

Eosinophil influx to the nasal airway after local, low-level LPS challenge in humans

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Background: Recent observations show that atopic asthmatic subjects have increased sensitivity to respirable endotoxin (or LPS) compared with normal persons. In vitro studies demonstrate that LPS enhances eosinophil survival. These observations suggest that the effects of inhaled LPS in asthmatic subjects may include increases in the number of airway eosinophils.

Objective: We sought to determine whether low-level nasal LPS challenge causes an increase in eosinophil numbers in the nasal airways of atopic or normal subjects.

Methods: Sixteen volunteers (10 atopic asthmatic subjects and 6 normal subjects) underwent 2 nasal challenge sessions. In one session, one nostril was challenged with saline and the other with 0.1 µg of LPS. During the second session, 0.3 µg and 1.0 µg of LPS was delivered to each nostril, respectively. Nasal lavage fluid was obtained from each nostril before challenge, as well as 4 and 24 hours after challenge, and examined for the percent of total cells that were eosinophils and neutrophils, as well as cytokine levels.

Results: LPS (1.0 µg) increased the percent of eosinophils in nasal lavage fluid 4 hours after challenge in atopic subjects only. There was also a correlation between constitutive nasal GM-CSF and eosinophil response to LPS in atopic subjects.

Conclusion: LPS challenge increases eosinophils in the airways of atopic subjects. (*J Allergy Clin Immunol* 1999;104:388-94.)

Key words: Endotoxin, air pollution, asthma, eosinophils, GM-CSF

Endotoxin (or LPS) from gram-negative bacteria is a commonly encountered air contaminant in a number of occupational settings, especially those in which exposure to animal waste (eg, swine confinement facilities or industrial chicken facilities) or plant matter (cotton dust or corn dust) occurs. LPS levels in such environments may exceed 20 µg/m³ ambient air and are associated with acute reductions in lung function, respiratory symptoms, and nasal inflammation in exposed persons.¹⁻⁶ Mean ambient air levels of LPS in the range of 0.25 µg/m³ have

Abbreviations used

BAL: Bronchoalveolar lavage
ECP: Eosinophil cationic protein
NLF: Nasal lavage fluid
OVA: Ovalbumin

been associated with increased reports of ocular and nasal symptoms by office workers, suggesting that even low levels of LPS encountered in office settings may cause airway symptoms and inflammation.⁷

Inhalation challenge studies have shown that LPS causes neutrophilic inflammation in nonatopic subjects, nonasthmatic atopic subjects, and asthmatic subjects.⁸⁻¹¹ In one study inhalation of 100,000 ng of LPS by 8 normal subjects resulted in a 100-fold increase in PMNs recovered in bronchoalveolar lavage (BAL) fluid when compared with PMNs in BAL fluid recovered after saline challenge.⁸ Another study, comparing the action of LPS in normal subjects and atopic subjects who did not have airway hyperreactivity, demonstrated that both atopic and nonatopic subjects have similar LPS-induced increases in airway PMN, TNF-α, IL-1β, IL-6, and IL-8 levels.⁹ Other recent studies demonstrate a dose response to inhaled LPS in the airways by using PMN numbers in induced sputum as a principal endpoint.¹¹ Nasal inflammation (as shown by PMNs and IL-8) is also increased in persons occupationally exposed to LPS.⁶ Thus inhaled LPS typically causes airway inflammation, which is typified by PMN influx and production of TNF-α, IL-1β, IL-6, and IL-8. This inflammation is associated with differing levels of symptoms in normal subjects, including some degree of bronchospasm, cough, sputum production, and increased nonspecific bronchial reactivity. Higher doses may lead to fever and toxic pneumonitis.¹

Recent observations by Michel et al¹² indicate that asthmatic subjects have responses to LPS challenge (decreased lung function and increased nonspecific airway reactivity) that are increased compared with those of nonasthmatic subjects. Disease severity of mite-sensitive asthmatic subjects has also been shown to correlate better with levels of endotoxin than mite allergen in samples of house dust collected from their homes. Although others have reported no increased sensitivity of asthmatic subjects to inflammatory or spirometric responses to LPS,¹⁰ these observations, as well as other challenge studies, suggest that atopic asthmatic subjects may have

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Received for publication Dec 31, 1998; revised Mar 19, 1999; accepted for publication Apr 6, 1999.

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0091-6749/99 \$8.00 + 0 1/1/99116

TABLE I. Skin test results for all atopic subjects (n = 10)

Test reagent	Skin test results for atopic subjects (all had nonreactive negative controls)									
	1	2	3	4	5	6	7	8	9	10
Histamine	3+	3+	2+	3+	3+	3+	2+	3+	3+	3+
<i>D farinae</i>	4+	4+	4+	3+	3+	4+	4+	4+	3+	3+
<i>D pteronyssinus</i>	4+	4+	3+	2+	3+	4+	4+	4+	3+	3+
Mold mix 1	—	—	2+	3+	2+	—	—	—	3+	2+
Mold mix 2	—	—	—	2+	2+	2+	—	—	3+	—
Cockroach	—	—	—	1+	2+	—	—	—	3+	—
Dog	3+	1+	1+	2+	2+	—	1+	2+	3+	1+
Cat	3+	3+	2+	3+	3+	4+	—	3+	3+	4+
Eastern trees (10)	4+	—	2+	4+	2+	4+	—	—	4+	3+
Weed mix	1+	—	2+	2+	2+	—	—	—	3+	—
Grass mix (7)	1+	—	2+	2+	2+	—	—	—	3+	2+

—, Negative response.

greater responsiveness to inhaled LPS than nonatopic subjects.^{13,14}

There is support for the hypothesis that T_{H2} inflammation characteristic of the type found in atopic asthmatic subjects may enhance responsiveness to LPS. Atopic asthmatic subjects have increased soluble CD14 (sCD14, a receptor for LPS) in BAL fluid after inhaled allergen challenge.^{15,16} In one study sCD14 levels in BAL fluid after allergen challenge correlated very well with allergen-induced eosinophils, suggesting a link between eosinophils and sCD14.¹⁶ Also, PBMCs and alveolar macrophages from asthmatic subjects have greater cytokine secretion after in vitro challenge with LPS than do cells from nonatopic subjects.¹⁷ Supernatants from LPS-stimulated monocytes from atopic subjects enhance in vitro eosinophil survival, and this activity is lost if supernatants are treated with anti-GM-CSF antibodies.^{18,19}

These reports suggest that LPS should have a proeosinophilic, as well as a proneutrophilic, effect in the airways of atopic subjects. However, there is little evidence of LPS enhancing airway eosinophilia in vivo in humans.^{1,10} On the other hand, recent observations in animals suggest that LPS may enhance eosinophil responses in challenged tissue. Skin challenge of guinea pigs with LPS doses ranging from 50 to 1000 ng revealed a dose-response influx of eosinophils 4 hours after challenge, which was diminished by inhibition of TNF- α .²⁰ LPS and ovalbumin (OVA) both induce pleural eosinophilia in OVA-sensitized mice. Interestingly, treatment with anti-IL-5 mAbs significantly blunted the allergen (OVA)-associated eosinophil influx but not that associated with LPS, suggesting that another cytokine was important in LPS-induced pleural eosinophilia in these animals.^{21,22}

The purpose of this pilot study was to determine whether nasal challenge of atopic and nonatopic subjects with 0, 0.1, 0.3, and 1.0 μ g of LPS would result in influx of eosinophils or neutrophils to the nasal airway. These doses were selected to model low-level ambient air LPS exposure. If individuals with a resting respiratory rate of 8 L/min are exposed to an atmosphere containing 0.25 μ g/m³ LPS (the level associated with the so-called "sick

building syndrome"), they would have an hourly airway exposure of 0.12 μ g of LPS. These estimates indicate that airway challenge with 1.0 μ g of LPS is a reasonable approximation of the airway exposure resulting from resting exposure to 0.25 μ g/m³ LPS for 8 hours.

METHODS

Subjects

A total of 16 (6 normal and 10 atopic) subjects were recruited into this study. With the exception of mild asthma and atopy, all subjects were otherwise healthy nonsmoking men or women between the ages of 18 and 50 years. Presence of asthma was based on one of the following criteria: symptoms of variable wheeze, shortness of breath, chest tightness, or cough for greater than 2 years. Absence of pregnancy was initially determined for women by medical history during the screening procedure, and before each challenge, urine testing was performed and menstrual history was obtained to substantiate the absence of pregnancy.

Ten of these subjects were atopic, as determined by positive epicutaneous skin test responses. A battery of 12 antigens common to this area were administered, and only subjects sensitive to *Dermatophagoides farinae* (house dust mite) were enrolled. Table I depicts the results of the screening allergy skin tests. A clinical history consistent with mild episodic or persistent asthma was also required for entry into the study. Seven of the 10 atopic subjects used inhaled corticosteroids for asthma. All subjects had to be free of symptoms of allergic or viral rhinitis for at least 4 weeks before entry into the study. All subjects reported an intermittent history of allergic rhinitis, which involved exposure to dust and pets (either dogs or cats); only 2 reported seasonal symptoms. One subject reported using oral antihistamines and nasal corticosteroids on an as-needed basis but had not used these medications for 5 months before the study. Subjects were not allowed to use nasal anti-inflammatory agents or antihistamines during the study, and none reported a need to do so. The remaining 6 subjects were healthy and nonatopic, with negative skin test responses and a negative history for asthma or atopy.

Screening examinations were conducted at the University of North Carolina Center for Environmental Medicine and Lung Biology. The protocols used in this study were reviewed and approved by the Committee for the Protection of the Rights of Human Subjects of the University of North Carolina School of Medicine. All volunteers gave informed consent before participation in these studies. All endotoxin and saline challenges of atopic subjects were performed between March 30, 1998, and April 21, 1998.

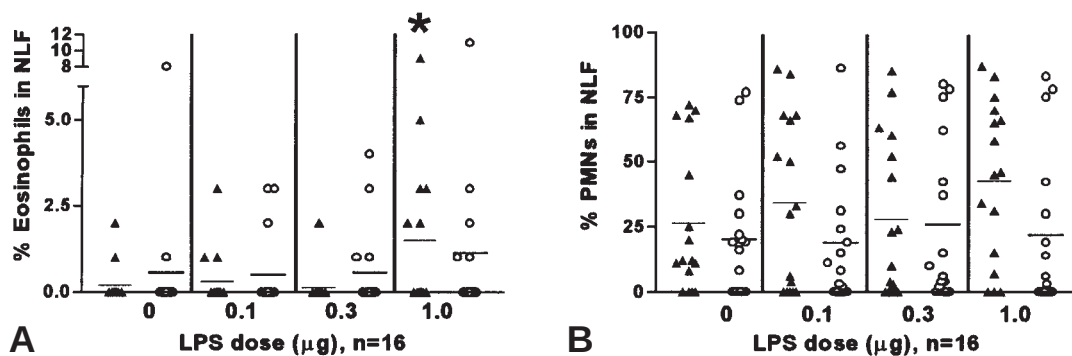


FIG 1. Effect of challenge with saline and 0.1, 0.3, and 1.0 µg of LPS on percentage of eosinophils (**A**) and percentage of PMNs (**B**) 4 and 24 hours after challenge in all subjects ($n = 16$). Closed triangles represent the percentage of eosinophils or the percentage of PMNs from a given subject 4 hours after challenge, and open circles represent the percentage of eosinophils or percentage of PMNs from a given subject 24 hours after challenge. Mean values are shown with a horizontal bar at each dose of LPS. Asterisks (*) depict findings that are significantly different from those seen after saline challenge at the same time after challenge.

Challenge protocol

Each subject underwent 2 challenge sessions. HPLC-purified *Salmonella typhimurium* LPS was used for endotoxin challenge (Sigma Chemical Co, St Louis, Mo). For each challenge, LPS was diluted into normal saline at an appropriate concentration and delivered to each nostril by means of two 100-mL sprays, as previously described.²³ In session 1, one nostril was challenged with saline and the other with 0.1 µg of LPS. During the second session, a dose of 0.3 µg LPS was delivered to one nostril and 1.0 µg was delivered to the other. The LPS exposure sessions were separated by no less than 2 weeks. Nasal lavage fluid (NLF) was obtained from each nostril before challenge and 4 and 24 hours after challenge.

Specific procedures

Nasal LPS challenge. LPS was diluted in saline and filtered through a 0.45-µm filter. Doses of 0.1 to 1.0 µg were used and were delivered by means of a hand-held nasal nebulizer, as previously described.²³

Nasal lavage. Nasal lavage was performed by using techniques previously described.^{23,24} Briefly, 4 mL of saline were sprayed into the nares by means of a hand-held nebulizer that delivered 100 mL/actuation. Each lavage was composed of 8 sets of 5 sprays. NLF was recovered by forceful expulsion into a specimen cup immediately after each set of 5 actuations. Cytospin preparations were prepared immediately, and supernatant samples were transported on ice to the laboratory and stored at -80°C until analysis.

Measurement of neutrophils and eosinophils in NLF. The cellular composition of the NLF was determined as previously described.²³ Differential cell counts were performed on cytospin preparations of the washed NLF cell suspensions, which were prepared in a cytocentrifuge and stained with Diff-Quik. Each slide was masked, coded, and shuffled before assessment of the differential count, with the reader (DBP) blinded to the identity of each slide.

Measurement of cytokine and eosinophil cationic fluid levels in NLF. Commercially available ELISAs were used to assay NLF for the cytokines TNF- α , IL-1 β , IL-8, and GM-CSF (Endogen, Cambridge, Mass) and for eosinophil cationic protein (ECP; Amersham, Arlington Heights, Ill).

Statistical analysis

For this study, the a priori principal endpoints were the percentages of total cells in recovered NLF that were eosinophils and PMNs. Intragroup (the entire group, atopic, or nonatopic cohorts)

comparisons of the percentage of eosinophils and the percentage of PMNs after challenge with 0, 0.1, 0.3, and 1.0 µg of LPS were conducted in 3 groups of NLF samples (ie, those collected at baseline and those collected 4 and 24 hours after challenge) by using repeated-measures ANOVA. The effect of each dose of LPS was compared with that of the saline control by using Dunnett's procedure.

The other endpoints (ECP, GM-CSF, IL-1 β , IL-8, and TNF- α) were considered as exploratory endpoints. The a priori analysis strategy for these endpoints was to only examine for effect of LPS at doses that had significantly affected eosinophil or PMN response (compared with the saline challenge) as outlined above by using paired t tests. Comparisons of a given response parameter between the atopic versus normal cohorts were made by using ANOVA and t tests with Welch's correction for nonequal variances as appropriate. It was expected that LPS would cause increases in each endpoint, and therefore 1-tailed P values were used, with significance set at .05.

Comparison of measures of constitutive levels of inflammation (percentage of eosinophils, percentage of PMNs, and levels of GM-CSF, IL-1 β , IL-8, and TNF- α in baseline NLF) in normal and atopic subjects was assessed by using t tests with Welch's correction for nonequal variances. Correlation between LPS-induced eosinophil and PMN responses and measures of constitutive inflammation were performed by using Pearson's correlation techniques. Prism 2 software was used for statistical calculations (GraphPad Software, Inc).

RESULTS

Effect of LPS on granulocyte influx in the entire group of subjects ($n = 16$)

Fig 1 depicts the effect of the dose range of LPS (0, 0.1, 0.3, and 1.0 µg) on the percentage of eosinophils (panel A) and the percentage of PMNs (panel B) in the entire group of subjects 4 and 24 hours after challenge. Four hours after challenge, only 1.0 µg of LPS induced a significant increase in the percentage of eosinophils when compared with the effect of saline challenge, with 6 of the 16 subjects demonstrating LPS-induced eosinophil influx. All 6 of these subjects were atopic. There was no significant effect of LPS on the percentage of eosinophils 24 hours after challenge when either the entire study group or the 6 that had eosinophil responses 4 hours after challenge were considered. No dose of LPS

had an effect on the percentage of PMNs found in NLF either 4 or 24 hours after challenge when compared with saline within the entire group.

Effect of LPS on eosinophil influx in atopic subjects (n = 10)

There were no eosinophils observed in NLF recovered from any of the 6 nonatopic subjects at baseline, as well as the NLF recovered 4 or 24 hours after either saline or challenge with any dose of LPS. Within the subset of atopic subjects (n = 10), the mean percentage of eosinophils in NLF after administration of saline were $2.40\% \pm 0.91\%$ after $1.0 \mu\text{g}$ of LPS versus $0.3\% \pm 0.21\%$ after saline (Fig 2). If the 6 atopic subjects with eosinophil responses 4 hours after administration of $1.0 \mu\text{g}$ of LPS (atopic subjects no. 1, 2, 3, 5, 6, and 10, the "eosinophil responders") are considered separately, the percentage of eosinophils in NLF 4 hours after challenge with $1.0 \mu\text{g}$ of LPS is $4.0\% \pm 1.1\%$ versus $0.17\% \pm 0.17\%$ 4 hours after saline challenge (Fig 2). Only $1.0 \mu\text{g}$ of LPS exerted an effect on the percentage of eosinophils in NLF in atopic or eosinophil-responsive subjects.

This study was conducted between March 30, 1998, and April, 21, 1998, during which time there would normally be airborne tree pollen. Four of the 6 eosinophil-responsive subjects also had positive skin test responses to eastern tree mix (subjects 1, 3, 6, and 10). None of these subjects had nasal symptoms before either challenge session. Prechallenge percentage of eosinophils for the atopic group and the eosinophil-responder group were similar, with the mean for eosinophil-responsive subjects being $0.6\% \pm 0.3\%$ eosinophils.

Effect of LPS on PMN influx into the nasal airway in atopic subjects (n = 10)

LPS did not have a significant effect on the percentage of PMNs in NLF recovered from either normal subjects or asthmatic subjects 4 or 24 hours after challenge with $1.0 \mu\text{g}$ LPS when compared with the percentage of PMNs found after saline challenge (Fig 3, A). However, NLF from the 6 subjects who had eosinophil responses 4 hours after challenge also showed a significant increase in the percentage of PMNs 4 (but not 24) hours after administration of $1.0 \mu\text{g}$ of LPS (Fig 3, B). This was not found in 10 subjects who did not have eosinophil responses to $1.0 \mu\text{g}$ of LPS (Fig 3, B). Baseline percentages of PMNs before any dose of LPS were similar for each group (range, $10.4\% \pm 4.8\%$ to $27.8\% \pm 10.8\%$ among all subjects, n = 16, data not shown).

Cytokine and ECP responses to LPS challenge in all subjects (n = 16)

When compared with saline, no dose of LPS had an effect on levels of $\text{TNF-}\alpha$, $\text{IL-1}\beta$, IL-8 , or ECP in NLF 4 or 24 hours after challenge in any subject group (data not shown). However, there was a trend for increased ECP levels 4 hours after challenge with $1.0 \mu\text{g}$ of LPS ($32.4 \pm 13 \text{ ng/mL}$ 4 hours after saline versus $46.3 \pm 16 \text{ ng/mL}$ 4 hours after $1.0 \mu\text{g}$ of LPS, n = 16). There were significant

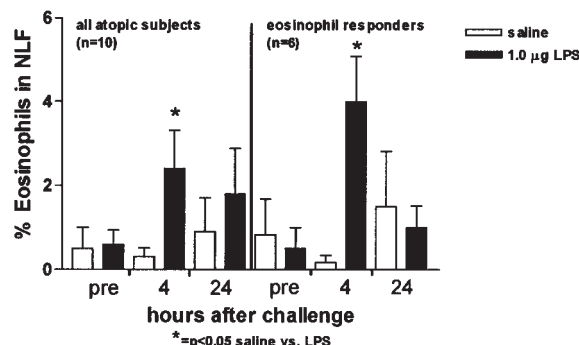


FIG 2. Effect of $1.0 \mu\text{g}$ of LPS on percentage of eosinophils in NLF in atopic subjects. The *left panel* depicts the effect of $1.0 \mu\text{g}$ of LPS and saline on the percentage of eosinophils in NLF at baseline (prechallenge), as well as 4 and 24 hours after challenge in the 10 atopic subjects. The *right panel* depicts these findings for the 6 atopic subjects who had eosinophil responses to LPS (eosinophil responders). Asterisks (*) denote significant differences between percentage of eosinophils recovered after challenge with saline or LPS at a given time ($P < .05$).

changes in GM-CSF associated with LPS challenge. Within the entire study group (n = 16), challenge and both 0.1 and $1.0 \mu\text{g}$ LPS yielded increases of GM-CSF in NLF 4 hours after challenge ($10.6 \pm 2.07 \text{ pg/mL}$ after saline vs $21.01 \pm 2.4 \text{ pg/mL}$ after $0.1 \mu\text{g}$ of LPS and $20.35 \pm 3.01 \text{ pg/mL}$ after $1.0 \mu\text{g}$ of LPS; $P < .01$ for both comparisons). The level of GM-CSF in NLF after challenge with $0.3 \mu\text{g}$ of LPS ($11.5 \pm 2.4 \text{ pg/mL}$) was not different from that found with the saline control. No amount of LPS induced a change in GM-CSF 24 hours after endotoxin when compared with that in NLF collected 24 hours after saline challenge.

Relationship between GM-CSF and other measures of inflammation after challenge with $1.0 \mu\text{g}$ LPS

Constitutive (baseline) levels of GM-CSF in NLF (obtained before challenge) correlate with the percentage of eosinophils present 4 hours after LPS challenge ($r = 0.55$, $P = .01$, n = 16). There was no correlation between GM-CSF in NLF collected 4 hours after challenge and the percentage of eosinophils at any time. NLF samples collected 24 hours after challenge had levels of GM-CSF that correlated well with the percentage of eosinophils ($r = 0.71$, $P < .001$, n = 16) and the percentage of PMNs ($r = 0.52$, $P = .02$, n = 16).

Relationship between IL-8 and other measures of inflammation after challenge with $1.0 \mu\text{g}$ of LPS

Constitutive levels of IL-8 correlate with the percentage of eosinophils present 24 hours after LPS challenge ($r = 0.44$, $P = .03$, n = 16) but not with the percentage of PMNs 4 or 24 hours after challenge. There were correlations between IL-8 at baseline and ECP in NLF at baseline ($r = 0.54$, $P = .03$, n = 16), 4 hours after LPS challenge ($r = 0.58$, $P = .02$, n = 16), and 24 hours after LPS challenge ($r = 0.7$, $P = .003$, n = 16).

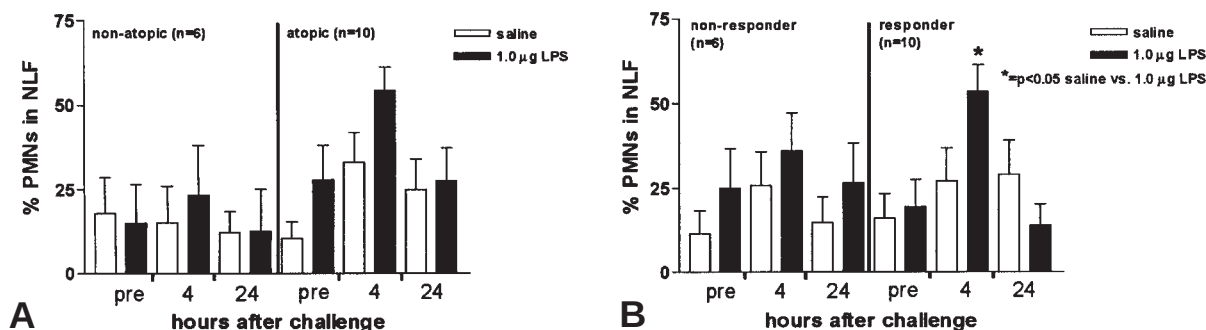


FIG 3. Effect of 1.0 µg of LPS on the percentage of PMNs in NLF in atopic subjects. **A.** The effect of 1.0 µg of LPS and saline on the percentage of PMNs in NLF in nonatopic (n = 6) and atopic subjects. **B.** The effect of 1.0 µg of LPS and saline on the percentage of PMNs in NLF of subjects with (eosinophil responders, n = 6) and without eosinophil responses (nonresponders, n = 10) to LPS. Asterisk (*) denotes significant differences between the percentage of eosinophils recovered after saline or LPS challenge at a given time ($P < .05$).

IL-8 present in NLF 4 hours after LPS challenge also correlates with both the percentage of eosinophils 24 hours after challenge ($r = 0.44$, $P = .03$) and ECP 4 and 24 hours after LPS challenge ($r = 0.9$, $P < .001$ and $r = 0.71$, $P = .002$, respectively). IL-8 in NLF collected 24 hours after challenge correlated with the percentage of eosinophils 24 hours after challenge and with ECP at baseline ($r = 0.49$, $P = .05$), 4 hours ($r = 0.69$, $P = .003$), and 24 hours after administration of LPS ($r = 0.72$, $P = .002$).

DISCUSSION

The primary (a priori) purpose of this pilot study was to determine whether low-level endotoxin (0.1 to 1.0 µg) would induce eosinophil influx to the nasal airways of atopic and normal subjects. We observed that challenge with 1.0 µg of LPS (but not 0.1 or 0.3 µg of LPS) caused an influx of eosinophils to the nasal airways in 6 of 10 mite-sensitive atopic subjects with a clinical history consistent with mild asthma. This influx was noted 4, but not 24, hours after challenge, even among those subjects who had an eosinophil response 4 hours after challenge (n = 6). LPS challenge did not induce influx of eosinophils in any of the normal subjects.

There was no significant effect of this dose range of LPS on nasal PMNs within either the entire group or the atopic subsets. However, if the eosinophil-responsive subset (n = 6) is considered separately, there is an increase in the percentage of PMNs present 4 hours after challenge with 1.0 µg of LPS.

Also examined on an exploratory basis was the effect of LPS on GM-CSF, IL-1β, IL-8, and TNF-α levels in NLF. Of the cytokines examined, acute challenge with 0.1 and 1.0 µg of LPS resulted in a significant increase of GM-CSF 4 hours after challenge when compared with responses after saline challenge in both atopic and normal subjects. Levels of none of the other cytokines measured in NLF were affected by any dose of LPS.

As an exploratory hypothesis, the influence of the baseline state of inflammation on cellular response to LPS in the nasal airway was examined. The effect of con-

stitutive levels of IL-8 and GM-CSF was of particular interest in this study because of other observations that these cytokines (which are increased with allergic inflammation) have reported actions on eosinophils, including priming, prevention of apoptosis, and chemotaxis.^{18,19,25-30}

Supporting the hypothesis that constitutive GM-CSF may enhance response to LPS are reports that GM-CSF restores systemic response to parenteral LPS challenge in rodents desensitized to LPS.³¹ GM-CSF has been shown to increase CD14 expression in neutrophils and myeloid tumor cell lines and to induce human peripheral blood monocytes to differentiate into macrophages with increased CD14 expression and the ability to act as antigen-presenting cells.^{32,33} GM-CSF also enhances LPS-induced IL-1β and TNF-α secretion by the THP-1 mononuclear cell line.³⁴ Thus GM-CSF may act in a number of ways to enhance inflammatory responses to LPS in atopic subjects. Consistent with that hypothesis, we observed that the percentage of eosinophils present 4 hours after challenge with 1.0 µg of LPS correlated well with constitutive levels of GM-CSF but not with levels present 4 or 24 hours after challenge.

Previous studies from our group support the notion that constitutive levels of IL-8 might enhance response to inflammatory stimuli, such as LPS. In these studies we found that levels of ECP and IL-8 in NLF were elevated in children with a history of symptomatic asthma but not in those with infrequent asthma symptoms or those who were nonatopic and nonasthmatic.²⁴ NLF was collected from these children at times when they were not symptomatic, suggesting that the elevations in ECP and IL-8 observed in those with a history of symptomatic asthma reflected constitutive airway inflammation rather than acute response to infection or allergen exposure. Increased asthma exacerbations, which occurred in the group of children with increased IL-8 and ECP, may have resulted from increased responses to acute inflammatory stimuli resulting from increased baseline inflammation. These results also suggest a relationship between IL-8 and eosinophil activation.

The hypothesis that IL-8 plays a role in asthma severity is further supported by observations by Shute et al,³⁵ who observed that IgA enhances the action of IL-8 on eosinophils, including chemotaxis and GM-CSF production. These same investigators also report that free IL-8, as well as that bound with IgA and IgG, are increased in serum, tissue, and lysates of mononuclear cells and granulocytes recovered from patients with severe asthma compared with similar samples recovered from patients with mild asthma or without asthma.³⁷ Furthermore, these same investigators show a marked correlation in serum IL-8 and ECP in asthmatic subjects, suggesting an impact of IL-8 on eosinophil activation in such subjects.³⁶

In this study we noted a near significant trend for an increase in ECP 4 hours after LPS challenge (compared with ECP in NLF collected after saline challenge). Despite having no increase in IL-8 levels after challenge with 1.0 μg of LPS, there were significant correlations between IL-8 and ECP in NLF collected at baseline and 4 and 24 hours after LPS challenge. This was particularly true of IL-8 and ECP levels observed 4 hours after challenge with 1.0 μg of LPS. There was also a correlation between constitutive levels of IL-8 and the percentage of eosinophils 24 hours after LPS challenge. These findings indicate that increased eosinophil responsiveness to IL-8 (as reflected in ECP levels) may be induced by LPS.

Although we had insufficient samples to assay for secretory IgA in nasal secretions from asthmatic subjects, it seems likely that LPS would enhance IgA secretion in the nasal airway. As suggested by Shute et al,^{35,36} increased levels of secretory IgA in the nasal airway could then enhance IL-8 responsiveness of eosinophils in the nasal airway, perhaps accounting for the correlation between IL-8 and ECP observed after LPS challenge in this study. Whether by this or other mechanisms, it does seem reasonable that LPS could alter cellular responsiveness to mediators already present in the airway mucosa as a result of allergic inflammation.

The maximum dose of LPS used in this study (1.0 μg) was selected to mimic an 8-hour exposure to low-level (0.25 $\mu\text{g}/\text{m}^3$) ambient air LPS. It is possible that the classical responses to inhaled LPS (secretion of IL-1, TNF- α , and increase in PMNs) are only apparent at higher doses in most persons. The subjects in this study who were "eosinophil responsive" to LPS may have had increased susceptibility to the proneutrophilic effects of LPS as a result of whichever process accounted for eosinophil increases after LPS challenge.

Future studies are planned to compare dose-response characteristics of both atopic and nonatopic subjects to LPS across a higher dose range (1.0 to 5.0 μg) to determine whether there are differing sensitivities of these groups to LPS. Likewise, studies of subjects stratified on the basis of constitutive GM-CSF and IL-8 will be undertaken to determine whether constitutive levels of these cytokines predict eosinophil and neutrophil response to LPS.

The proeosinophilic effect of LPS in the nasal airways

of atopic subjects, as well as the suggestion that asthmatic subjects (eosinophil responders) are more susceptible to the neutrophil response to LPS than nonasthmatic subjects, are similar to previous findings by our group of the effect of ozone on nasal and bronchial eosinophilic inflammation in atopic asthmatic subjects.^{23,37-40} As with LPS, ozone is generally known as an inducer of neutrophilic inflammation, with effects on nasal eosinophils being apparent only in atopic subjects.^{23,37} Other reported similarities between ozone and LPS include marked increases in IL-8 and increased nonspecific airway reactivity after in vivo exposure.^{4,5,40} Also, decreases in lung function occur with both irritants, with true bronchospasm being reported only in asthmatic subjects.³⁷

It also seems probable that LPS would alter acute response to inhaled or topically applied allergen in atopic subjects. Other environmental agents, such as ozone, nitrogen dioxide, and diesel exhaust particles, have been shown to enhance allergen-induced eosinophilic or $\text{T}_{\text{H}2}$ inflammation in the nasal airway or increases in immediate airway response to inhaled allergen.^{23,37}

Conversely, allergen-induced inflammation might enhance response to LPS. Of the 6 eosinophil-responsive subjects in our study, 4 were also allergic to tree pollen. Our study was conducted during our local tree pollination season. It is possible that chronic allergic response to ambient air tree antigens may have contributed to enhanced eosinophil and PMN responses to LPS in the eosinophil-responsive subjects. Indeed, increased eosinophil response to LPS may simply be a component of enhanced nonspecific airway responses to inflammatory and pharmacologic stimuli observed with allergic airway inflammation.

Consistent with that argument are observations that acute allergen challenge increases levels of sCD14 in BAL fluid.^{15,16} If chronic allergic inflammation has a similar effect on sCD14, then it would be logical that chronic allergic inflammation would enhance response to LPS because of the action of this LPS response mediator. Likewise, increased GM-CSF expression, reported to occur with acute allergen exposure,²⁸⁻³⁰ may also enhance response to LPS.

In summary, we report our pilot observations that challenge with 1.0 μg of LPS induces eosinophils to the nasal airways of mite-sensitive atopic subjects but not nonatopic subjects. Those subjects with eosinophil responses also had PMN responses to low-level LPS challenge. This challenge also resulted in increases in GM-CSF recovered in NLF of both atopic and nonatopic subjects. Constitutive (baseline) GM-CSF levels correlated positively with eosinophil response to LPS. There was also a nearly significant increase in nasal ECP after LPS challenge, which correlated well with levels of IL-8 in the nasal airway. These findings indicate that low doses of LPS can enhance eosinophilic inflammation and may be an important environmental cofactor in exacerbation of allergic diseases of the airway. Furthermore, these data suggest that PMN responses are enhanced in the nasal airways of eosinophil-responsive subjects and that GM-

CSF and response to IL-8 may play an important role in the inflammatory responses of atopic subjects to LPS. Future studies will examine the relationship between allergen-induced inflammation and LPS-induced inflammation in allergic subjects.

We thank Susan Burkett, BS, for technical assistance in completion of the studies outlined in this manuscript.

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