Allergen provocation augments endotoxin-induced nasal inflammation in subjects with atopic asthma

Marlowe W. Eldridge, MD,[a,b,c,d] and David B. Peden, MD,[a,b] *Chapel Hill and Research Triangle Park, NC*

Background: Recent epidemiologic and in vivo studies have suggested that inhaled endotoxin plays an important role in asthma pathogenesis.
Objective: The present study examines the effect of nasal allergen provocation on subsequent endotoxin challenge in subjects with atopic asthma.
Methods: By using a split-nose randomized crossover design, individual nares of 12 asthmatic subjects underwent challenge and lavage as follows. Immediately after a baseline nasal lavage, one nares received normal saline, and the other received dust mite antigen. Four hours later, both nares were exposed to either saline or endotoxin. Dust mite antigen *(Dermatophagoides farinae)* and endotoxin *(Escherichia coli* 026:B6) doses were 100 AU and 1000 ng, respectively. Postchallenge lavages were done at 8 and 24 hours after the initial challenge. The subjects then returned a minimum of 3 weeks later for crossover to the study arm. Nasal lavage fluid was analyzed for total and differential cell counts, IL-8, IL-6, intercellular adhesion molecule 1, GM-CSF, eosinophil cationic protein, myeloperoxidase, and soluble CD14.
Results: A significant increase in the total inflammatory cell count was seen at 8 hours for the dust mite/endotoxin exposure compared with the saline/saline and saline/endotoxin exposures. Differential cell counts revealed a similar neutrophilic and eosinophilic inflammation for the dust mite/endotoxin exposure at 8 hours.
Conclusions: These data demonstrate an interaction between allergen and endotoxin exposure in asthmatic subjects, suggesting that a prior allergen challenge significantly augments the endotoxin-induced inflammation. Moreover, these data provide further evidence that concomitant exposure to allergen and endotoxin may be an important factor in asthma pathogenesis. (J Allergy Clin Immunol 2000;105:475-81.)

Key words: *Endotoxin, dust mite antigen, asthma, eosinophils, neutrophils, allergy*

Abbreviations used
BAL: Bronchoalveolar lavage
ECP: Eosinophil cationic protein
ICAM-1: Intercellular adhesion molecule 1

Asthma is characterized by bronchial hyperresponsiveness and airway inflammation and is a leading chronic illness among children and adults.[1] Acute exacerbations of asthma are associated with an acute increase in airway inflammation. Epidemiologic studies[1] indicate that increases in asthma-related morbidity and mortality rates are associated with a number of environmental factors, many of which exert an inflammatory effect on the airway. These stimuli include viral respiratory tract infections,[2,3] tobacco smoke,[3,4] and a number of air pollutants.[5,6]

Endotoxin (LPS) from gram-negative bacteria is an air contaminant found in a number of occupational, as well as domestic settings. Exposure to ambient air LPS is associated with airway symptoms and inflammation in both normal and asthmatic subjects. However, asthmatic subjects have been reported to have increased responsiveness to inhaled LPS challenge (ie, decreased lung function and increased nonspecific airway reactivity) than nonasthmatic subjects.[7] In a study of subjects with atopic asthma who were sensitized to house dust mite allergens, asthma severity was reported to correlate with the concentration of endotoxin in household dust recovered from their homes.[8] These findings indicate that even low-level endotoxin exposure may enhance airway disease in susceptible groups, such as subjects with atopic asthma.

Other environmental agents, such as ozone and diesel exhaust particles, have been shown to augment airway inflammation in asthmatic subjects. Additionally, these agents have been shown to enhance responses to inhaled allergens in asthmatic subjects. The interaction between allergen- and pollutant-induced inflammation may be an important mechanism by which pollutants induce asthma exacerbation.[5] Recent studies by our group and others provide a rationale for the hypothesis that airway exposure of subjects with atopic asthma to both allergen and LPS might result in greater inflammatory responses than those observed with either stimulus alone.[8-11]

To test the hypothesis that exacerbation of allergic inflammation by acute allergen exposure increases inflammatory responses to LPS, we used a split-nose

From [a]the Center for Environmental Medicine and Lung Biology, [b]the Department of Pediatrics, Division of Pulmonary Medicine and Allergy, [c]the General Clinical Research Center, University of North Carolina School of Medicine, and [d]the National Institute of Environmental Health Sciences, Chapel Hill and Research Triangle Park.
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Reprint requests: Marlowe W. Eldridge, MD, Center for Environmental Medicine and Lung Biology, University of North Carolina, Chapel Hill, 104 Mason Farm Road, CB#7310, Chapel Hill, NC 27599-7310.

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TABLE I. Summary of nasal lavage measurements

	Saline/saline			Dust mite/saline		
	Baseline	8 h	24 h	Baseline	8 h	24 h
Weight (g)	2.5 ± 0.2	2.6 ± 0.2	2.7 ± 0.2	2.6 ± 0.2	2.9 ± 0.2	2.7 ± 0.2
Viability (%)	61.1 ± 8.8	63.9 ± 7.7	74.9 ± 17	68.3 ± 6.6	70.0 ± 7.1	63.2 ± 10.3
TCC ×1000	50.0 ± 10.4	210.3 ± 70.8	200.3 ± 40.7	180.6 ± 40.7	440.1 ± 150.4	270.1 ± 80.6
PMN (%)	42.3 ± 5.0	66.2 ± 4.9	52.8 ± 4.9	54.9 ± 6.8	57.7 ± 6.3	40.3 ± 7.8
Eosinophils (%)	31.9 ± 5.8	25.8 ± 4.7	34.8 ± 4.6	23.2 ± 5.1	35.1 ± 6.8	49.3 ± 7.7‡
Monocytes (%)	23.0 ± 4.3	7.2 ± 1.5‡	11.0 ± 2.4	16.4 ± 10.3	4.2 ± 1.2	3.3 ± 1.2
Lymphocytes (%)	2.8 ± 2.1	0.8 ± 0.3	1.3 ± 0.5	2.7 ± 1.8	0.6 ± 0.3	1.1 ± 0.4
TP (μg/mL)	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.7 ± 0.2	0.5 ± 0.1
ECP (μg/L)	73.7 ± 20.2	53.4 ± 12.7	121.4 ± 37	76.5 ± 16.9	105 ± 28.3	90.2 ± 13.7
Myeloperoxidase (ng/mL)	21.4 ± 7.8	28.9 ± 9.8	28.3 ± 8.3	23.8 ± 9.5	25.4 ± 8.8	44.9 ± 21.4
GM-CSF (pg/mL)	58.5 ± 8.2	51.5 ± 7.1	38.7 ± 6.8	49.8 ± 8.4	46.1 ± 11.2	64.8 ± 10.7
IL-6 (pg/mL)	41.1 ± 2.8	36.6 ± 4.4	33.7 ± 2.6	28.6 ± 2.4	40.8 ± 5.7	69.9 ± 10.7*†‡
IL-8 (pg/mL)	839 ± 286	681 ± 178	1153 ± 275	944 ± 275	1561 ± 393	1903 ± 490
sICAM-1 (pg/mL)	ND	ND	ND	ND	ND	ND
sCD14 (pg/mL)	2805 ± 436	5157 ± 572	2794 ± 432	3095 ± 1005	6044 ± 992	4618 ± 582

Data presented as means ± SEM.
TCC, Total cell count; *PMN,* polymorphonuclear leukocyte; *TP,* total protein; *ND,* not detected.
Significant differences ($P < .05$) from *saline/saline, †saline/LPS, ‡baseline, §24 hours, and ‖8 hours.

crossover design to investigate the effect of prior dust mite allergen provocation on subsequent endotoxin-induced inflammation in mite-sensitive asthmatic subjects. The split-nose design allowed for examination of the following 4 conditions in two challenge sessions: allergen challenge followed by LPS (representing the effect of sequential challenge with house dust mite allergen and LPS), allergen followed by saline (representing the effect of house dust mite allergen alone), saline followed by LPS (representing the effect of LPS alone), and saline followed by saline control condition (saline alone). Nasal inflammation was evaluated by means of total and differential inflammatory cell counts and proinflammatory cytokine and soluble (s)CD14 levels in sequentially collected nasal lavage fluid.

METHODS

Subjects

Twelve nonsmoking adults (7 women and 5 men; age, 30.6 ± 7.8 years [mean ± SD]) with stable atopic asthma were recruited from the general and campus population by means of newspaper advertisement and public postings. All subjects had a diagnosis of asthma established symptomatically by episodic wheezing, chest tightness, and/or dyspnea and objectively confirmed by methacholine airway hyperresponsiveness ($PC_{20} < 8$ mg with $FEV_1 \geq 70\%$ of predicted value). All the subjects had mild-to-moderate airway hyperresponsiveness to methacholine. The subjects were all atopic, as determined by positive epicutaneous skin test responses. A standard battery of 10 antigens (*Dermatophagoides farinae, Dermatophagoides pteronyssinus*, mold mix 1, mold mix 2, cockroach, dog, cat, eastern [10], weed mix, and grass mix [7]) common to this region were administered. All subjects were sensitive (≥3+) *to D farinae* (house dust mite), as well as 3 or more other antigens. Although all of the subjects reported an intermittent history of allergic rhinitis, all were free of allergic or viral rhinitis for at least 3 weeks before a challenge session. Furthermore, none of the subjects had been treated with inhaled or nasal steroids or had evidence of respiratory infection or an exacerbation of asthma symptoms in the previous 3 weeks. The study was approved by the Committee for the Protection of the Rights of Human Subjects of the University of North Carolina School of Medicine, and all subjects gave written informed consent.

Study design

Subjects attended the laboratory for 3 visits. At the first visit, entry criteria were assessed by means of a questionnaire, limited physical examination, spirometry, a methacholine inhalation test, and allergy skin tests. Spirometry was performed with a Koko spirometer and software (Pulmonary Data Service Instrumentation, Inc, Louisville, Colo). Baseline pulmonary function measurements were made according to American Thoracic Society criteria.[12] Methacholine inhalation challenges and allergen skin tests were performed by using standard procedures.[13,14]

At the second visit, by using a split-nose randomized crossover design, individual nares of the subjects underwent challenge and lavage as follows. Immediately after a baseline nasal lavage, one nares received normal saline, and the other received dust mite antigen. Four hours later, both nares were exposed to either saline or endotoxin (LPS). Dust mite antigen (*D farinae*; Greer Laboratories, Hickory, NC) and LPS (*Escherichia coli* 026:B6; Sigma, St. Louis, Mo) doses were 100 AU and 1000 ng, respectively. By using a limulus amebocyte lysate endotoxin assay kit (BioWhittaker, Walkersville, Md), we measured the endotoxin concentration in the dust mite challenge solution. The assay revealed an endotoxin concentration of 0.034 ng/mL; with this concentration the subjects were exposed to 0.007 pg/dose of endotoxin with the dust mite challenge. The endotoxin level in the saline solution was undetectable. The antigen and endotoxin were delivered to the nares by using a manual hand-held nebulizer similar to that described previously.[15] Postchallenge nasal lavages were done at 8 and 24 hours after the initial challenge. The subjects then returned a minimum of 3 weeks later for crossover to the study arm.

Nasal lavages were performed by using a technique previously described.[15,16] Briefly, 4 mL of normal saline was sprayed into the nares by using a manual hand-held nebulizer (source) that delivers 100 μL per actuation. Each lavage consisted of 8 sets of 5 sprays.

Saline/endotoxin			Dust mite/endotoxin		
Baseline	**8 h**	**24 h**	**Baseline**	**8 h**	**24 h**
2.5 ± 0.2	2.6 ± 0.2	2.5 ± 0.2	2.6 ± 0.2	2.6 ± 0.3	2.4 ± 0.2
59.5 ± 6.1	83.1 ± 4.8	75.9 ± 5.4	63.6 ± 7.2	76.5 ± 5.2	71.4 ± 6.3
90.6 ± 30.6	290.3 ± 80.7	190.3 ± 50.8	100.8 ± 50.0	590 ± 130*†‡§	190.7 ± 80.4
38.3 ± 3.8*	66.8 ± 3.3‡	55.9 ± 4.3	54.2 ± 6.6	76.8 ± 3.1	61.1 ± 6.7
42.3 ± 4.9	27.4 ± 4.0	36.5 ± 4.0	30.8 ± 5.7	18.7 ± 3.4	30.4 ± 6.2
17.8 ± 3.9	5.6 ± 1.1	4.4 ± 0.9‡	14.3 ± 3.8	4.4 ± 1.3	7.6 ± 1.6
1.6 ± 0.5	0.2 ± 0.1	3.1 ± 0.9	0.7 ± 0.3	0.2 ± 0.2	0.9 ± 0.4
0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.06
125.3 ± 37.9	116.5 ± 27.4	77.7 ± 16.6	114.2 ± 35.2	194.5 ± 54*	163.4 ± 37
8.5 ± 2.1	25.6 ± 14.3	20.9 ± 11.1	15.2 ± 6.8	21.1 ± 7.4	22.1 ± 8.4
52.8 ± 8.3	51.3 ± 10.5	50.6 ± 8.0	63.1 ± 13	43.8 ± 7.3	48.3 ± 10.7
43.0 ± 5.4	41.6 ± 4.6	30.8 ± 3.6	36.8 ± 5.6	36.5 ± 2.8	55.8 ± 5.0*†‡
1371 ± 574	1265 ± 386	1655 ± 487	2113 ± 796	1381 ± 411	1643 ± 400
ND	ND	ND	ND	ND	ND
3493 ± 1017	5967 ± 1091	4448 ± 1022	4146 ± 1091	4791 ± 1214	3940 ± 850

The lavage fluid was recovered by forceful expulsion into a specimen cup immediately after each set of 5 actuations. The samples were then transported to the laboratory on ice and processed immediately.

Nasal lavage fluid examination for cell count and soluble mediators

The nasal lavage fluid was weighed and treated with a 0.1% volume of dithiothreitol (Sputalysin 10%; Calbiochem Corp, San Diego, Calif) equal to two times the weight (in milligrams) followed by the addition of an equal volume of Dulbecco's PBS. The cell suspension was filtered through a 70-μm nylon filter (Falcon cell strainer #2350; Becton Dickinson Labware, Franklin Lakes, NJ), and the resulting suspension was used for total cell count and cell viability. Cytospin specimens were prepared (Shandon III Cytocentrifuge; Shandon Southern Instruments, Sewickley, Pa), and the slides were stained with a modified Wright-Giema stain (Hema 3, Biochemical Sciences Inc., Swedesboro, NJ) for differenial cell counts. The remaining cell suspension was centrifuged, and the supernatant was aspirated and stored at –80° C for later assay.

Supernatant concentrations of IL-6, IL-8, GM-CSF, soluble intercellular adhesion molecule 1 (sICAM-1), myeloperoxidase, and soluble (s)CD14 were determined by using commercially available ELISA kits (Endogen Inc, Woburn, Mass; Bioxytech, Oxis International Inc, Portland, Ore; R & D Systems, Minneapolis, Minn). The limits of detection for IL-6, IL-8, sICAM-1, GM-CSF, myeloperoxidase, and sCD14 were 1.0 pg/mL, 2.0 pg/mL, 0.3 ng/mL, 2.0 pg/mL, 1.5 ng/mL, and 125 pg/mL, respectively. Eosinophil cationic protein (ECP) concentrations were determined by using a sensitive RIA (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden) with a minimum detection limit of 2.0 μg/L. Supernatant protein concentrations were determined by using a commercially available microassay kit (Bio-Rad Laboratories, Hercules, Calif) and compared with a standard curve prepared with BSA (Sigma). All assays were done in duplicate on thawed supernatant.

Data analysis

Total cell counts and absolute differential cell counts were normalized by logarithmic transformation, and differences in the cell counts among the 4 exposure conditions and 3 time points were compared with a repeated-measures ANOVA. Multiple pairwise comparisons were made with the Tukey-Kramer HSD (Honestly Significant Difference) procedure, with the overall α level set at .05. Data are presented as means ± SEM unless otherwise noted.

RESULTS

All subjects tolerated the exposure combinations without complication, and adequate samples were obtained from each subject at each time point. A summary of all the assays performed and the results are presented in Table I.

Total and differential cell counts

A significant increase in the total inflammatory cell count (cells per milligram of nasal lavage fluid) was seen at the 8-hour time point for the dust mite/LPS combination (248.8 ± 70.9) compared with the saline/saline (91.9 ± 35.7, $P < .01$) and saline/LPS (118.2 ± 35.9, $P < .05$) combinations (Fig 1). The dust mite/LPS combination was also the only exposure combination that showed a significant increase from the baseline values. Total inflammatory cell counts returned to baseline values by 24 hours.

Differential cell counts revealed a significant neutrophilic inflammation (Fig 2) for the dust mite/LPS exposure (182.9 ± 52.0) at 8 hours compared with the saline/saline (67.7 ± 29.9, $P < .01$) and saline/LPS (80.3 ± 24.3, $P < .05$) exposures. By 24 hours after exposure, the neutrophilic inflammation had resolved. Likewise, there was a similar significant increase in eosinophil numbers in nasal lavage fluid 4 hours after LPS exposure when such exposure was preceded by allergen challenge (55.7 ± 18.7) compared with saline/saline (20.0 ± 6.2, $P < .05$), dust mite/saline (22.0 ± 5.0, $P < .05$), and saline/LPS (23.0 ± 4.4, $P < .05$) exposures (Fig 3). No

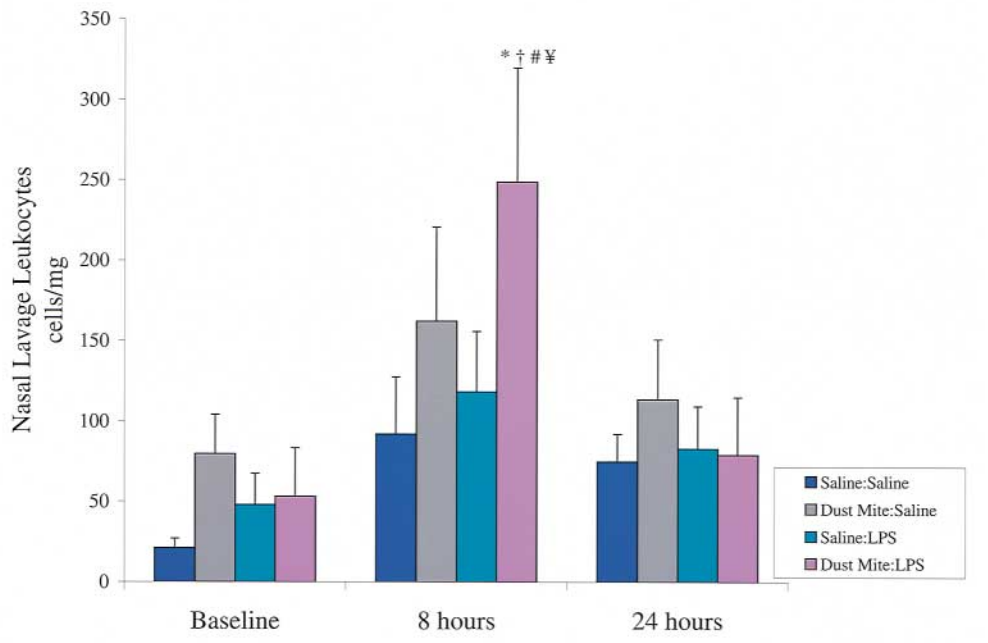

FIG 1. Effect of each exposure condition on the inflammatory cell numbers (cells per milligram of nasal lavage fluid) at each time point ($P < .05$; differs significantly from *saline/saline, †saline/LPS, #baseline, and ¥24 hours).

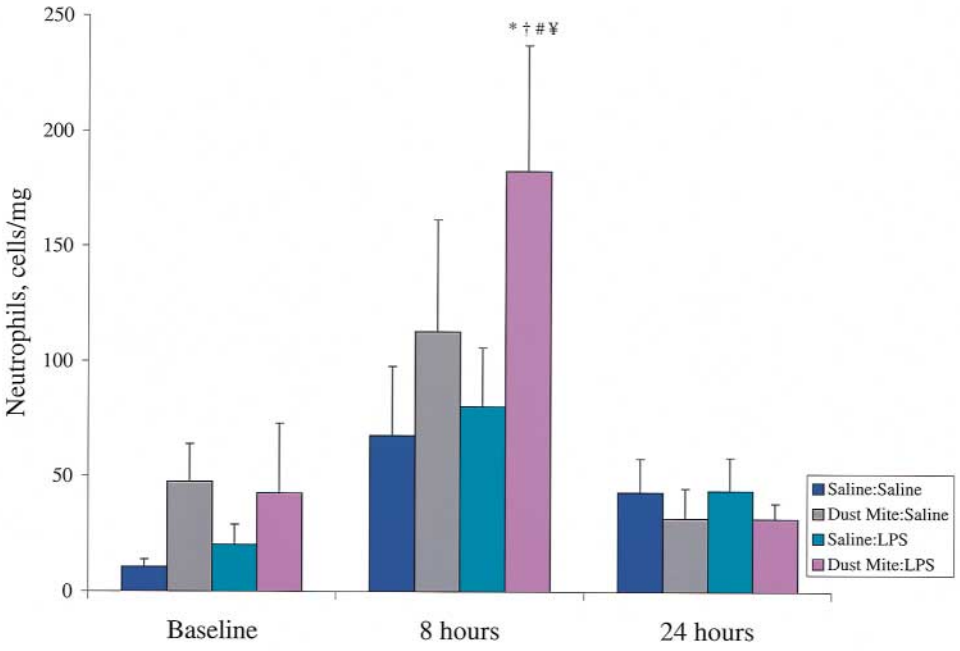

FIG 2. Effect of each exposure condition on the nasal neutrophilic inflammation expressed as neutrophils per milligram of nasal lavage fluid at each time point ($P < .05$; differs significantly from *saline/saline, †saline/LPS, #baseline, and ¥24 hours).

significant effect on mononuclear cell numbers was observed.

Soluble mediators and sCD14 levels

Nasal lavage fluid concentrations of IL-6 were significantly elevated at the 24-hour time point for both the dust mite/saline and the dust mite/LPS exposures (Table I). In addition, the ECP concentration was significantly elevated at the 8-hour time point only for the combined allergen and LPS exposure compared with the saline/saline exposure (Fig 4). No other exposure combination resulted in a significant change in any of the other

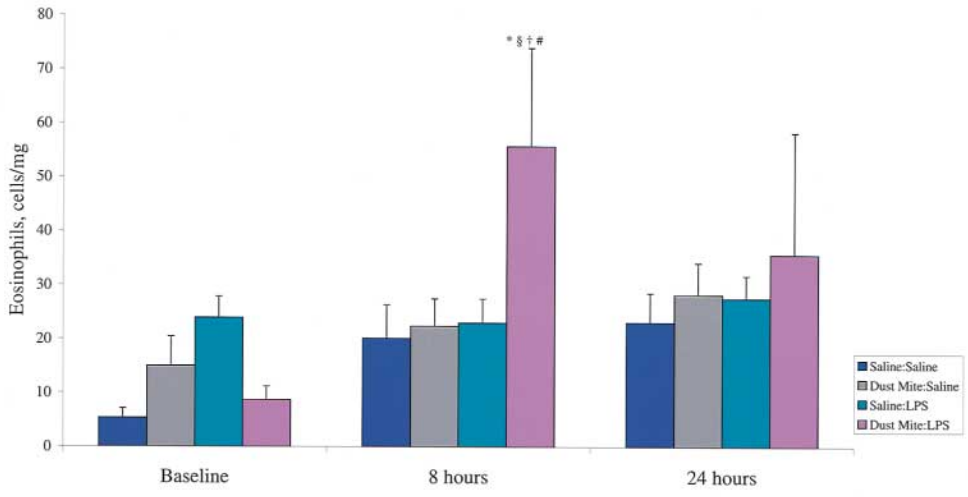

FIG 3. Effect of each exposure condition on the nasal eosinophilic inflammation expressed as eosinophils per milligram of nasal lavage fluid at each time point ($P < .05$; differs significantly from *saline/saline, §dust mite/saline, †saline/LPS, #baseline, and 24 hours).

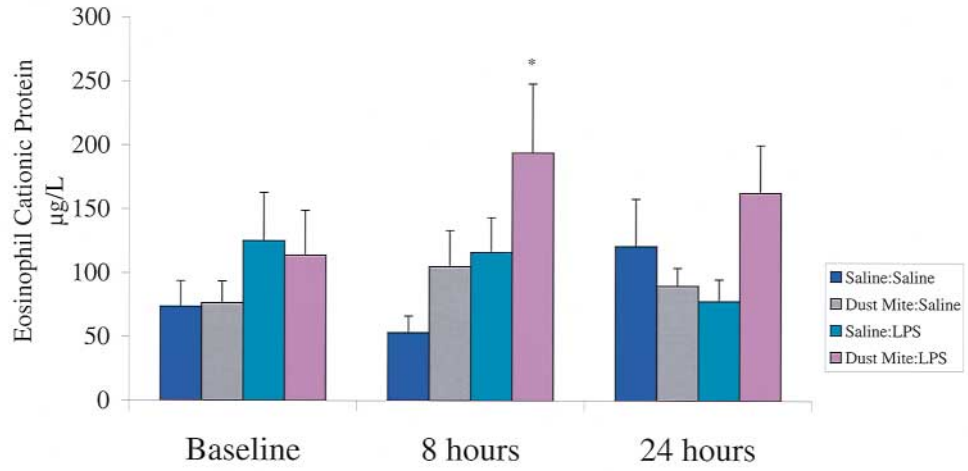

FIG 4. Effect of each exposure condition on nasal eosinophilic cationic protein concentrations at each time point ($P < .05$; differs significantly from *saline/saline).

soluble mediators (IL-8, ICAM-1, GM-CSF, myeloperoxidase, and sCD14) assayed when compared with levels obtained with the control condition (saline challenge followed by a second saline challenge). Furthermore, we were unable to demonstrate a significant relationship between mediator levels and the cellular responses. These results are all depicted in Table I.

DISCUSSION

In high concentrations inhaled endotoxin can induce asthma-like airway reactivity and inflammation in both asthmatic and nonasthmatic subjects.[17,18] The LPS-induced inflammation reported in these studies is primarily neutrophilic, regardless of whether the subject is atopic without asthma, has atopic asthma, or is a healthy control subject. Indeed, significant differences between asthmatic and nonasthmatic subjects in these studies have not been easily identified. These results are not unlike many initial reports of the effect of ozone in asthmatic subjects.[5,15,19,20] However, more recent reports suggest that atopic or asthmatic subjects have either an enhanced neutrophilic response to ozone or manifest an eosinophil response to ozone.[15,21-23] Moreover, ozone has also been shown to enhance both the immediate and late-phase response to allergen in allergic subjects with asthma.[15] Given the apparent similarities between the effects of ozone and inhaled LPS in the airway, coupled with reports that LPS may modulate asthma severity, it seems reasonable to believe that stimulation of the nasal airway with allergen and LPS should yield an exaggerated inflammatory response.

We tested the hypothesis that allergen challenge would enhance the inflammatory response of subjects with

atopic asthma to subsequently applied endotoxin. The findings reported herein support that hypothesis. When compared with a control condition of two sequential saline challenges, there was a significant increase in inflammatory cells recovered in nasal lavage fluid when mite-sensitive asthmatic subjects were nasally challenged with 1000 ng of LPS 4 hours after local challenge with 100 AU of dust mite antigen. In contrast, neither challenge with LPS after an initial saline challenge (designed to assess the effect of LPS alone) nor house dust mite allergen followed by subsequent saline challenge (to assess the effect of mite allergen alone) yielded increased cellularity in nasal lavage fluid when compared with the control condition.

Although the cellular inflammation yielded by the mite allergen/LPS sequential challenge was dominated by neutrophils, the number of eosinophils in the lavage fluid also increased significantly after the endotoxin exposure. These data demonstrate an interaction between allergen and endotoxin exposure in asthmatic subjects, suggesting that acute allergen exposure augments both endotoxin-induced neutrophilic and eosinophilic nasal inflammation in atopic subjects.

In individuals allergic to mites, inhalation of mite allergen induces an acute increase in airway microvascular permeability[24] and airway wall edema, followed by infiltration of activated eosinophils, neutrophils, and mononuclear cells. This late-phase response also includes the accumulation of inflammatory cytokines that further modulate the inflammatory response. It has been proposed that acute allergen-induced responses allow for extravasation of LPS response mediators, such as LPS-binding protein and sCD14 into the airway.[11] Such changes could result in increased levels of soluble mediators, such as sCD14, accounting at least in part for increased responsivness to LPS.

Supporting this idea are observations by Dubin et al[9] who have revealed that inhaled allergen challenge does increase levels of sCD14 in bronchoalveolar lavage (BAL) fluid. A second study, by Virchow et al,[11] also found that levels of sCD14 were increased in BAL fluid after allergen challenge. Postallergen challenge sCD14 levels in BAL fluid correlated very well with the number of allergen-induced eosinophils, suggesting a link between response to allergen (as indicated by eosinophilia) and sCD14. Whether increases in the amount of LPS-response mediators, such as sCD14 in BAL fluid, are simply a result of increased vascular permeability or whether they are due to influx of cells that bear sCD14 and release it into the local environment remains unclear.

It is also possible that increased responsiveness to LPS after allergen challenge reflects a general enhancement of cellular responsiveness to inflammatory agents. Indeed, PBMCs and alveolar macrophages from asthmatic subjects have greater cytokine secretion after in vitro challenge with LPS than do cells from nonatopic subjects.[25,26] Likewise, our recent examination of the effect of LPS on nasal inflammation in 10 atopic and 6 nonatopic subjects demonstrated that baseline (prechallenge) levels of GM-CSF in nasal lavage fluid correlated well with eosinophil influx after challenge with 1000 ng of LPS.[10] This eosinophil response was observed in 6 of the 10 mite-sensitive asthmatic subjects. Four of these 6 were also allergic to tree pollen, and this study was conducted during tree pollination season. GM-CSF is known to promote eosinophil survival in tissues and is induced by allergen challenge. Taken together, these observations, which are not likely to involve immediate increases in sCD14 from plasma sources, suggest that acute or chronic allergen exposure could induce other changes in the local milieu that could facilitate inflammatory responses to LPS.

In the present study we examined levels of both CD14 and acute response mediators (IL-8, GM-CSF, myeloperoxidase, and ECP) in nasal lavage fluid to try to determine whether these mediators were increased as a result of allergen challenge. We did not observe a significant increase in any of these mediators at levels above those found in our control challenge (saline followed by saline) after sequential challenge with allergen and LPS, allergen and saline, or saline and LPS.

However, our design called for an initial application of allergen, followed 4 hours later by the second stimulus (LPS or saline). To avoid disrupting any allergen-induced priming of the response to LPS, no lavages were performed between the time of allergen and LPS challenge. It seems plausible that any mediators that could account for allergen-induced priming of response to LPS 4 hours after allergen challenge might have been present at the time of the LPS challenge but not when the post-LPS nasal lavage was obtained (8 hours after the initial allergen challenge). Consequently, our inability to observe any acute allergen-induced increases in either sCD14 or inflammatory cytokines should not be viewed as evidence against their potential role as mediators of the observed allergen-enhanced responsiveness to LPS.

Certainly some subjects are likely more sensitive to allergen than others. Subjects who proved not to have increased response to LPS may have had relatively muted responses to allergen. Most subjects experienced little or no symptoms after nasal allergen challenge, and for these subjects, this was a subthreshold dose of allergen. It is possible that if every subject had undergone a graded allergen challenge to the point of having overt symptoms, there might have been a more robust response to LPS. Use of a graded allergen challenge (to induce symptoms) might result in better priming of subsequent responses to LPS. Likewise, studying persons with seasonal allergy during and outside of their relevant allergen season (when ambient allergens cause symptoms) may also be an approach to study the effect of allergen on LPS response. Coupling such approaches with more frequent sampling might provide a better understanding of the overall inflammatory process by which allergen and LPS interact.

However, it is important to point out that use of the relatively small doses of allergen and LPS used in this study also might be advantageous. The allergen dose of 100 AU was selected so that a consistent dose could be adminis-

tered to each subject. Such a dose may better reflect low-level exposure to indoor allergens and may be a better model of real-life allergen exposure than larger doses, which may have a more robust effect. Likewise, the dose of 1000 ng of LPS roughly mimics the cumulative exposure of a worker breathing at rest for 8 hours in an environment containing 250 ng/m^3 LPS (a level associated with ocular and nasal symptoms in office settings). Therefore despite not finding a clear mechanism for the effect of allergen challenge on LPS response, we contend that our findings are of practical interest because an enhancement of LPS-induced inflammation by allergen was observed at relatively small doses that likely mimic true ambient air exposures of both irritants.

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