Challenge with environmental tobacco smoke exacerbates allergic airway disease in human beings

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Background: Despite widespread perceptions that environmental tobacco smoke (ETS) is a potent risk factor for allergic airway disease, epidemiologic studies studying this have been equivocal. There is a clear need for experimental studies to address these questions.
Objective: We directly tested the hypothesis that ETS could interact with allergen in human beings to alter immune responses and promote changes associated with allergic airway disease.
Methods: In a randomized, placebo-controlled crossover study, 19 nonsmoking volunteers with ragweed allergy underwent nasal lavage followed by controlled chamber exposures to 2 hours ETS or clean air followed by another nasal lavage. Subjects immediately randomly received nasal challenge with either ragweed allergen or placebo (300 μL saline). Lavages were also performed 10 minutes, 24 hours, and 4 and 7 days after challenge and IgE, cytokines, and histamine measured. The other arms of the study were spaced at least 6 weeks apart.
Results: Environmental tobacco smoke promoted the production of allergen-specific IgE, the hallmark of allergic disease in nasal lavage fluid. Four days after exposure to ETS/ragweed, levels were on average 16.6-fold higher than after clean air/ragweed challenge. In addition, ETS (vs air) promoted the induction of a T_H2-cytokine nasal milieu (increased IL-4, IL-5, and IL-13 and decreased IFN-γ production), characteristic of an active allergic response. Moreover, nasal histamine levels were 3.3-fold greater after ETS/ragweed challenge than after clean air/ragweed challenge.
Conclusion: These studies provide the first experimental evidence that secondhand smoke can exacerbate allergic responses in human beings.
Clinical implications: The studies suggest that patients with allergies should avoid tobacco smoke. (J Allergy Clin Immunol 2006;118:441-6.)

Key words: *Environmental tobacco smoke, IgE, pollution, histamine, allergic airway disease*

There is broad agreement that allergic airway disease prevalence has risen dramatically over the period of the last 200 years, especially in the last few decades.[1,2] The causes of this increase are the source of much discussion and controversy.[3,4] Epidemiologic studies have been useful in demonstrating associations between allergy and/or asthma and diet, childhood infections, allergen levels, and indoor and outdoor pollutants.[5-8] Despite the many strengths of these studies, they have been less useful in demonstrating causality and possible mechanisms. There is a clear need for experimental studies to address these questions.

Maternal smoking has been identified in multiple epidemiologic studies as a risk factor for allergic disease.[9-15] These studies have shown associations between environmental tobacco smoke (ETS; commonly referred to as *second-hand smoke* or *passive smoking*) and increased skin test reactivity, serum IgE, and prevalence of eosinophilia in children. Yet despite claims that with regards to allergy, ''tobacco smoke is by far the most important single environmental factor,''[16] this is still a matter of contention, and very few studies have tested this supposition by direct experimentation. Pioneering studies by Bascom[17] showed that exposure to sidestream smoke (the main component of ETS) can cause a spectrum of individual responsiveness including nasal resistance and congestion in normal healthy individuals. None to our knowledge have examined atopic subjects and the interaction of allergen with ETS.

Here we use human exposures in an environmentally controlled chamber to test the hypothesis that ETS can interact with allergen to modulate immune responses in the upper airway. We demonstrate for the first time that short-term exposure of subjects with allergy to ETS leads to an enhanced allergic response characterized by specific allergic antibody (IgE) production against an inhaled protein allergen and the local formation of a T_H2-type cytokine milieu, characteristic of and critical to allergic inflammation.

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Abbreviations used
DEP: Diesel exhaust particle
ETS: Environmental tobacco smoke
PM: Particulate matter

METHODS

Study design

We performed a randomized, placebo-controlled crossover study. Nasal lavages were obtained from subjects with ragweed allergy. Fifteen minutes later, they entered the inhalation exposure chamber. The subjects were randomized to receive 2-hour exposure to either clean air or ETS. After the 2-hour exposure, subjects were removed from the chamber, and immediately afterward, nasal lavages were again performed. On completion of nasal lavage, subjects immediately randomly received nasal challenge with either ragweed allergen or placebo (300 μL saline), a process that took less than 1 minute. Lavages were also performed 10 minutes, 24 hours, and 4 and 7 days after challenge. Subsequent visits to perform the other arms of the study were spaced at least 6 weeks apart.

Subjects

A total of 19 nonsmoking volunteers (7 men and 12 women) age 20 to 34 years were recruited in Los Angeles, Calif. All had been shown to have an allergy history consistent with allergic rhinitis and a positive skin prick test (Multitest; Lincoln Diagnostics, Decatur, Ill) to short ragweed. Although all subjects showed positive skin tests for other allergens, all were asymptomatic, and none complained of symptoms during the course of the study or 1 week before. In addition, all subjects who were challenged intranasally with the ragweed allergen Amb a 1 displayed immediate allergic symptoms such as sneezing, runny nose, and ocular itching. The subjects did not take any medication for the 3 days before or during the duration of the study. None of the volunteers cohabited with smokers or had any known extensive or extraordinary exposure to pollutants. Salivary cotinine measurements confirmed that none were active smokers. Short ragweed was used as the antigen in the nasal challenges because it is not present in the Los Angeles area, and the cross-reacting western ragweed is a minor allergen. Ragweed IgE levels in nasal lavages were very low or undetectable before challenge. The research was approved by the Human Subject Protection Committees of the University of California at Los Angeles and Los Amigos Research and Education Institute, Rancho Los Amigos National Rehabilitation Center, Los Angeles. All subjects provided written consent.

Generation of ETS and controlled exposure

The exposure chamber (700 ft^3) maintained controlled temperature (70°F), humidity (50%), and ventilation (8 exchanges/h) during the exposures, which lasted for 2 hours. During this time, subjects rested and refrained from vigorous activity. ETS was generated from the side-stream smoke of 1R4F cigarettes (University of Kentucky Tobacco and Health Research Institute, Lexington, Ky). ETS consists primarily (95%) of side-stream smoke (emitted from the burning zone) and also (4%) smolder stream smoke (emitted from the puffing zone).[10] Each of these filtered reference cigarettes contains 9.2 mg tar and 0.8 mg nicotine.[18] To maintain proper moisture levels, the cigarettes were stored in a sealed plastic bag at 4°C. They were immediately brought to room temperature 15 minutes before needed and lit in a RM G1 Borgwaldt smoking machine (Hamburg, Germany). The smoking machine was placed in the chamber at a distance of at least 2 feet from the subjects. This automated smoking machine was set to conform to the Federal Trade Commission guidelines for the generation of side-stream smoke: 1 inhalation every 55 seconds with an inhalation/exhaust cycle of 5-second duration. Mainstream smoke was captured, scavenged by charcoal filters, and excluded from entering into the system. A total of 5 cigarettes were smoked by the machine in each 2-hour period.

The levels of carbon monoxide in the chamber were continuously monitored and were never higher than 5 ppm during the exposure. The mean particulate matter (PM) level was 310 $\mu g/m^3$. Measurements in the chamber showed a uniform distribution of particulates throughout the chamber beyond a distance of 2 feet from the smoking machine. Subjects were mobile but excluded from approaching within 2 feet of the machine. The ETS exposure was well tolerated by all subjects. Five of the subjects reported mild irritation of the throat and eyes during exposure, but these symptoms did not persist for more than 5 minutes. No other adverse symptoms were noted. Clean air exposures were performed in the same manner with the absence of smoke generation. The mean PM level during clean air exposure was 46 $\mu g/m^3$. All exposures were performed at the same time of day (between 9 and 11 AM) to avoid diurnal variation.

Allergen challenge and nasal lavage

Allergen challenge was performed as previously detailed.[19,20] Briefly, at least 30 days before each clean air/ETS exposure, a dose of allergen that would elicit allergic symptoms was established for each subject. This was done by spraying the nose of allergic subjects with increasing doses of an extract of ragweed containing a known amount of the antigen Amb a 1 (Hollister Stier/Baxter, Irwindale, Calif). The starting dose was 10 AU, and this was increased until a symptom score of 5 (out of 12) was achieved in our allergic symptom severity score system.[19,20] Subjects then returned for 2 subsequent visits that were spaced at least 6 weeks apart. In these visits, allergen challenge was performed after either clean air or ETS exposure. Subjects were challenged with the established allergen dose immediately after the nasal lavage performed after clean air/ETS exposure.

Nasal lavage is a well established procedure which has been used for more than 20 years to study the effect of pollutants on the upper airway,[21] and was performed as previously described.[22,23] Briefly, 5 mL normal saline was delivered into each nostril of the subjects, and after 10 seconds, the wash fluid was collected. The subjects then performed 4 subsequent nasal washes. The samples were pooled and tubes were centrifuged at 350*g* for 10 minutes at 4°C and the aqueous supernatants separated from the cell pellets and stored at −20°C until needed.

Immunoassays

The levels of IgE, IgG, IgG_4, and IgA in the supernatants were measured by isotype specific ELISAs as previously described with minor modifications.[19,22,24] All samples were run in duplicate and repeated if there was more than a 10% variation between the duplicates. Ragweed-specific IgE, IgG, and IgG_4 were determined as previously reported using the same procedure as for total Ig isotypes except that an amplification system previously described was used with minor modifications.[19] Cytokines were measured by using commercial ELISA kits (BD Pharmingen, San Diego, Calif) as per the manufacturer's instructions. Because many baseline levels are under the detection limit of the assays, samples were concentrated 20-fold (Centricon concentrators; Amicon, Bedford, Mass). Quality control tests were performed by spiking saline and lavage samples and demonstrated that recovery was greater than 95%, was reproducible, and was the same for all cytokines measured. If a signal was still not apparent, we used an ELISA amplification system (Gibco, Rockville, Md), which uses nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) enzyme conversion to increase sensitivity as much as 20-fold. If no signal was still seen, the samples were considered nondetectable.

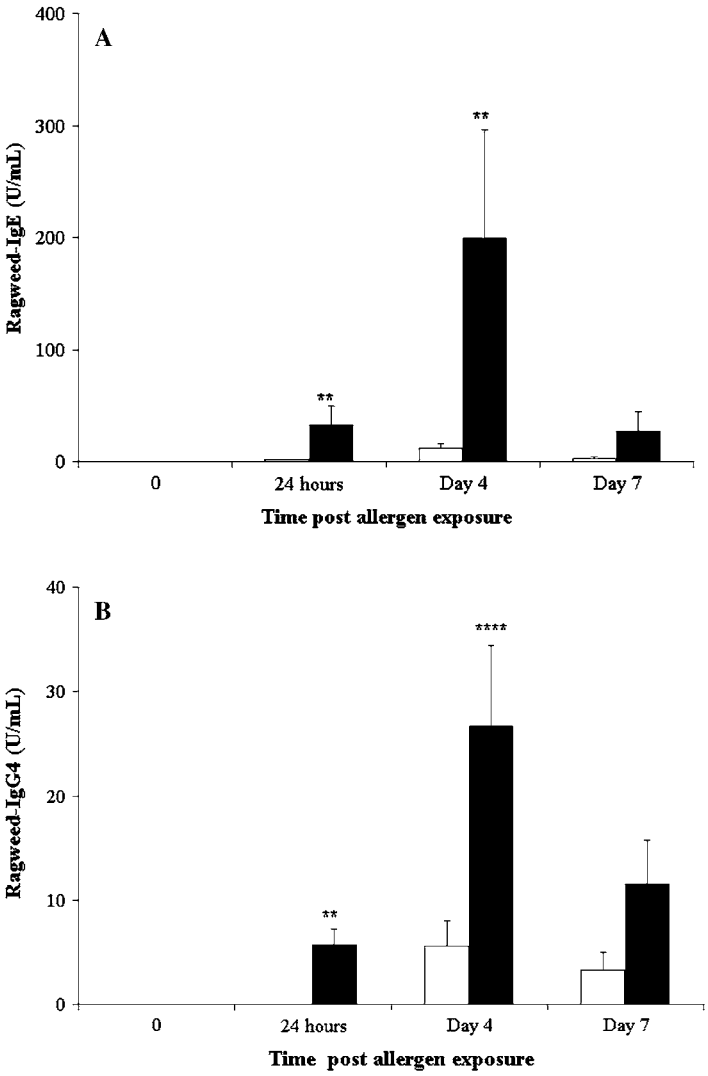

FIG 1. ETS interacts with allergen to enhance allergen specific IgE and IgG_4 levels. Subjects were challenged with ragweed allergen after ETS *(black bars)* or clean air *(white bars)* exposure. The mean ± 1 SE ragweed IgE **(A)** or IgG_4 **(B)** levels in nasal washes for 19 subjects is shown. $^{**}P < .01$ vs challenge with clean air/ragweed.

Histamine was measured in nasal lavages performed before and immediately after ETS/clean air exposure and 10 minutes after allergen exposure. Histamine levels in nasal washes 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.

Statistical methods

Samples that were below the lower limit of detection for each assay were assigned a value of lower limit of detection/$\sqrt{2}$ for use in analyses. Comparisons were performed by using paired *t* tests. All analyses were conducted by using SAS software (SAS Institute, Cary, NC), and all reported *P* values are based on a 2-sided alternative hypothesis.

RESULTS

ETS enhances allergen-induced allergic antibody responses

Allergen-specific IgE is the hallmark of allergic disease. Fig 1 shows that ETS promoted the production of ragweed-specific IgE. As expected, challenge with ragweed after clean air (control) exposure resulted in a significant increase in ragweed-specific IgE at 24 hours and at days 4 and 8. However, the IgE levels detected in nasal lavage fluids after ragweed challenge after ETS exposure significantly exceeded these responses. Four days after exposure to ragweed plus ETS, levels were as much as 65-fold higher in a subject than after clean air/ragweed challenge (mean, 199 U/mL ± 96 U/mL vs 12 U/mL ± 4 U/mL). This resulted in highly significant differences between the 2 challenge protocols ($P < .005$ and $P < .001$ for 24 hours and day 4, respectively; paired *t* test).

Similar results were observed for IgG_4, an antibody thought to be closely coregulated with IgE. Levels of ragweed specific-IgG_4 were significantly higher after ETS/ragweed exposure than clean air/ragweed exposure (eg, after 4 days mean levels were 26.7 U/mL ± 7.8 U/mL vs 5.6 U/mL ± 2.4 U/mL). In contrast, no differences

TABLE I. Cytokine levels in nasal lavages 24 hours after nasal provocation challenge†

	Clean air (n = 10)	ETS (n = 10)	Clean air/ragweed (n = 19)	ETS/ragweed (n = 19)
IL-4 (pg/mL)	ND	ND	ND	2.54 (1.24)**
IL-5 (pg/mL)	ND	ND	0.31 (0.30)	4.82 (0.51)**
IL-13 (pg/mL)	ND	ND	0.14 (0.09)	1.06 (0.49)*
IFN-γ (pg/mL)	1.22 (0.45)	0.89 (0.34)	0.91 (0.22)	0.45 (0.08)*

ND, Nondetectable.
*$P < .05$ vs clean air/ragweed challenge.
**$P < .01$ vs clean air/ragweed challenge.
†Mean cytokine levels (SE).

were observed between the 2 exposure regimes in levels of other antibody types (data not shown). Thus, for both ragweed-specific IgG and IgA, levels measured in nasal washes were the same after challenge with ETS/ragweed or with clean air/ragweed ($P > .05$; paired t test). As expected, exposure to ETS with placebo and not allergen did not result in the formation of any allergen-specific antibodies at any time point (data not shown).

ETS enhances allergen-induced T_H2 cytokine production

Environmental tobacco smoke interacted with allergen to produce a local T_H2 cytokine milieu, a response characteristic of an enhanced allergic response and critical to allergic inflammation. After challenge of allergic subjects with clean air/allergen, we observed little or no change in nasal cytokine levels compared with clean air alone (Table I). In contrast, if allergen challenge was performed after ETS exposure, there was a rise in IL-4, IL-5, and IL-13 levels in nasal washes obtained 24 hours later. The levels of these cytokines were significantly greater at this time than after challenge with allergen/clean air (eg, IL-5 mean, 4.8 pg/mL vs 0.3 pg/mL; $P < .01$). In contrast, IFN-γ levels were significantly inhibited by ETS plus allergen exposure. ETS exposure alone did not significantly enhance IL-4, IL-5, IL-13, or IFN-γ in lavages obtained 24 hours later or at day 4.

ETS augments allergen-induced histamine release

Histamine is the principal mediator involved in immediate hypersensitivity responses to allergen. We measured histamine levels in nasal lavage fluid obtained 10 minutes after challenge with ragweed allergen. Significantly higher levels were measured when subjects were pre-exposed to ETS than clean air (Fig 2). Baseline levels of histamine were virtually identical in all challenge days. After clean air/ragweed challenge, there was a 7.7-fold rise in mean histamine from baseline values (4.02 nmol/L ± 0.9 nmol/L vs 0.52 nmol/L ± 0.3 nmol/L). In contrast, this was significantly less than the 25.1-fold increase observed after ETS/ragweed challenge. In the absence of allergen provocation, no changes in histamine levels were observed. Thus, 2 hours of ETS or clean air exposure alone did not result in elevation of histamine above baseline levels.

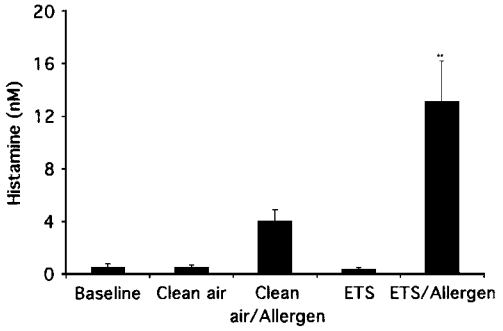

FIG 2. ETS increases allergen-induced histamine release in nasal lavages. The concentration of histamine in nasal lavage fluid was determined before (baseline) and immediately after ETS or clean air exposure and 10 minutes after challenge with allergen. Allergen challenge was performed after ETS or clean air. The mean ± 1 SE for 19 subjects is shown. **$P < .01$ vs clean air/allergen.

No differences in histamine levels between groups was observed in lavages performed 24 hours after exposure.

DISCUSSION

This is the first report using a human *in vivo* model to demonstrate definitively that secondhand smoke can interact with allergen to exacerbate acute allergic responses. It is now clear that severity of allergic airway diseases such as allergic rhinitis and asthma is a consequence of the interplay between genes and environment.[25-27] Underpinning these diseases are the formation of a T_H2 cytokine environment in the airways and the production of allergen-specific IgE antibodies.

Environmental tobacco smoke has been implicated in various diseases including middle ear effusion, bronchitis, and pneumonia in children.[10] Several epidemiologic studies have also found associations between household exposure of ETS and airway disease in children.[28-30] These studies have suggested that emergency department visits, asthma symptom severity, and medication usage are all increased if children with asthma are exposed to parental smoking.[13,31-33] Indeed, the US Environmental Protection Agency in its health review document on passive smoking concluded that ETS was a major cause of childhood asthma exacerbation that worsened the condition of between 200,000 and 1 million children with asthma in the

US.[34] Most studies have focused on childhood because this is when asthma often begins, ETS exposure may be assessed more readily, and confounders such as active smoking are few. A few studies that have studied adult populations have seen associations between asthma severity and ETS exposure. Eisner,[35] using data obtained from the National Health and Nutrition Examination Surveys (NHANES III), showed that ETS is associated with decreased pulmonary function in adults and that this is especially apparent in patients with asthma.

The previous assertions are based primarily on observational studies with few studies considering the mechanism underlying these results. Most of these studies postulate that the ETS effect is primarily mechanical as a direct irritant causing a gross inflammatory process that leads to airway damage and a subsequent impairment of airway caliber or bronchial responsiveness. Here, we provide evidence that in human beings, ETS can work through an additional mechanism of adjuvancy: the interaction with allergen to alter the immune system and enhance allergic responses. In our model, ETS/allergen resulted in a noticeably increase in nasal levels of IL-4, IL-5, and IL-13 and a decrease in IFN-γ. Thus, ETS can cause a shift to a T_H2-dominated local cytokine milieu. Some other nasal provocation studies using allergen alone have seen a similar change in cytokine levels. We did not, presumably because our subjects received allergen enough to elicit only mild symptoms. One caveat to these findings is that cytokine levels were very low, necessitating concentration. However, we would argue that they are important because they are an indicator of the general milieu. Moreover, although the results are a sample of the whole nasal cavity, it is likely that local cytokine levels or hotspots are of importance.

Support for the notion of ETS as an adjuvant comes from 3 sources: epidemiologic studies, murine models, and work using other particulate pollutants. As noted before, ETS exposure in children has been associated with increased serum IgE and skin test reactivity (presumably because of heightened histamine release). However, other studies have failed to observe any association.[36] We and others have shown that ETS exposure exerts an adjuvant effect in mice characterized by an increase in antigen-specific IgE, elevated T_H2 responses, and influx of eosinophils into the lungs.[37,38] These studies have also shown that ETS can augment primary sensitization to an innocuous protein.

Tobacco smoke consists of approximately 6000 known chemical components. Other particulate pollutants resulting from incomplete combustion of organic materials such as diesel exhaust particles (DEPs) contain many of the main constituents of ETS such as polyaromatic hydrocarbons and also activate phase I and II detoxifying enzymes.[27,39-42] Human and murine *in vitro* and *in vivo* studies have demonstrated that DEP and polyaromatic hydrocarbons can also induce IgE, increase T_H2 cytokine production, select against T_H1 cytokines, and augment histamine release (see review[43-45]). The mechanism by which ETS can induce adjuvancy is still unknown; however, important clues can be gleaned from the literature of similar effects of DEP and other PM. Many investigators have focused on the ability of PM to generate reactive oxygen species and oxidative stress, which can activate redox-sensitive transcription factors such as nuclear factor-κB and activation protein 1, which regulate expression of many proinflammatory cytokines.[46] The role of oxidative stress in asthma and allergic disease has received much attention of late.[47] Because cigarette smoke is an extremely potent oxidant mixture with 10^{14} oxidant radicals produced per puff,[48] we propose it is likely that similar pathways are involved and that induction of oxidative stress and formation of reactive oxygen species results in activation of transcription factors that regulate T_H2 cytokines directly or indirectly. Current studies are underway to test this hypothesis.

The ability of ETS to augment allergen-induced histamine release and IgE has important clinical implications. Release of chemicals such as histamine from mast cells and basophils mediate allergic airway disease and are the key factor in symptom severity.[49] Allergen-induced histamine release is dependent on the density of IgE receptors on cell surfaces, which in turn are dependent on circulating IgE levels.[50] The central role of IgE in allergic disease is highlighted by the results of clinical studies treating subjects with asthma with monoclonal anti-IgE antibody.[51] This treatment results in the drastic reduction of free serum IgE levels and the inhibition of both early-phase and late-phase responses and symptoms to allergen inhalation in subjects with asthma.[52,53] We also observed that ETS augmented allergen-induced IgG_4 but not IgA levels. This is unsurprising because IgE and IgG_4 are to a large part (but not completely) regulated by common mechanisms, and high levels of allergen-specific IgG_4 are often observed in patients with allergy.[54,55]

In conclusion, although cigarette smoking has generally declined in the Western world, it is still a considerable problem worldwide. Estimates of daily exposure of children to ETS in the home range as high as 40% to 60% in some reports.[56-58] The demonstration that ETS can augment allergic airway responses is therefore potentially a public health issue of extreme importance.

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