Effect of inhaled endotoxin on airway and circulating inflammatory cell phagocytosis and CD11b expression in atopic asthmatic subjects

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Background: In a cohort of 8 normal and 10 allergic asthmatic volunteers, we previously reported that inhalation of 5 μg of endotoxin (LPS) induced airway inflammation that correlated with CD14 expression that was, in turn, correlated with eosinophil numbers in the airway. Macrophage and neutrophil functions have been reported to be modified by endotoxin in vitro and in vivo, and response to endotoxin is mediated largely by airway phagocytes and related to allergic inflammation.
Objective: We sought to examine functional and cell-surface phenotype changes in phagocytes recovered from atopic asthmatic subjects after endotoxin challenge.
Methods: Sputum and peripheral blood from 10 allergic asthmatic subjects was recovered after saline and LPS challenge. Assessment of phagocytosis and cell-surface phenotype (CD11b, CD14, and CD64) was performed on phagocytes obtained from sputum (n = 7) and blood samples (n = 10).
Results: Phagocytosis of blood and sputum phagocytes was blunted after LPS challenge in a fashion that correlated with the increase in airway neutrophils after LPS challenge. Cell-surface expression of CD14 (membrane-bound CD14) was increased in sputum cells, whereas CD11b was decreased in sputum and circulating phagocytes. Baseline expression of CD11b in blood correlated with the magnitude of the neutrophil response after LPS inhalation, as well as (inversely) with baseline airway eosinophil levels.
Conclusions: Inhalation of endotoxin at levels adequate to induce a neutrophil influx to the airways (but not systemic symptoms) results in decreased phagocytosis in both airway and circulating cells and modifies CD11b expression in a way that implicates its involvement in phagocyte responsiveness to inhaled LPS. (J Allergy Clin Immunol 2003;112:353-61.)

Key words: *Endotoxin, phagocytosis, CD11b, neutrophil response*

Endotoxin (LPS) responsiveness in the lung is essential for host defense but, when dysregulated, also contributes significantly to acute lung injury and acute respiratory distress syndrome (ARDS),[1] agricultural occupational lung disease, and asthma.[2] Recognition of endotoxin is mediated by a number of molecules, including the pattern recognition receptors CD14 (membrane-bound CD14 [mCD14] and soluble CD14 [sCD14]) and toll-like receptor 4. Other molecules important in innate immune responses include CD11b-CD18 (the complement receptor 3 [CR3] receptor), which mediates complement-associated phagocytosis[3-6] and plays a role in airway response to pathogens. It has also been suggested that the presence of ongoing airway inflammation modifies airway responsiveness to endotoxin. Consistent with this idea is the observation that CD14 levels are increased 10-fold in plasma and airway fluids in ARDS.[1,7]

Airway CD14 is also modified in asthma. Dubin et al[8] and Virchow et al[9] reported that atopic volunteers had enhanced expression of CD14 in the airway after allergen challenge, and Viksman et al[10] observed that endobronchial allergen challenge not only enhanced expression of CD14 in the airway but also induced increased expression of CD11b and CD64 (FcγRI) on airway inflammatory cells. These data suggest that inflammatory cells in these allergic subjects might be more responsive to endotoxin and other inflammatory stimuli.[10] Consistent with the idea that allergic inflammation enhances response to endotoxin are nasal studies by our group in which we observed that allergen challenge followed by endotoxin results in increased neutrophils (PMNs) and eosinophils than either stimulus alone.[11]

Although we have observed that allergen challenge enhances endotoxin-induced neutrophil influx in the nasal airway, Michel et al[12] have reported that atopic subjects have decreased circulating phagocyte function after inhaled endotoxin challenge. Studies by our group have found that in sputum macrophages obtained from asthmatic patients with airway eosinophilia, expression of CD11b and CD64 and phagocytosis were all decreased when compared with that seen in asthmatic patients without airway eosinophilia.[13] In normal volunteers Sand-

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TABLE I. Subject characteristics and pulmonary function data

	Asthmatic subjects
N	10
Age (y)	25.3 ± 6.0
Height (cm)	165.9 ± 9.1
Weight (kg)	67.1 ± 9.2
FVC, % predicted	112.9 ± 15.5
FEV_1, % predicted	102.0 ± 13.3
FEV_1/FVC, % predicted	92.8 ± 10.1
PC_{20}, mg/mL	2.9 ± 5.5
PMN/mg sputum before LPS challenge	376 ± 88
PMN/mg sputum, after LPS challenge	1987 ± 388

Data are presented as means ± SEM.
FVC, Forced vital capacity.

Abbreviations used
ARDS: Acute respiratory distress syndrome
CR3: Complement receptor 3
MFI: Mean fluorescence intensity

strom et al[14,15] observed that challenge with 100 μg of endotoxin was associated with increases in neutrophils and blunted macrophage phagocytic activity. Taken together, these observations suggest that atopic individuals might have enhanced inflammation (neutrophil response) but decreased innate host defense capacity (decreased phagocytosis, CD11b, and CD64) after endotoxin challenge.

To determine the effect of atopy on response to endotoxin and the effect of endotoxin inhalation on phagocyte function, we challenged a group of 10 atopic asthmatic patients and 8 normal volunteers with 5 μg of inhaled endotoxin and saline in a double-blind, placebo-controlled fashion and examined induced sputum for changes in airways neutrophil content in all 18 subjects. In the 10 asthmatic subjects we also examined the ability of airway and circulating phagocytes (monocytes, macrophages, and neutrophils) to phagocytize IgG-opsonized zymosan and express mCD14, CD11b (a component of the CR3 receptor), and CD64 (the FcγRI receptor). In our initial report from these challenges, we described that 5 μg of endotoxin did induce a neutrophil influx to the airway and that this influx correlated with CD14 expression on sputum macrophages before challenge.[16]

We now extend our observations in the 10 atopic asthmatic patients who underwent these challenges to examine the effect of endotoxin inhalation on airway and circulating phagocyte function, airway and circulating cell-surface markers of interest (mCD14, CD11b, and CD64), the relationship between baseline levels of these markers and changes in phagocytosis, and the relationship between baseline sputum eosinophilia and phagocyte function and cell-surface marker expression after endotoxin. Peripheral blood samples were obtained in 10 subjects, whereas adequate sputum samples for flow cytometric analysis were recovered from 7 volunteers.

METHODS

Subjects

Subject characteristics, baseline pulmonary function data, and pre-endotoxin and postendotoxin challenge neutrophil counts in recovered sputum (previously reported by Alexis et al[16]) are shown in Table I. Nonsmoking patients with mild atopic asthma between the ages of 18 and 40 years were recruited for the study. A medical screening examination that included a medical history, physical examination, blood tests, methacholine challenge tests, and allergy scratch tests were completed for all subjects. Ten atopic asthmatic subjects who were a subset of the volunteer cohort we recruited for our previous report[16] were also studied in this current examination of the effect of endotoxin inhalation challenge on airway and circulating phagocyte biology.

These volunteers all met the National Heart, Lung, and Blood Institute criteria for mild asthma and were enrolled in the study.[17] All subjects had a positive asthma history (wheezing, chest tightness, and reversible airflow obstruction) and a bronchodilator-withheld FEV_1/forced vital capacity of greater than 60%. Subjects were free of oral or inhaled corticosteroid treatment within the previous 6 months, had no hospital admissions for asthma within the previous year, and were able to withhold antihistamines for 48 hours before study days. Subjects with seasonal atopic asthma were studied outside their allergy season, and none were taking cromolyn or leukotriene modifiers. All subjects were asked to refrain from anti-inflammatory use for 48 hours before the study days. The subjects used inhaled bronchodilators as needed. This study was approved by the Committee for the Protection of the Rights of Human Subjects at the University of North Carolina School of Medicine. All subjects provided written informed consent.

Study design

Subjects attended the laboratory 5 times. At the first visit, questionnaire, limited physical examination, spirometry, a methacholine inhalation test, and allergy skin tests were used to assess entry criteria. At the second visit, the subjects were randomized to inhale nebulizer sterile saline or 5 μg of endotoxin (*Escherichia coli* 026:B6, Sigma), and pre-exposure spirometry, induced sputum, and blood draws were performed. The subjects returned the following day for the third visit and inhaled saline or endotoxin through a closed-circuit ultrasonic nebulizer (De Vilbus 99). The total fluid volume nebulizer was 10 mL. Hourly FEV_1 and forced vital capacity tests were performed. At 5 hours after exposure, a methacholine challenge was preformed, and at 6 hours after exposure, blood was drawn, and induced sputum was collected. A minimum of 3 weeks later, the subjects returned to cross over to the other arm of the study, during which on visit 4 they had prechallenge measures and on visit 5 they were challenged with the opposite treatment of what they received on visit 3.

Sputum induction and processing

Sputum induction and processing were carried out as previously outlined.[13,16,18] Briefly, the induction procedure involved 3 separate 7-minute inhalation periods of 3%, 4%, and 5% hypertonic saline administered after baseline spirometry. At the end of each 7-minute inhalation, the subjects performed a 3-step cleansing procedure before the cough attempt. The subject was then instructed to perform a chesty-type cough without clearing the back of the throat. The sample was expectorated into a sterile specimen cup that was placed on ice throughout the procedure.

Sample processing began immediately after sputum was collected. In brief, mucus plugs were manually selected and weighed, incubated (15 minutes at room temperature) in 0.1% dithiothreitol (Calbiochem Corp), washed with Dulbecco's PBS, and gravity fil-

TABLE II. Mean ± SEM total and differential cell counts in sputum 6 hours after inhaled saline and endotoxin (5000 ng/mL) challenge in asthmatic subjects

	TCC/MG	PMN (%)	Eosinophils (%)	Macrophages (%)	Lymphocytes (%)
Saline (n = 10)	2490 (695)	26* (2)	6 (2)	66* (4)	1 (0.3)
Saline (n = 7)	3199 (934)	29* (1)	8 (3)	62* (3)	1 (0.3)
Endotoxin (n = 10)	3340 (617)	59 (4)	4 (1)	36 (4)	0.2 (0.2)
Endotoxin (n = 7)	4640 (1686)	59 (6)	4 (1)	37 (6)	0 (0)

TCC, Total cell count; *MG,* milligrams of sputum.
*$P < .05$ versus endotoxin condition.

tered through a 48-μm-pore mesh filter (BBSH Thompson). Total cell counts were performed with the use of a Neubauer Hemocytometer. Visually identifiable squamous epithelial cells were neither counted nor included in the total cell count. Cell viability was determined by using Trypan blue exclusion staining. Differential leukocyte analysis of nonsquamous cells (Diff Quik stained) was performed on a minimum of 300 cells provided that squamous epithelial cells were less than 40% of the total cells. Differential cell counts (lymphocytes, neutrophils, eosinophils, monocytes, and macrophages) were expressed as a percentage of total nonsquamous nucleated cells.

Flow cytometry

Flow cytometry was performed with a FACSORT (Becton Dickinson) by using an Argon-ion laser (wavelength, 488 nm). Gain and amplitude settings were set for blood and sputum samples from the same subject, thus allowing for establishment of reference gates for leukocyte identification. Settings were consistent throughout the study for each subject. The FACSORT was calibrated with Calibrite (Becton Dickinson) beads (noncolor, green, and red) before each use, and 10,000 events were counted for all sample runs. Gating of healthy macrophages, monocytes, neutrophils, eosinophils, and lymphocytes in sputum was based on light-scatter properties and positive expression for CD45 (panleukocyte marker), as well as using reference gates from the whole-blood leukocyte preparations. Discrete populations of inflammatory cell types were observed. FITC- and phycoerythrin-conjugated nonspecific antibodies of the same isotope as the receptor antibodies were used as controls to establish background fluorescence and nonspecific antibody binding. The (arithmetic) mean fluorescence intensity (MFI) of the cells stained with control antibody was subtracted from the MFI of the cells stained with receptor antibodies to provide a measure of receptor-specific MFI. Relative cell size and density-granularity were quantified by analyzing light-scatter properties, namely forward scatter for cell size and side scatter for cell density-granularity, and recording the mean fluorescent intensities for each.

Cell-surface immunofluorescence staining

Cell-surface immunofluorescence staining was previously described.[16]

Phagocytosis assay

Saccharomyces cerevisiae zymosan A BioParticles (Molecular Probes, Inc) conjugated to FITC were opsonized with opsonizing reagent (IgG) for 45 minutes at 37°C and then washed with RPMI 1640 two times before adjusting the particle concentration to 2×10^6/mL. Purified (Percoll separated) blood mononuclear cells (2×10^6/mL) and neutrophils (2×10^6/mL) and sputum cells (2×10^6/mL) from the same subject were exposed to the yeast cell walls at a ratio of 1:10 for 1 hour at 37°C in the presence of human serum (20 μL) before tubes were placed on ice. Next, 200 μL of 2% paraformaldehyde was added to each tube, and the tubes were stored at 4°C in the dark and analyzed by means of flow cytometry (FACSORT) within 24 hours of particle exposure. Particle uptake was identified and displayed on histogram plots as a rightward shift in side scatter (side scatter x axis) in the phagocyte populations. Phagocytosis was determined by assessing the proportion of cells in the zymosan-exposed population showing increased mean fluorescence compared with cells that had not been exposed (control population) to zymosan particles.

Data analysis

Assays for phagocytosis and cell-surface marker expression of macrophages, monocytes, and lymphocytes were expressed as MFI, and differences in these end points after saline or endotoxin challenge were expressed as differences in MFI assayed. For a given response measure, differences between saline and endotoxin were compared by using nonparametric tests (Wilcoxon signed-rank test), with the overall α level set at .05. Data are presented as means ± SEM unless otherwise noted. The Spearman signed-rank procedure was used to examine correlations between any 2 end points.

RESULTS

Pulmonary function and systemic response to inhaled endotoxin

All the subjects tolerated the endotoxin inhalation without incident. No subject experienced any systemic responses (fever, myalgia, malaise, shortness of breath, chest tightness, or hypotension). Furthermore, pulmonary function testing and methacholine hyperresponsiveness were not significantly different after endotoxin inhalation compared with before challenge or saline inhalation.

Inflammatory cell response to inhaled endotoxin

As previously reported,[16] low-dose endotoxin had a significant effect on neutrophil counts but not on monocyte or macrophage counts (cells per milligram of sputum), with a 5.3-fold increase over pre-exposure in airway neutrophils after endotoxin exposure (376 ± 88 to 1987 ± 388 cells/mg, $P = .002$, Table I) compared with a 1.3-fold increase after saline challenge. Compared with postexposure saline, percentages of neutrophils were significantly increased and percentages of macrophages were decreased for both 10 and 7 specimens, respectively (Table II). No other cell type was modified after endotoxin challenge.

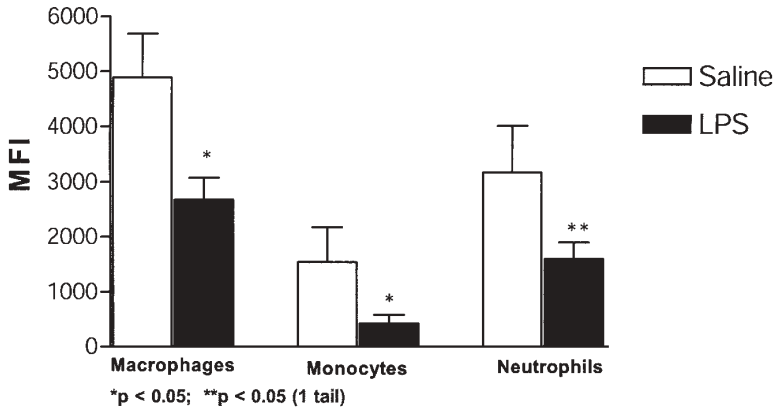

FIG 1. Mean (± SEM) phagocytosis of opsonized zymosan particles (MFI) of recovered sputum (airway) macrophages, monocytes, and neutrophils in atopic asthmatic individuals 6 hours after endotoxin *(closed bar)* and saline *(open bar)* inhalation challenge.

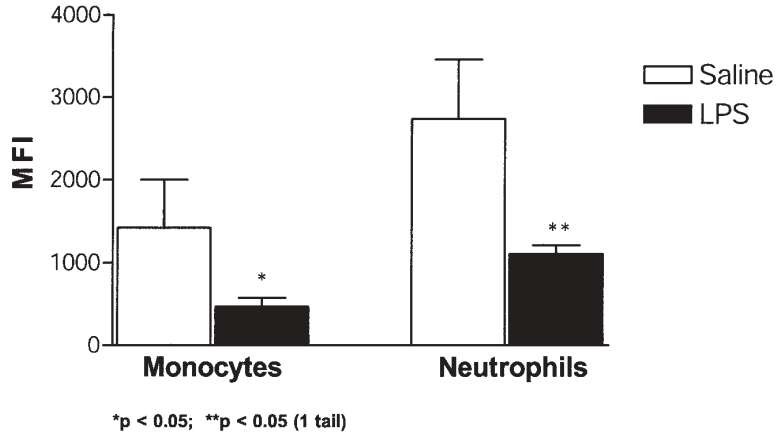

FIG 2. Mean (± SEM) phagocytosis of opsonized zymosan particles (MFI) of recovered circulating (blood) monocytes and neutrophils in atopic asthmatic individuals 6 hours after endotoxin *(closed bar)* and saline *(open bar)* inhalation challenge.

Phagocytic response of inflammatory cells recovered from sputum (n = 7) and peripheral blood (n = 10) to inhaled endotoxin

Macrophages, monocytes, and neutrophils recovered from the airway in 7 subjects showed decreased ability to phagocytose IgG-opsonized zymosan 6 hours after challenge with 5 μg of endotoxin compared with the results after saline challenge (Fig 1). Peripheral blood monocyte and neutrophil samples collected simultaneously from these subjects also showed blunted phagocytosis after endotoxin challenge (Fig 2). We also examined the relationship between the degree of the neutrophil response in the airway (percentage difference between sputum neutrophils per milligram after endotoxin and saline challenge) and phagocytosis activity of recovered airway macrophages and circulating monocytes (Fig 3). We observed similar significant positive correlations between the percentage increase in sputum PMNs per milligram and percentage decrease in sputum macrophage (R = –0.80, P = .05) and circulating monocyte (R = –0.75, P = .05) phagocytosis 6 hours after endotoxin challenge.

CD11b response to inhaled endotoxin

As an estimate of baseline cell-surface marker status, postsaline values were used for all cell types. As shown in Fig 4, we observed a significant correlation between endotoxin-induced PMN influx to the airway and CD11b expression on circulating monocytes (Fig 4, *A*) and neutrophils (Fig 4, *B*). However, there was no correlation between baseline airway cell CD11b expression and PMN influx to the airway after endotoxin challenge. In addition, we observed correlations between baseline eosinophils (cells per milligram of sputum) in sputum and CD11b expression on circulating monocytes (trend:

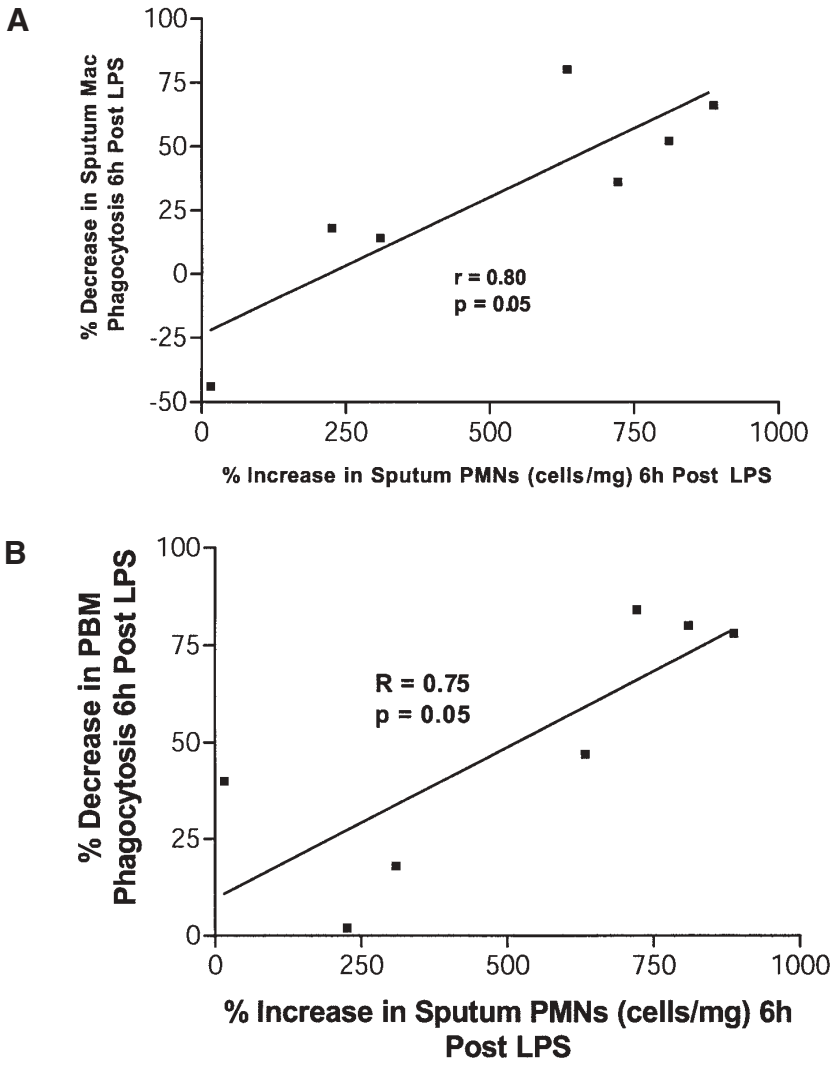

FIG 3. A, Relationship between degree of neutrophilic inflammation in the airways, expressed as percent change in sputum neutrophils (post-endotoxin–post-saline PMN counts in sputum) and the percent decrease in sputum macrophage phagocytosis (difference between post-endotoxin–post-saline × 100%) after endotoxin challenge in 7 atopic asthmatic individuals. **B,** Relationship between degree of neutrophilic inflammation in the airways, expressed as percent change in sputum neutrophils (post-endotoxin–post-saline PMN counts in sputum) and the percent decrease in peripheral blood monocyte *(PBM)* phagocytosis (difference between post-endotoxin–post saline × 100%) after endotoxin challenge in 7 atopic asthmatic individuals.

$r = 0.6$, $P = .10$) and neutrophils ($r = 0.75$, $P = .03$), as well as with the percentage of neutrophils in the airway after endotoxin challenge ($r = 0.8$, $P = .01$). Fig 5 shows that compared with saline, endotoxin caused a significant decrease in CD11b expression in circulating monocytes. Interestingly, when we obtained peripheral blood monocytes from volunteers for in vitro assessment of the effect of LPS, CD11b expression was increased.

mCD14 response to inhaled endotoxin

There was a significant increase in mCD14 expression (MFI) after endotoxin versus saline challenge on sputum monocytes (100 ± 12 vs 27 ± 5, $P < .05$) and a similar trend for sputum macrophages (49 ± 12 vs 6 ± 2, $P < .10$; Fig 6). Consistent with our original report,[16] we observed a significant correlation between mCD14 expression on airway macrophages and PMN influx after endotoxin challenge ($r = 0.7$, $P = .05$).

Sputum fluid-phase constituents (n = 8)

Airway sputum supernatants from 8 volunteers were adequate for assessment of GM-CSF, total protein, eosinophil cationic protein, myeloperoxidase, IL-6, IL-8, IL-1β, and sCD14, with no changes being observed between samples collected after saline and endotoxin inhalation challenge (Table III).

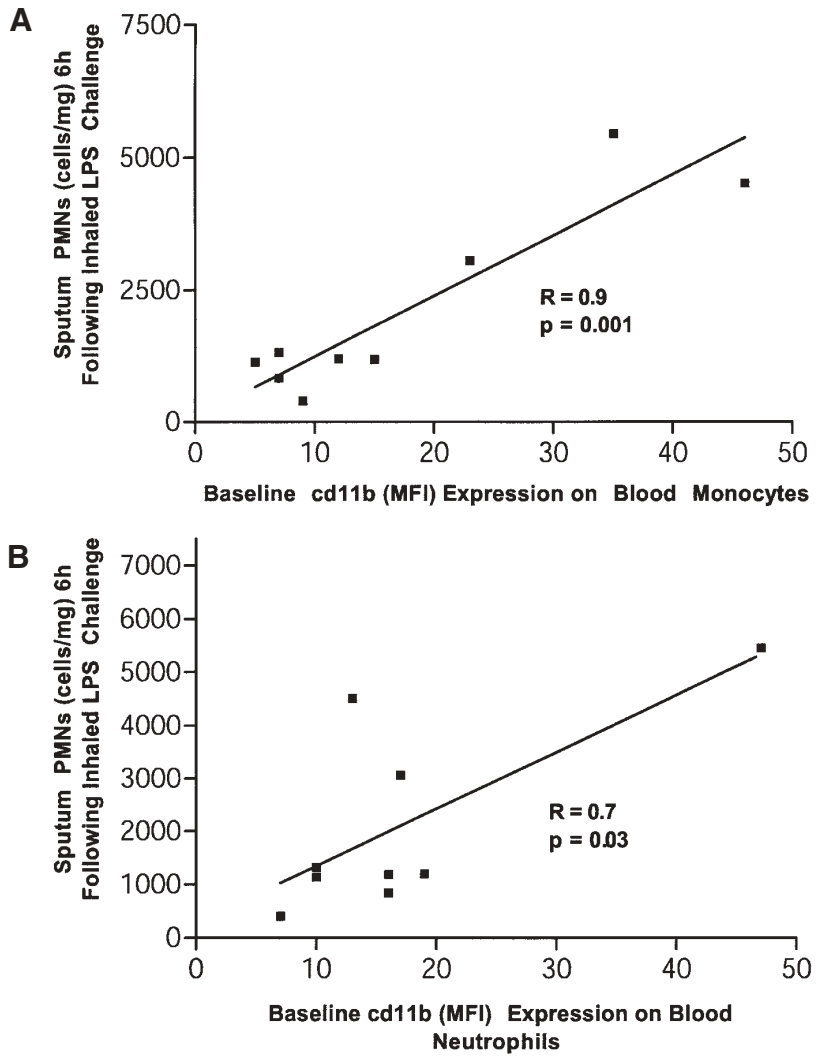

FIG 4. Relationship between constitutive CD11b expression (MFI) on blood monocytes **(A)** and neutrophils **(B)** and the degree of neutrophilic inflammation in the airways 6 hours after endotoxin inhalation challenge in 10 atopic asthmatic individuals. Spearman correlation coefficient: (R) = 0.9, *P* = .001 **(A)**; (R) = 0.7, *P* = .03 **(B)**.

TABLE III. Mean ± SEM postexposure values for fluid-phase constituents in 8 atopic asthmatic subjects

	GM-CSF (pg/mL)	TP (mg/mL)	ECP (ng/mL)	MPO (ng/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	IL-1β (pg/mL)	sCD14 (pg/mL)
After LPS	20 (9)	1.3 (0.3)	622 (204)	226 (93)	190 (42)	3195 (244)	387 (313)	22,870 (3682)
After saline	65 (26)	2 (0.8)	448 (180)	267 (128)	327 (187)	3348 (721)	196 (161)	31,390 (8537)

TP, Total protein; *ECP,* eosinophil cationic protein; *MPO,* myeloperoxidase.

DISCUSSION

We have previously reported that inhalation of 5 μg of endotoxin induces neutrophil influx, which correlates with airway sCD14 and mCD14 expression at baseline on airway macrophages in a cohort of 8 healthy and 10 asthmatic volunteers. In this initial report we also observed a correlation between baseline eosinophil numbers, baseline sCD14 expression, and neutrophil influx associated with endotoxin challenge.[16] These observations suggest that eosinophilic inflammation enhances immediate response to endotoxin through modification of CD14-facilitated signal transduction.

However, other processes in addition to those involving CD14 likely play a role in response to endotoxin. CD11b, through its dimerization with CD18, forms the CD11-CD18 complex (CR3) on neutrophils and monocytes. This complex is crucial to migration of phagocytes from the circulation to tissues, and abnormalities of the CD11b-CD18 complex are associated with significant infections.[19] In animal models treatment with neutralizing antibodies directed against CD11b-CD18 increases mortality caused

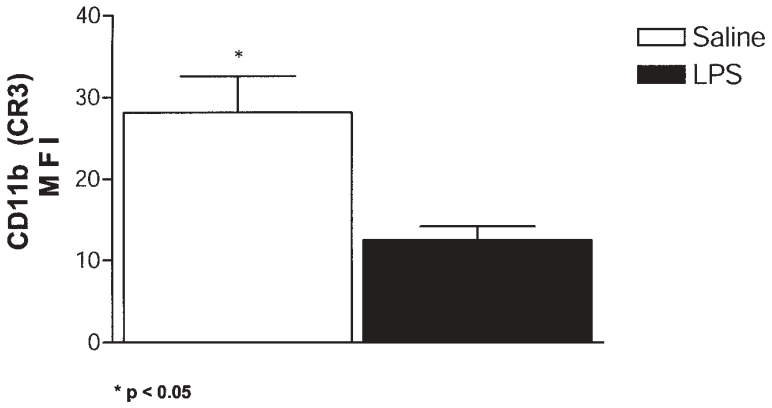

FIG 5. Mean (± SEM) CD11b expression (MFI) on blood monocytes in atopic asthmatic individuals 6 hours after inhaled endotoxin (LPS; *closed bar*) and saline *(open bar)* challenge.

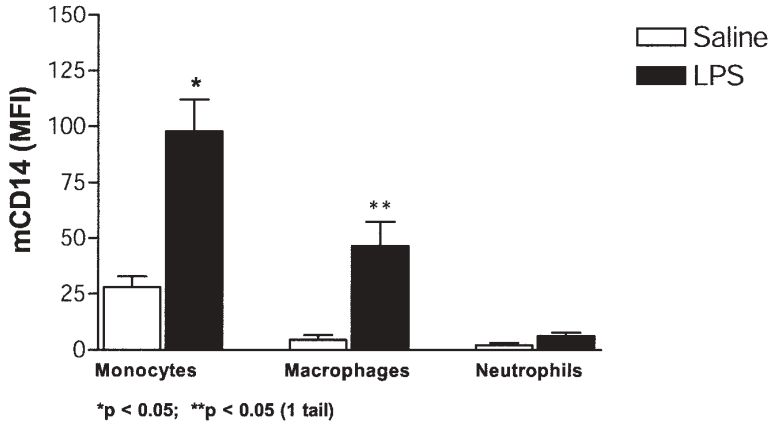

FIG 6. Mean (± SEM) mCD14 expression (MFI) on sputum monocytes, macrophages, and neutrophils in atopic asthmatic individuals 6 hours after inhaled endotoxin (LPS; *closed bar*) and saline *(open bar)* challenge.

by sepsis.[20] CD11-CD18 has also been shown to play a role in ARDS and trafficking of neutrophils to the airway.[4,5,21] It also appears that CD14 and CD11b-CD18 share a common signal transduction capability for the LPS component Lipid A, and they act together to allow phagocyte activation and binding to LPS-bound erythrocytes.[3,22] CD11b is also involved in complement-mediated phagocytosis.[6] These observations suggest that CD11b, like CD14, participates in endotoxin-induced airway inflammation and is induced on airway inflammatory cells after allergen challenge.[10,23]

In 10 atopic asthmatic volunteers we measured CD11b and mCD14 expression on airway and circulating phagocytes and phagocytosis of opsonized zymosan by airway and circulating phagocytes. We observed that mCD14 expression on airway macrophages, monocytes, and neutrophils was enhanced by endotoxin challenge, and CD11b expression on blood monocytes was decreased. We also observed that baseline CD11b expression on blood phagocytes correlated well with endotoxin-induced PMN influx. These observations suggest that CD11b is a determinant of the ability of monocytes and neutrophils to be activated and leave the circulation after endotoxin challenge. We also observed that the degree of baseline eosinophilic inflammation correlated to CD11b expression on blood neutrophils (with a trend for a correlation on monocytes), suggesting that ongoing allergic airway inflammation promotes expression of this molecule. We have also recently reported that asthmatic patients pretreated with inhaled corticosteroids demonstrate decreased airway eosinophilia along with decreased PMN response to endotoxin.[18] Thus CD11b in the circulation might be a marker of risk for endotoxin-induced exacerbation of asthma.

We assessed the ability of airway and circulating monocytes, macrophages, and neutrophils to phagocytose IgG-opsonized zymosan in airway phagocytes from 7 asthmatic volunteers and peripheral blood from all 10 asthmatic volunteers enrolled in the study. To our knowledge, this is the first study that examines the phagocyte activity in sputum monocytes, macrophages, and neutrophils in allergic asthmatic patients after inhaled endotoxin challenge. We

observed that inhalation challenge with 5 μg of endotoxin resulted in a decrease in airway macrophage, monocyte, and neutrophil phagocytosis capability when compared with that observed after saline challenge. We also observed that the change in the number of neutrophils present in the airway 6 hours after endotoxin compared with saline challenge correlated with the change in phagocytosis of zymosan by airway macrophages.

These results are consistent with those by Sandstrom et al[14] in which blunted phagocytosis was observed in cells recovered from bronchoalveolar lavage fluid from normal volunteers after challenge with a 20-fold greater dose of endotoxin than that used in this study. Others have reported that phagocytosis is blunted in circulating neutrophils recovered from patients with sepsis and from animals subjected to experimental sepsis.[24-26] It seems reasonable that inhaled endotoxin challenge might affect subsequent phagocyte function by the actions of LPS-induced cytokine release. IL-10 has been shown to blunt LPS-induced inflammation in animal studies[27] and inhibits pro-inflammatory cytokine production by both LPS-stimulated neutrophils and those undergoing phagocytosis.[28] It has also been shown that TNF-α and IL-1β, which are primary cytokine products of endotoxin-stimulated monocytes, will inhibit phagocytic responses of neutrophils.[29] Interestingly, in this study we observed a trend toward increased IL-1β levels after endotoxin challenge versus saline challenge that was not statistically significant.

The clinical significance of the association between the degree of increased airways inflammation and phagocytosis is not completely clear. Interestingly, it has been reported that endotoxin treatment of the airway before experimental bacterial infection enhances clearance of the bacteria from the airway.[30] However, it has also been suggested that although circulating phagocytes initially have an activated phase, this is followed by a later period of blunted phagocytic function in the context of systemic sepsis or endotoxinemia.[1,25,31] Likewise, there is evidence that apoptotic neutrophils and IL-10 will decrease monocyte and macrophage activity.[32] IL-10 has also been reported to play a role in alveolar macrophage deactivation in experimental bacterial peritonitis.[33] These observations suggest that ongoing acute inflammation blunts macrophage and monocyte activity, perhaps through the action of IL-10, supporting our hypothesis that recruitment of activated neutrophils greatly modifies the local airway milieu in a way that negatively affects phagocytosis.

Although asthmatic patients are not known clinically to have increased occurrence of bacterial airway infections, there is an increase in bacterial sinusitis in persons with allergic upper airway disease, suggesting that T_H2 inflammation might blunt local phagocytic responses.[34] A more extreme example of this might lie in hyper-IgE syndrome, in which persons with markedly increased IgE and peripheral eosinophilia have increased airway and skin infections and have been shown to have a defect in neutrophil chemotaxis and circulating monocyte function.[35,36] The potential for markedly advanced T_H2 inflammation to impair phagocyte function is consistent with both our observations and those of Michel et al[37] regarding response of atopic subjects to inhaled endotoxin.

In summary, macrophages are the first cells of the airway to encounter bioaerosols that contain endotoxin and bacteria. Airway and circulating monocyte and macrophage cytokine responses to endotoxin are enhanced in asthmatic patients when compared with those in nonasthmatic patients,[38] and any modification of the function of these cells by allergic inflammation might have significant effects on their response to pollutants and pathogens. Given the interaction between allergen- and endotoxin-mediated responses in the airway, continued study of the biology of airway cells and the modulation of airway inflammation in allergic asthmatic patients is needed to further understand the effect of environmental endotoxin in asthma. This study is a first step in examining the effect of lower levels of endotoxin that might prime or otherwise modify the airway in asthmatic subjects. In addition, future research will examine the effect of allergen challenge in response to endotoxin and whether asthmatic subjects respond differently than nonasthmatic subjects to repeated challenge with endotoxin.

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