles.

Materials

DEPs were the kind gift of Dr Masaru Sagai (National Institute for Environmental Studies, Tsukuba, Japan) and were obtained by collecting the exhaust from a 4JB1-type, light-duty, 2.74-L, 4-cylinder Isuzu diesel engine under a load of 10 torque onto a cyclone impactor equipped with a dilution tunnel constant volume sampler system. The particles were collected, processed, and preserved as previously described.⁹ Immediately before the in vivo challenge, these nonextracted DEPs were sonicated for 10 minutes in a 50-W sonic disrupter (Tekmar, Cincinnati, Ohio). Carbon black, which was the control for the in vivo experiments, was treated in exactly the same way. The soluble organic chemical fraction of DEP (DEP-SOC) was obtained by means of dichloromethane extraction of the DEPs, as previously described,¹⁰ and used in the in vitro mast cell degranulation experiments.

Nasal challenge and symptom scores

The severity of allergic symptoms in response to an allergen challenge were measured before and after nasal challenge by using a similar system to that previously reported by Togias et al.¹¹ The severity of nasal itching, nasal congestion, and rhinorrhea were rated by subjects on a scale ranging from 0 to 3 (no symptoms or mild, moderate, or severe symptoms). In addition, the number of sneezes were enumerated and scored from 0 to 3 (none, 1-2, 3-4, or >5 sneezes). Thus subjects could have a maximum symptom score of 12. A significant correlation has previously been shown between symptom scores and rhinoscopic evaluation.¹²

We performed a single-blind, randomized, placebo-controlled crossover study. The noses of allergic subjects were sprayed with increasing doses of an extract of house dust mite containing a known amount of the antigen Der p 1 (Hollister Stier/Baxter, Irwindale, Calif). The starting dose was 10 AU, and this was increased until a symptom score of 5 (see above) was achieved. Subjects then returned for 3 subsequent visits, each spaced at least 6 weeks apart. A single-blind crossover study design was used with subjects ran-

domly receiving nasal challenge with allergen and either 0.3 mg of DEPs (in 300 μ L of saline), 0.3 mg of carbon black, or placebo (in 300 μ L of saline). Dust mite allergen challenge was performed immediately afterward as before, and the dose of allergen now needed to obtain a symptom score of 5 was recorded. We have previously shown that if there is a gap of 30 days or more between allergen or DEP challenges, there is no priming or accumulative effect.

In a second study, during 4 visits spaced 6 weeks apart, subjects were challenged intranasally in a randomized, blinded, crossover fashion with allergen alone, allergen plus placebo, allergen plus 0.3 mg of carbon black, or allergen plus 0.3 mg of DEPs. The dose of allergen used was one tenth that previously determined to induce a symptom score of 5. Symptom scores were noted after nasal challenge. Nasal lavage specimens were performed immediately before and 5 minutes after challenge, as previously described.^{9,13} Briefly, for nasal lavage, 5 mL of normal saline was delivered into each nostril of the subjects, and after 10 seconds, the wash fluid was collected. The tubes were centrifuged at 350g for 10 minutes at 4°C, and the aqueous supernatants were separated from the cell pellets and used for histamine determination. Histamine levels in nasal wash specimens were measured by using a commercial assay (Immunotech, Brea, Calif), as per the manufacturer's instructions. The sensitivity of the assay was 0.5 nmol/L.

Mast cell line degranulation

A murine mast cell line (MMC-34) was used because the only appropriate human mast cell lines reported (eg, HMC-1) do not express Fc ϵ RI. MMC-34 cells were suspended (4×10^4 cells/well) in quadruplicate in a 96-well round-bottom plate (Nunc, Roskilde, Denmark) in Tyrode's solution containing 137 mmol/L NaCl, 2.7 mmol/L KCl, 1.8 mmol/L CaCl₂, 1.1 mmol/L MgCl₂, 25 mmol/L HEPES, 5.6 mmol/L D-glucose, 11.9 mmol/L NaHCO₃, and 0.4 mmol/L NaH₂PO₄. Cells were incubated with mouse IgE (0.5 μ g/mL) for 1 hour at 37°C. The cells were then washed, and anti-mouse IgE (R35-72; Pharmingen, San Diego, Calif) was added at 0.5 μ g/mL with different concentrations of DEP-SOC. After 1 hour of incubation at 37°C, the supernatant was harvested and spun for 10 minutes at 300g to sediment any free-floating cells. Granule release was measured by assaying for histamine as above or β -hexosaminidase activity by the release of *p*-nitrophenol from *p*-nitrophenol-D-2-acetamido-2-deoxyglucopyranoside (Sigma, St Louis, Mo) as per Schwartz et al.¹⁴ It was quantified by the amount of absorbance at 405 nm and compared with a *p*-nitrophenol standard (Molecular Probes, Eugene, Ore). The diluent used for DEP extraction was used as the control for these experiments. Histamine and β -hexosaminidase activity was identical between the diluent control and an extract of carbon black with dichloromethane and treated in the same fashion as the DEPs. Various clones of KU812, a human basophil cell line that variably expresses Fc ϵ RI, did not show reproducible histamine release under control conditions.

Statistical analysis

The Statview II computer package (Abacus concepts, Berkeley, Calif) for the Macintosh was used for all analysis. Comparisons of symptom scores were performed by using the Wilcoxon signed-rank test. In vivo histamine levels were analyzed by using paired *t* tests, and mediator release in vitro was analyzed by using the Mann-Whitney *U* test.

RESULTS

Symptom response to nasal challenge

Coadministration of DEPs significantly increased the symptom response to nasal challenge with allergen (Fig

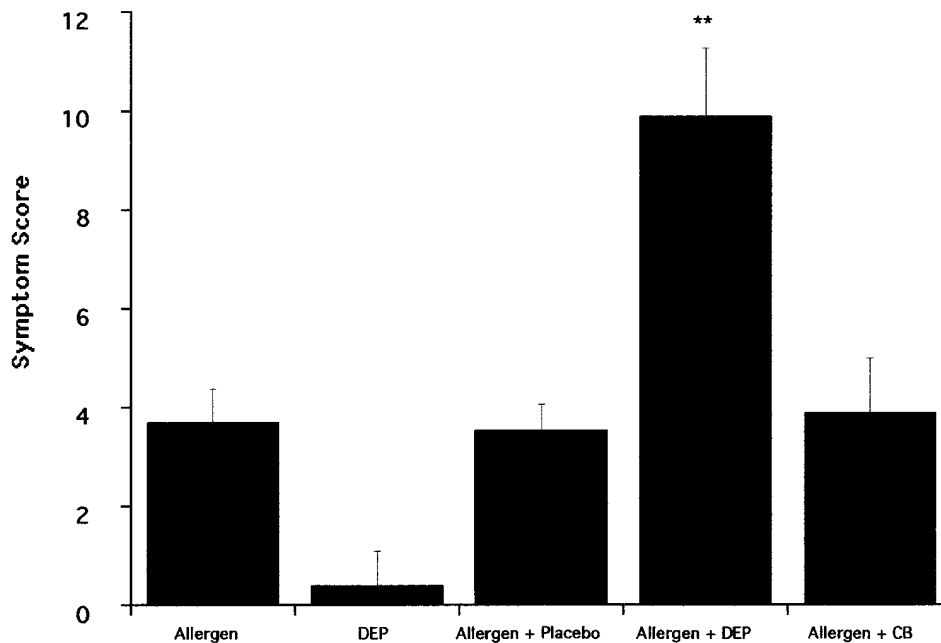


FIG 1. DEPs increase allergen-induced symptom scores. Subjects were challenged with a one-tenth dose of allergen (Der p 1) previously determined to induce a total symptom score of 5 (out of a possible 12). The mean $\pm$ 1 SD symptom scores for 10 subjects allergic to house dust mites are shown. CB, Carbon black. ** $P < .01$ versus allergen control.

1). Symptom severity in 4 categories (nasal itching, nasal congestion, rhinorrhea, and sneezing) was scored by dust mite-sensitive subjects after nasal challenge with either allergen (dust mite extract) alone, DEPs alone, allergen plus placebo, or allergen plus DEPs. Although DEPs alone did not elicit symptoms, they significantly increased symptoms induced by allergen. The mean total symptom score with allergen alone was 3.7, but when patients were coexposed to DEPs plus allergen, this rose to 9.9. This increase was observed in all individual symptoms, especially sneezing, with a mean of 2.2 sneezes recorded after allergen challenge and 6.4 sneezes after DEPs plus allergen. That DEPs were not exacerbating symptoms by acting simply as a particulate irritant was shown by the use of carbon black. Carbon black is composed of elemental carbon and is devoid of any chemicals. When subjects were challenged with carbon black plus allergen, no increase in symptom scores was observed over that of allergen alone.

In addition, DEPs significantly lowered the allergen threshold at which nasal symptoms occurred ($P < .05$, Fig 2). A total symptom score of 5 (out of a possible 12) was regarded as indication of moderate nasal symptoms. The amount of allergen required to elicit this score varied between subjects but was consistent for each subject, regardless of whether he or she was challenged with allergen alone or allergen in combination with placebo. In contrast, after exposure to both allergen and DEPs, on average only one fifth the amount of allergen was needed to generate the symptoms seen with allergen alone.

Histamine release after nasal challenge

Nasal provocation with DEPs plus allergen (Fig 3) induced significantly higher histamine levels in nasal lavage fluid than challenge with allergen alone ($P < .01$). Baseline levels of histamine were virtually identical in all challenge days. As expected, after challenge with allergen, there was a significant rise in histamine from baseline values (0.66 vs 4.6 nmol/L). However, this increased nearly 3-fold to 13.5 nmol/L when subjects were challenged with DEPs immediately before allergen. Potentiation of the allergen-induced histamine response was not observed when subjects were prechallenged with either placebo or carbon black (histamine level of 3.8 nmol/L and 5.6 nmol/L, respectively). Nasal provocation with placebo did not result in elevation of histamine above baseline levels. DEPs and carbon black alone also had no effect (data not shown).

Mast cell granule release

To determine whether the chemicals in DEPs could act directly on mast cells, a mouse mast cell line (MMC-34) was used (Fig 4). Levels of both β -hexosaminidase and histamine, preformed granule constituents in mast cells, were significantly higher after cross-linking of the high-affinity IgE receptor (Fc ϵ RI) by the combination of IgE and anti-IgE as a mimic of antigen binding, as previously reported by others.¹⁵ Addition of the soluble organic chemicals extracted from DEPs (DEP-SOC) to IgE/ α -IgE-activated cells augmented the release of both β -hexosaminidase and hista-

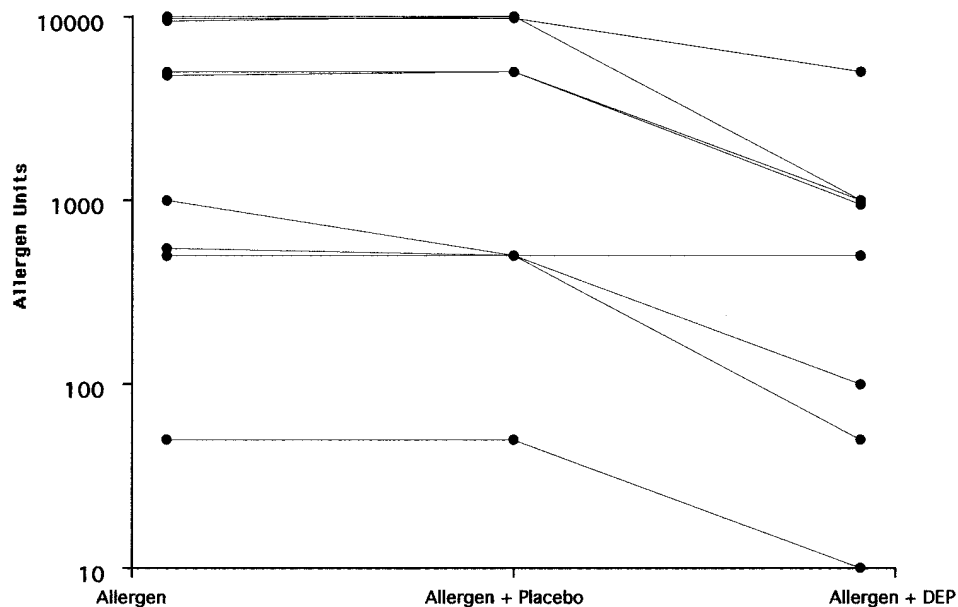


FIG 2. DEPs lower the allergen threshold for symptoms to occur. Subjects were challenged with increasing doses of allergen (Der p 1) until a symptom score of 5 (out of a possible 12) was achieved. The amount of allergen needed (in allergen units) for each of 10 subjects is shown.

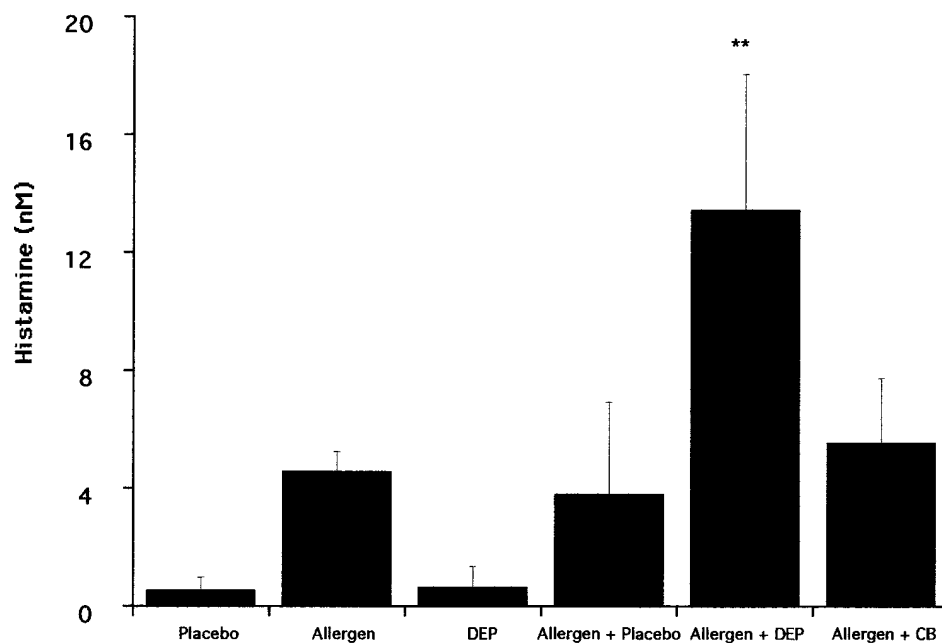


FIG 3. DEPs increase allergen-induced histamine release in nasal wash specimens. The concentration of histamine in nasal lavage fluid before and after nasal challenge with a one-tenth dose of allergen (Der p 1) previously determined to induce a total symptom score of 5 (out of a possible 12) was measured. The mean $\pm$ 1 SD for 10 subjects is shown. CB, Carbon black. ** $P < .01$ versus allergen control.

mine in a dose-dependent manner. At 100 ng/mL or higher concentrations of DEP-SOC, degranulation was significantly greater than in cells stimulated with IgE/ α -IgE alone ($P < .01$, Mann-Whitney U test). At 100 ng/mL, release of

β -hexosaminidase and histamine was 49% and 72% higher, respectively, than release from cells stimulated with IgE/ α -IgE alone. DEP-SOC alone did not alter the release of either mediator, even at concentrations as high as 2000 ng/mL.

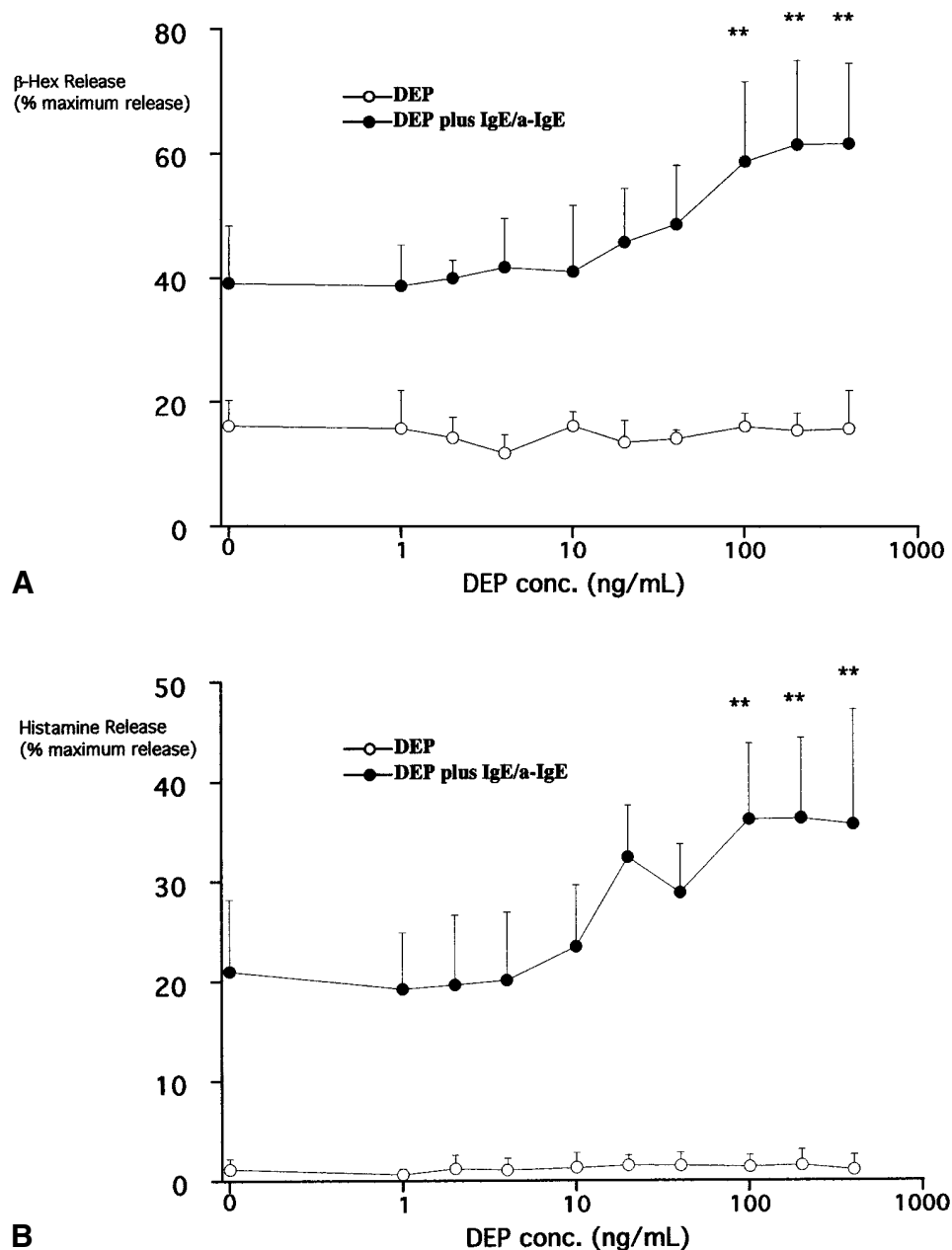


FIG 4. DEPs induce degranulation of Fc ϵ RI cross-linked murine mast cells. MMC-34 mast cells were incubated with DEPs in the presence or absence of IgE/ α -IgE cross-linking of the high-affinity IgE receptor. The amount of β -hexosaminidase (β -Hex, **A**) and histamine (**B**) released in the supernatant was measured and expressed as percentage degranulation. Each point represents the mean $\pm$ 1 SD of 7 (**A**) or 3 (**B**) independent experiments performed in triplicate. ** $P < .01$ versus no DEP control.

DISCUSSION

This study is the first to demonstrate that the important air pollutant DEPs can act on mast cells to enhance the immediate-phase response to allergen. It suggests that symptoms will occur on encounter of allergen plus DEPs at concentrations of antigen that alone would not elicit a response. This is particularly true for high-dose short-term exposure rather than chronic DEP exposure.

In addition to the release of preformed mediators, activation of mast cells causes production and release of newly synthesized lipid products, such as PGD₂, which have been recognized as important mediators of allergic asthma¹⁶ and cytokines, notably IL-4, IL-6, TNF- α , and IL-13.^{17,18} IL-4 is central to the formation of IgE and the development of T_H2 effector cells, both features of an allergic response. We have previously shown that in our

human model, combined intranasal challenge with DEPs plus allergen induce a large increase in the local production of allergen-specific IgE. Moreover, in allergic subjects this increase was associated with a T_H2 -type cytokine profile, including enhanced IL-4 and IL-13 production and decreased production of IFN- γ .^{13,19} In this model DEPs plus allergen induce CD117⁺ (c-kit⁺) cells (primarily mast cells) in the nasal mucosa to be the primary contributors in the early or initial production of IL-4.²⁰ We proposed that this initial burst of IL-4 expands the repertoire of IL-4-producing cells in the local microvicinity until other cells (predominantly CD4⁺ cells) become the predominant source of IL-4 and a T_H2 response is established.

DEPs have also been shown to induce IL-4 and IL-6 production from bone marrow-derived mast cells in vitro.²¹

DEPs consist of particles consisting of an elemental carbon core surrounded by a host of chemicals, including polyaromatic hydrocarbons and quinones. Both particles and chemicals have been implicated in the adjuvant properties of DEPs. It has been reported that both carbon black (elemental carbon) and pyrene (a polyaromatic hydrocarbon) can increase specific IgE production in mice.^{22,23} In vitro, DEP-SOC and phenanthrene, a major component of the chemicals, can enhance IgE production from B cells. In this study we show that carbon black had no effect on either symptoms or histamine production. Furthermore, DEP-SOC directly enhanced MMC-34 mast cell degranulation, suggesting that it is the organic chemicals in the DEPs that are the key players in this effect. This is in agreement with studies on the mechanisms of action of DEPs, where it recently was shown that DEP-SOC can activate transcription of genes containing antioxidant response elements²⁴ by rapidly inducing the production of reactive oxygen species.²⁵ Kumagai et al²⁶ also demonstrated that DEP extracts induce superoxide and hydroxyl radical generation when coincubated with lung microsomal preparations. The role of oxidative stress on DEP-induced mast cell histamine release is as yet unknown, and further studies in this area are warranted.

DEPs are a major air component of inhaled particulate pollution in the industrialized world. DEPs and their polycyclic aromatic hydrocarbon components have been demonstrated to enhance IgE production in animals and human subjects both in vivo and in vitro.^{4-7,9,22,27} Their role in enhancing the worldwide increase in allergic disease has been the focus of much debate.²⁸ Recently, DEP-SOC was also shown to increase expression of histamine H1 receptor in cultured human epithelial cells and increase histamine-induced IL-8 and GM-CSF production from these same cells.²⁹ These results suggest that DEP exposure should lead to enhanced inflammation. Indeed, when healthy human volunteers are exposed to diesel exhaust an increase in inflammatory cells and markers, as well as an increase in histamine, is observed in airway lavage specimens.³⁰

Yet DEPs are not unique among xenobiotics in their ability to directly induce mast cell activation and degranulation. Mercuric chloride is a compound used in mining

and manufacturing that has similar effects to DEPs in that it has recently been shown to cause granule release from murine mast cells and the secretion of the cytokines IL-4 and TNF- α .³¹ In both rats and mice mercuric chloride can induce potent specific and polyclonal IgE production³² through induction of IL-4.³³ In contrast to DEPs, mercuric chloride alone induced mast cell degranulation and IL-4 production. In our study DEPs alone had no effect on either in vivo histamine production or symptom scores or in vitro degranulation. In addition, we have previously seen that in vivo IL-4 production from mast cells requires both DEPs and allergen. In this respect DEPs resemble adenosine, a naturally occurring purine nucleoside, which potentiates IgE-dependent histamine release from human lung mast cells in vitro.³⁴ Nasal challenge with adenosine 5'-monophosphate will increase symptoms and histamine release in allergic individuals but not in nonallergic control subjects.³⁵ Adenosine-induced symptoms of rhinitis can be blocked by the use of antihistamines.³⁶ Whether increased histamine release is due to the ability of DEPs to increase intracellular cyclic adenosine 5'-monophosphate is currently being studied.

In conclusion, we have now shown that DEPs can activate mast cells directly to have at least 2 important immunologic effects in concert with allergen. In the immediate term it can augment allergen-induced degranulation, increasing histamine release and leading to worsening symptoms. In the intermediate term it can increase IL-4 production and thereby deviate the immune system toward a T_H2 environment, enhancing the likelihood in sensitization and exacerbation of allergic disease. Oil and engine manufacturers have made a concerted effort to decrease the emissions from diesel engines in the last few years. As a consequence, the mass of DEPs emitted has declined by almost 6-fold; however, some reports suggest that this decrease has been accompanied by a rise in the number of particles and perhaps a reduction in particle size.³⁷ Given the dramatic increase in truck traffic in recent years (620% increase from 1950-1997)³⁸ and the predominance of diesel as the fuel of choice in trucks, we believe that the potential of DEPs to exacerbate allergic symptoms may represent an important health issue.

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