

Blunting airway eosinophilic inflammation results in a decreased airway neutrophil response to inhaled LPS in patients with atopic asthma: A role for CD14

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Recent data demonstrate that atopic inflammation might enhance airway responses to inhaled LPS in individuals with atopic asthma by increasing CD14 expression on airway macrophages. We sought to determine whether blunting airway eosinophilic inflammation decreases CD14 expression and the subsequent airway polymorphonuclear neutrophil (PMN) response to inhaled LPS in subjects with atopic asthma. Twelve such subjects underwent a 2-week, placebo-controlled trial of inhaled steroid (440 µg fluticasone propionate [FP] twice per day); this was followed 48 hours later by an inhaled LPS (5 µg) challenge. A comparison of LPS-induced inflammatory cells in sputum, CD14 expression, and methacholine responsiveness with FP or placebo was conducted. Flow cytometry was used to analyze membrane-bound CD14 expression (mean fluorescence intensity) on sputum macrophages. We report that 48 hours before inhaled LPS challenge (baseline), FP significantly blunted airway eosinophils (cells per milligram; $P = .04$) and mCD14 expression (mean fluorescence intensity; $P = .03$) but did not decrease the number of PMNs (cells per milligram). Six hours after LPS challenge, airway PMNs and mCD14 expression were significantly decreased for FP in comparison with placebo ($P = .04$). Our data suggest that decreasing airway allergic inflammation with corticosteroids results in both decreased expression of CD14 on airway monocytic cells and a decreased PMN response to inhaled LPS. (*J Allergy Clin Immunol* 2001;108:577-80.)

Key words: CD14, LPS, eosinophilic inflammation, inhaled steroid

In atopic asthma, airway eosinophils correlate with disease severity, bronchospasm, and bronchial hyperresponsiveness.¹⁻³ Recent observations in human beings demonstrate that LPS enhances eosinophil responses in allergic disease⁴ and that allergen challenge of the airway induces eosinophilic responses to LPS.⁵ Moreover, allergic asth-

Abbreviations used

AMMs: Airway macrophages and monocytes
ECP: Eosinophil cationic protein
PMN: Polymorphonuclear neutrophil

matic individuals appear to have increased expression of CD14 in their airways in comparison with nonallergic controls.⁴ These studies suggest that atopy plays an important role in enhancing CD14-mediated airway responses to inhaled LPS in allergic asthmatic individuals.

We previously reported that low-dose (5 µg) inhalation challenge with LPS in subjects with asthma induces polymorphonuclear neutrophil (PMN) influx into the airways without changes in pulmonary function or symptoms.⁶ This response correlated with prechallenge eosinophil numbers and CD14 expression on airway macrophages, suggesting that atopic airway inflammation might modulate the response to LPS challenge.

To further determine the underlying role of eosinophilic inflammation and LPS, we treated a new group of subjects with atopic asthma with either 440 µg of fluticasone propionate (FP) or placebo twice per day for 2 weeks in a double-blinded crossover fashion; this was followed by LPS challenge. Endpoints of interest were inflammatory cell response to LPS, membrane CD14 (mCD14) expression of airway macrophages and monocytes (AMM), and methacholine responsiveness. We hypothesized that FP would blunt eosinophil numbers and CD14 in the airways with a resultant reduction in the PMN response to inhaled LPS.

METHODS

Subjects

Twelve allergic subjects who had mild, intermittent asthma (based on current NIH Guidelines⁷; 22 to 50 years; percent of predicted forced vital capacity, $105.6\% \pm 4.8\%$; FEV₁, $93.5\% \pm 3.7\%$) who were not taking any chronic controller medications were recruited to this study, which was approved by the University of North Carolina Committee on the Protection of the Rights of Human Subjects. All subjects (male, $N = 3$; female, $N = 9$) were otherwise healthy, had not smoked for at least 1 year, had positive skin test results to house dust mite, and had positive methacholine challenge results (PC₂₀ FEV₁, <5 mg/mL; mean $\pm$ SEM, 1.88 ± 0.7 mg/mL).

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TABLE I. Sputum differential cell counts

Treatment condition	48 h before LPS challenge			6 h after LPS challenge		
	PMN	EOS	MAC	PMN	EOS	MAC
Drug (fluticasone propionate)	636 ± 149	9 ± 4	1971 ± 601	722 ± 259	3 ± 2	540 ± 287
Placebo	655 ± 213	69 ± 26*	1276 ± 432	1662 ± 403*†	100 ± 57	527 ± 256

Data are presented as means ± SEMs and expressed in cells/mg.

PMN, Polymorphonuclear neutrophil; EOS, eosinophil; MAC, macrophage.

*Significantly different from drug ($P < .05$)

†Significantly different from pre-exposure condition (48 h before LPS challenge; $P < .05$).

TABLE II. Sputum fluid phase components

Treatment condition	48 h before LPS challenge				6 h after LPS challenge			
	IL-6	IL-8	TNF- α	ECP	IL-6	IL-8	TNF- α	ECP
Drug (fluticasone propionate)	353 ± 68	3725 ± 452	39 ± 26	410 ± 157	369 ± 64	2953 ± 816	38 ± 10	163 ± 60
Placebo	227 ± 69	3158 ± 1077	7 ± 5	413 ± 192*	459 ± 78	2939 ± 888	24 ± 10	426 ± 207*

Data are presented as means ± SEMs and expressed in pg/mL (*ng/mL).

ECP, Eosinophil cationic protein.

Study protocol

During the screening visit, informed consent was obtained and a medical history was taken; this was followed by spirometry and determination of skin test reactivity, methacholine reactivity, and ability to induce sputum.

The investigation was a randomized, double-blinded, placebo-controlled crossover study in which subjects were treated with inhaled FP or placebo twice per day for 12 days; this was followed by an inhaled LPS challenge (5 μ g *Escherichia coli* #L-2262; Sigma, St Louis, Mo) on day 14, with crossover occurring no sooner than 2 weeks after completion of the initial treatment. On day 12 of each treatment, induction of sputum was performed to assess baseline airway inflammation and AMM CD14 expression. An LPS dose of 5 μ g was used; this dose induces PMN influx to the airway without the systemic symptoms (fever, chills) that are observed at doses of >50 μ g.⁸ Spirometry was administered hourly for 5 hours after LPS challenge; this was followed immediately by methacholine challenge. Sputum induction followed 30 minutes after the completion of the methacholine challenge. The sputum induction and processing methods used in this study have been described in detail in a previous publication.⁶

Measurement of cytokine levels in sputum

Cytokines (IL-8, IL-6, and TNF- α) were measured through use of commercially available ELISAs (Endogen, Inc, Woburn, Mass; Bio-Rad, Hercules, Calif; R&D Systems, Minneapolis, Minn; Oxis International, Portland, Ore). Eosinophil cationic protein (ECP) was measured through use of an RIA (Pharmacia Diagnostics, Columbus, Ohio). Cytokine and protein content were expressed in terms of weight per milliliter of airway fluid.

Flow cytometry and immunofluorescence staining

The flow cytometric and immunofluorescence staining methods used in this study have been described in detail in a previous report.⁹ CD14 expression was expressed in terms of receptor-specific mean fluorescence intensity.

LPS challenge

The method of delivering nebulized inhaled LPS at a 5- μ g dose is based on the method of Michel et al¹⁰ and has been used previously in our laboratory.⁶

Statistics

Paired parametric *t* tests were used to compare induced sputum and pulmonary function endpoints between the drug and placebo conditions. A *P* value of .05 was considered statistically significant.

RESULTS

FP affected sputum cell counts before and after LPS inhalation. As shown in Table I, FP significantly attenuated airway eosinophils ($P = .04$), but not PMNs, 48 hours before LPS challenge in comparison with placebo. FP treatment was also shown to significantly blunt LPS-induced airway PMN influx in comparison with the placebo condition ($P = .04$). Indeed, only the placebo treatment was associated with a significant increase in PMNs from day 12 to day 14 (post-LPS, $P = .03$; Table I). A trend ($P = .05$; 1-tail *t* test) toward decreased eosinophils (in cells per milligram) was also observed 6 hours after LPS challenge with FP in comparison with placebo (Table I). In addition, FP attenuated the proportion of airway PMNs 6 hours after LPS challenge ($47\% \pm 7\%$ vs $69\% \pm 7\%$; $P = .03$) in comparison with placebo (data not shown).

We observed no significant effect of drug in the mean concentration of fluid-phase components of induced sputum (IL-6, IL-8, TNF- α , ECP) 48 hours before or 6 hours after LPS challenge (Table II). However, a trend toward decreased mean ECP concentration after LPS challenge was observed with FP treatment (163 ± 60 ng/mL vs 426 ± 207 ng/mL; $P = .05$, 1-tail *t* test; Table II). No increase in fluid-phase components of sputum induced by LPS was seen, regardless of treatment.

Expression of the LPS surface receptor CD14 on AMM was modified by FP. The mean mCD14 expression (mean fluorescence intensity) was significantly decreased on sputum monocytes ($P = .03$) and macrophages ($P = .03$) 48 hours before inhaled LPS challenge (Fig 1, A) and 6 hours after LPS challenge (for monocytes, $P = .001$; for macrophages, $P = .01$). There

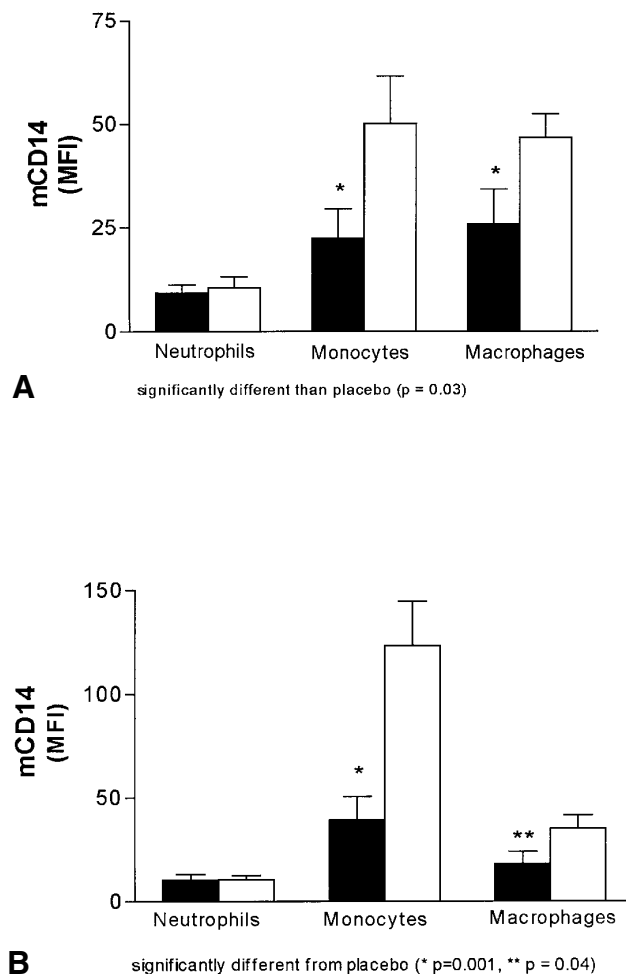


FIG 1. A, Effect of FP (solid bar) and placebo (open bar) on mCD14 expression on sputum neutrophils, monocytes, and macrophages 48 hours before LPS (5 μ g) inhalation challenge in 12 subjects with atopic asthma. **B,** Effect of FP (solid bar) and placebo (open bar) on mCD14 expression on sputum neutrophils, monocytes, and macrophages 6 hours after LPS (5 μ g) inhalation challenge in 12 subjects with atopic asthma.

was no significant difference in post-LPS airway responsiveness (mean $\pm$ SEM PC₂₀ FEV₁ mg/mL) between FP (4.34 $\pm$ 1.6 mg/mL) and placebo (2.15 $\pm$ 1.07 mg/mL) treatments.

DISCUSSION

Studies correlating CD14 expression in the airways with airway eosinophilia suggest that atopic inflammation might modulate CD14-associated responses to inhaled LPS.⁴⁻⁶ Our results support this hypothesis. We observed that FP treatment of these subjects with atopic asthma decreased baseline eosinophilic inflammation and constitutive expression of mCD14 on AMM as well as reducing LPS-induced PMN response and mCD14 expression on AMM observed in sputum obtained 6 hours after LPS challenge. CD14, in conjunction with Toll-like receptors, mediates nonspecific inflammatory responses to LPS *in vivo*.¹¹

FP did not cause a decrease in the number of baseline PMNs or in IL-8 levels, suggesting that blunted LPS-induced neutrophil responses are not explained by either a decrease in pre-LPS neutrophils or a single key neutrophil chemotactic factor. However, both mCD14 expression and airway eosinophils remained significantly depressed 6 hours after LPS inhalation, a possible indication of decreased activation of these cells with an associated inhibition of T_H2 cytokine secretion. Although for technical reasons we could not measure IL-4 or IL-5, we did observe a trend toward decreased ECP levels after LPS with FP in comparison with placebo (163 $\pm$ 60 ng/mL vs 426 $\pm$ 207 ng/mL), suggesting an FP effect on the T_H2 response to LPS. Taken together, our data indicate that FP, through its action on airway atopic inflammation as well as its action, either direct or indirect, on CD14, decreases the response of an individual with allergic asthma to inhaled LPS; this further supports the hypothesis that atopic inflammation affects CD14 expression and response to LPS.

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