

protection against development of T_H2 -immune responses during childhood and exacerbation of asthma in persons who already have allergic airway inflammation.¹ Occupational and experimental inhalation exposures to endotoxin have been reported to induce bronchospasm in both asthmatic and nonasthmatic persons, but it has been postulated that asthmatic persons have increased airway reactivity to endotoxin.¹ Michel et al² observed that asthma severity in mite-allergic asthmatic persons correlated better with levels of endotoxin than with mite allergen in dust samples recovered from their homes. Our group has demonstrated that allergen-induced inflammation enhances the response to endotoxin in the nasal airways and that baseline sputum eosinophilia is associated with neutrophilic response to inhaled endotoxin.^{3,4} These studies suggest that allergen and endotoxin may act synergistically in the airway. We have previously reported that ozone exposure enhances immediate airway response to inhaled allergen and hypothesized that inhaled endotoxin would have a similar effect.⁵

Teeuw et al⁶ reported increased symptoms in office workers exposed to ambient levels of endotoxin (100 to 408 ng/m³) that are considerably lower than those present in some occupational exposures (>5000 ng/m³ in very contaminated workplaces) or used in most human challenge experiments. We thought that it was important to determine if exposure to an endotoxin concentration approximating these indoor environments enhances response to inhaled allergens in mildly mite-sensitive asthmatic persons. Participants underwent double-blinded 4-hour chamber exposures to clean air and airborne particulate-associated endotoxin (approximate concentration, 500 ng/m³) in randomized order separated by at least 3 weeks. Approximately 1 hour after each exposure, they underwent a graded-dose inhaled allergen challenge, then were monitored by spirometry until stable and admitted to the General Clinical Research Center (GCRC) for overnight observation. The study was approved by the Committee for the Protection of the Rights of Human Subjects and the Clinical Research Advisory Committee of the GCRC of the University of North Carolina School of Medicine.

Gram-negative bacterial (*E agglomerans*) endotoxin adsorbed onto microgranular cellulose particles (FMC Corporation, Philadelphia, Pa) was aerosolized into a plexiglass chamber for the exposures. The particles were introduced into the exposure chamber through a port after being entrained into an airstream from a groove on a turntable by a suction tube over the groove. Airborne dust concentration was monitored continuously during the exposure with the use of a Portable Continuous Aerosol Monitor (PCAM) optical particle counter (PPM Inc, Knoxville, Tenn). The rate of dust generation was varied as required to maintain the desired dust concentration in the chamber. Dust samples for analysis to verify the airborne dust and endotoxin concentration during the exposures were collected on membrane filters in open-faced cassettes. Endotoxin concentration per milligram of dust was measured by a chromogenic modification of the

Low-dose airborne endotoxin exposure enhances bronchial responsiveness to inhaled allergen in atopic asthmatics

To the Editor:

Endotoxin exposure has been associated with both

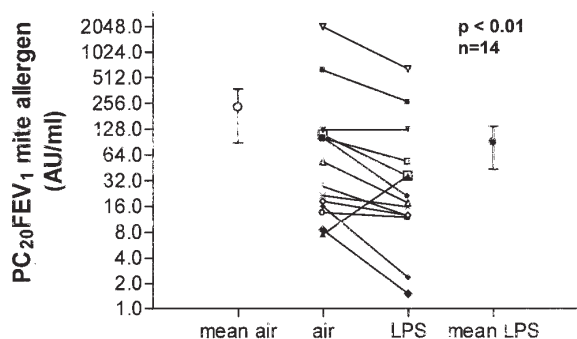


FIG 1. Effect of endotoxin on PC₂₀ FEV₁ to *D Farinae* allergen after 4-hour exposure to 500 ng/m³ of endotoxin and clean air.

Limulus amoebocyte assay after extraction in pyrogen free water for 1 hour at room temperature. Mean airborne dust concentration for the endotoxin exposures was 0.312 mg/m³, with an endotoxin content of 1548 ng/mg, giving a mean endotoxin concentration across all exposures of 496 ng/m³. The mass median aerodynamic diameter of the particles was measured as 4.9 μ m.

We studied 14 mildly allergic, nonsmoking asthmatic persons 18 to 46 years of age who were sensitive to house dust mite by skin testing and who responded to inhaled allergen challenge. All had airway hyperresponsiveness defined as a 20% fall in FEV₁ (PC₂₀ FEV₁) at a methacholine concentration of ≤ 10 mg/mL on a standard graded-dose bronchoprovocation test. Volunteers were excluded if they had a physician-directed emergency treatment for an asthma exacerbation or used systemic corticosteroid therapy within the preceding 12 months, used inhaled steroids or cromolyn within the past month, or used theophylline on a daily basis within the past month. Subjects were also disqualified if they were currently using medications that might alter the response to methacholine or antigen challenge (including anti-inflammatory and antihistamine agents), or were unable to withhold inhaled or oral bronchodilating medications for 12 hours before the exposure. Current cigarette smoking or occupational exposure to organic dust containing endotoxin, for example, textile mill work, and recent respiratory tract infections were also disqualifying.

The subjects' peak expiratory flow rate and exhaled NO excretion rate was measured before entering the chamber. Peak flow rate and respiratory symptoms were also recorded each hour in the chamber. Approximately 1 hour after completion of the exposure (5 hours after starting exposure), subjects underwent a graded-dose inhalation challenge with aerosolized mite allergen to determine the dose causing a 20% fall in FEV₁ (the PC₂₀ FEV₁ allergen). The allergen challenge procedure used was similar to that previously used by our group.⁵ Increasing concentrations of house dust mite allergen (Greer Laboratories, Hickory, NC) were administered from DeVilbiss No. 646 nebulizers through a dosimeter, with concentrations of *D farinae* extract expressed as allergen units per milliliter (AU/mL). After administration of saline diluent, doubling concentrations of allergen

were administered, starting with 0.156 AU/mL up to a maximum of 2560 AU/mL. FEV₁ was measured 8 minutes after each aerosol inhalation, and the challenge was terminated when a decline of $\geq 20\%$ was reached or the highest dose of allergen had been given. Subjects were then monitored by spirometry until stable. When indicated, a bronchodilator (Albuterol) was given by metered-dose inhaler. Subjects were then admitted to the GCRC for peak flow monitoring to determine the course of any delayed phase reaction and for treatment if clinically necessary. The subjects remained in the GCRC overnight, with routine monitoring of vital signs.

None of the subjects reported respiratory symptoms or had a drop in peak expiratory flow rate during any chamber session. As shown in Figure 1, airway responsiveness to allergen was increased by endotoxin exposure in 12 of the 14 subjects. The mean concentration of allergen required to reach the end point (PC₂₀ FEV₁ allergen) after endotoxin exposure (89 AU/mL) was more than a doubling dose lower than that required after the air exposure (231 AU/mL). This difference in responses was statistically significant on a paired *t* test ($P < .01$), using the difference in PC₂₀ FEV₁ allergen between air and endotoxin on a log scale. Linear models showed that preexposure methacholine responsiveness and NO excretion rate did not explain this difference. The order in which air and endotoxin exposures had been given also had no significant effect ($P = .25$).

In summary, we observed that a 4-hour exposure to a level of endotoxin that may be encountered in indoor air enhances airway response of persons with allergic asthma to inhaled allergen. The effect of endotoxin on the response to allergen appears similar to that previously reported for ozone. Future studies by this laboratory will examine whether these and other airborne agents share common mechanisms that may enhance airway response to allergen and disease severity in asthma.

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