

Association between airway hyperreactivity and bronchial macrophage dysfunction in individuals with mild asthma

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Alexis, Neil E., Joleen Soukup, Stefan Nierkens, and Susanne Becker. Association between airway hyperreactivity and bronchial macrophage dysfunction in individuals with mild asthma. *Am J Physiol Lung Cell Mol Physiol* 280: L369–L375, 2001.—Little is known about the functional capabilities of bronchial macrophages (BMs) and their relationship to airway disease such as asthma. We hypothesize that BMs from asthmatics may be modulated in their function compared with similar cells from healthy individuals. BMs obtained by induced sputum from mild asthmatics ($n = 20$) and healthy individuals ($n = 20$) were analyzed using flow cytometry for CD16, CD64, CD11b, CD14, and human leukocyte antigen-DR expression, phagocytosis of IgG opsonized yeast, and oxidant production. Asthma status was assessed by lung function [percent predicted forced vital capacity and forced expiratory volume in 1 s (FEV_1)], percent sputum eosinophils, and nonspecific airway responsiveness [provocative concentration that produces a 20% fall in FEV_1 (PC_{20,FEV_1})]. Asthmatics with >5% airway eosinophils (AEo+) had decreased BM CD64 expression and phagocytosis compared with asthmatics with <5% eosinophils (AEo−). Among asthmatics, a significant correlation was found between CD64 expression and BM phagocytosis ($R = 0.7$, $P < 0.009$). Phagocytosis was also correlated with PC_{20,FEV_1} ($R = 0.6$, $P < 0.007$), lung function (%predicted FEV_1 , $R = 0.7$, $P < 0.002$) and percent eosinophils ($R = -0.6$, $P < 0.01$). In conclusion, BM from asthmatics are functionally modulated, possibly by Th2 cytokines involved in asthma pathology.

asthma; bronchial macrophages; induced sputum; flow cytometry analysis of surface receptors; CD64; CD11b; phagocytosis

THE IMMUNOPATHOLOGY OF ALLERGIC asthma is characterized by peribronchial infiltration of macrophages, eosinophils, and T cells in stable and symptomatic periods of disease (2, 5, 37, 39). These cells may all be functionally involved in asthma symptomatology. Among them, macrophages are critical in removing inhaled particulates and microbes that have the potential to exacerbate asthma (6, 11, 43). Previous studies have suggested a link between decreased phagocytic function and asthma (26, 27) as well as enhanced superoxide generation (10, 20) and mediator release

(31) by airway cells in asthmatics. These studies, however, examined peripheral blood phagocytes, not bronchial macrophages (BMs) proximal to the site of disease pathology. T cells at the site of allergic-asthmatic disease have been shown to preferentially produce the cytokines interleukin (IL)-4 and IL-5 (31). IL-4 has been shown to modulate surface receptors on macrophages in vitro (3), while IL-5 promotes the survival and activation of eosinophils both in vivo and in vitro (1). Among the receptors modulated by IL-4 are human leukocyte antigen (HLA)-DR and the phagocytic receptor FcγRI, i.e., CD64. HLA-DR expression is increased while CD64 expression is decreased following exposure to IL-4 (3). Decreased expression of CD64 and CD11b is associated with decreased phagocytosis (3, 53). On the other hand, exposure to interferon-γ (IFN-γ), also found to be increased in asthmatics (38), upregulates CD64 and HLA-DR while decreasing CD11b expression and phagocytosis (3).

Induced sputum has proven to be a safe and relatively noninvasive procedure that obtains cells and fluid phase components from the bronchial airways (33–35). As a result, induced sputum is well suited to examine bronchial airway cells from asthmatic subjects. Sputum eosinophilia, described cytologically as >2–5% eosinophils (18, 22), can serve as a reliable inflammatory marker of asthma severity in asthmatics with airway eosinophilia (32, 46, 49). However, many asthmatics do not have airway eosinophilia even following acute exacerbations (18, 23, 47). Asthmatics with eosinophilic sputum have been reported to have increased symptoms of coughing and wheezing, greater airway obstruction, and more severe airway hyperresponsiveness compared with asthmatics without sputum eosinophilia (18). Few studies have attempted to investigate the characteristics and functions of cells obtained by the induced sputum technique (19). In this study, we wanted to determine whether, in mild atopic asthmatics, lung function and sputum eosinophilia, which have been shown to be associated, also correlate with modulated macrophage phenotype and function. Flow cytometry was used to assess the expression of

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cell surface receptors known to be modulated by environmental factors as well as by phagocytosis in 20 mild asthmatic subjects and 20 controls.

The data obtained suggest an intriguing correlation between eosinophilic inflammation, lung function, and decreased BM function, associated with decreased CD64 expression, in subjects with mild asthma.

METHODS

Subjects. Subject characteristics are shown in Table 1. Nonsmoking volunteers between 18 and 40 yr of age were recruited for the study. A medical screening exam that included a medical history, psychological questionnaire, physical exam, blood tests, and allergy scratch tests was performed on all subjects on a separate day before the study. All asthmatics and 10 healthy subjects received a methacholine challenge test to assess nonspecific airway responsiveness. The maximum concentration of methacholine used was 40 mg/ml. Seven of ten healthy subjects were able to achieve a nonspecific airway responsiveness [provocative concentration that produces a 20% fall (PC_{20}) in forced expiratory volume in 1 s (FEV_1 ; PC_{20,FEV_1})] by the maximum concentration. Pulmonary function tests were performed between 8:00 and 9:00 AM on all subjects, and with the exception of one subject, none of the asthmatics had taken any β_2 -agonist within 24 h of pulmonary function testing.

A total of 20 healthy and 20 nonsmoking mild, well-controlled atopic asthmatic subjects underwent successful sputum induction. Healthy subjects had no history of asthma or allergic disease and were free of any symptoms of acute respiratory illness for at least 4 wk. They had a ratio of FEV_1 to forced vital capacity (FVC; FEV_1/FVC) $\geq 75\%$, as well as FVC and $FEV_1 > 90\%$ of predicted normal values. Healthy subjects who underwent methacholine challenge and achieved a PC_{20,FEV_1} ($n = 7$) had a geometric mean (range) $PC_{20,FEV_1} = 11.9$ mg/ml (5–25 mg/ml) (Table 1). One healthy subject had a PC_{20} in the asthmatic range (< 8 mg/ml) of 5.0 mg/ml. Three healthy subjects who did not reach a PC_{20,FEV_1} were not included in the mean analysis for PC_{20,FEV_1} .

Mild asthmatic subjects were limited to persons who met the National Heart, Lung, and Blood Institute (12) criteria for mild asthma. Subjects had a positive asthma history (wheezing, chest tightness, and reversible airflow obstruction) diagnosed by their family physician and confirmed by our study physician and a bronchodilator-withheld FEV_1 /

FVC $> 60\%$. With one exception, all subjects demonstrated nonspecific bronchial responsiveness to methacholine (defined as a minimum of a 20% decrement in FEV_1 , above that of a diluent alone) after inhaling ≤ 8 mg/ml of aerosolized methacholine. Subjects had to be free of oral corticosteroid treatment within the previous 6 mo, no hospital admissions for asthma within the previous year, and be able to withhold antihistamines for 48 h before being tested. Except for asthma, subjects had to be in good general health, with no cardiovascular disease or acute respiratory illness within 4 wk. All seasonal atopic asthmatics were studied outside their allergy season, and none were taking cromolyn or leukotriene medicines. Both healthy and asthmatic subjects were asked to refrain from taking any anti-inflammatory medication 48 h prior to study. All asthmatic subjects used inhaled bronchodilators on an as needed basis, and no asthmatic subjects used inhaled steroids within the previous 6 mo. This study was approved by the Committee on the Protection of the Rights of Human Subjects at the University of North Carolina, Chapel Hill.

Induced sputum. The induction procedure of Pin et al. (32) was followed with some modifications. Three 7-min inhalation periods of 3, 4, and 5% hypertonic saline were administered following baseline spirometry. All asthmatics were premedicated with inhaled β_2 -agonist before saline inhalation, and FEV_1 was checked following each saline dose. At the end of each 7-min inhalation period, subjects performed a three-step cleansing procedure before a cough attempt as follows: 1) the mouth was rinsed and gargled with water, 2) the back of the throat was cleared (without coughing), and 3) the nose was blown. 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 according to the method of Pizzichini et al. (34). In brief, mucus plugs were manually selected and weighed, incubated (15 min at room temperature) in four times the weight (in μ l) of the selected plug (in mg) in 0.1% dithiothreitol (Calbiochem, San Diego, CA), washed with four times the plug weight (in μ l) in Dulbecco's PBS, and gravity filtered through a 48- μ m-pore mesh filter (BBSH Thompson, Scarborough, Ontario). Total cell counts were performed with the use of a Neubauer hemocytometer. Visually identifiable squamous epithelial cells were not counted or included in the total cell count. Cell viability was determined using trypan blue exclusion stain-

Table 1. Subject characteristics and baseline lung function

Subject	Age, yr	Sex	Atopy	β_2 -Agonist Use	Inhaled Steroid Use in Past 6 Months	%Predicted FEV_1	%Predicted FVC	PC_{20,FEV_1} , mg/ml
Control	27 $\pm$ 2.0	M = 13 F = 7	0	0	None	112 $\pm$ 2.0	116 $\pm$ 2.0	11.9 (5–24) (n = 7)
Asthmatic	27.2	M = 14 F = 6	20	20	None	104 $\pm$ 2.0 ^a	109 $\pm$ 1.6 ^a	1.48 ^b (0.02–20) (n = 20)
AEo+	28 $\pm$ 1.0	M = 7 F = 2	9	9	None	101 $\pm$ 2.0 ^c	106 $\pm$ 1.0 ^d	0.4 ^e (0.02–3.9) (n = 9)
AEo–	27 $\pm$ 2.0	M = 7 F = 4	11	11	None	108 $\pm$ 2.0	113 $\pm$ 2.0	2.3 ^a (0.2–20) (n = 11)

Values are means $\pm$ SE for age, %predicted forced expiratory volume in 1 s (FEV_1), and %predicted forced vital capacity (FVC) and are geometric mean (range) for nonspecific airway responsiveness [provocative concentration that produces a 20% fall in FEV_1 (PC_{20,FEV_1})]. M, male; F, female; AEo+, eosinophilic; AEo–, noneosinophilic. ^a $P < 0.05$, significantly different from control. ^b $P < 0.001$, significantly different from control. ^c $P < 0.003$, significantly different from AEo–. ^d $P < 0.002$, significantly different from AEo–. ^e $P < 0.001$, significantly different from control and AEo–.

ing. Differential leukocyte analysis of nonsquamous cells (Diff-Quik stained) was performed on a minimum of 400 cells, provided that squamous cells were <40% of the total cells. Differential cell counts (lymphocytes, neutrophils, eosinophils, monocytes, and macrophages) are expressed as a percentage of total nonsquamous nucleated cells.

Analysis of fluid-phase cytokines and eosinophil cationic protein. Supernatants from the soluble fraction of the spun-down sputum sample were collected and analyzed for IL-4, IL-5, IL-8, IFN- γ , and eosinophil cationic protein (ECP). Cytokines were measured by specific and sensitive human immunoassays (ELISA, Quantikine; R&D Systems, Minneapolis MN). The lower limits of detection for IL-4, IL-5, IL-8, and IFN- γ were as follows: 35, 3, 10, and 3 pg/ml, respectively. ECP levels in sputum supernatant were measured by means of specific radioimmunoassay (Pharmacia RIA, Uppsala, Sweden). This method has been proven highly specific and sensitive (lower detection limit <2 μ g/l), with high intra- and interassay reproducibility.

Flow cytometry. Flow cytometry was performed with a FACSORT (Becton Dickinson) using an argon-ion laser (wavelength = 488 nm). Gain and amplitude settings were set so as to analyze both blood and sputum samples from the same subject to establish reference gates for sputum leukocyte identification. Settings were kept the same throughout the study for each subject. The FACSORT was calibrated with Calibrite (Becton Dickinson) beads (no color, green, and red) before each use. Ten thousand events were counted for all sample runs. Gating of white cells in sputum was based on a combination of light-scatter characteristics and positive/negative expression for relevant surface markers, i.e., lymphocytes CD3⁺/CD14⁻, monocytes CD14⁺/CD3⁻, neutrophils CD16⁺/HLA-DR⁻, macrophages HLA-DR⁺/CD14⁺, and the use of reference gates based on isolated (Percoll-separated) leukocytes from whole blood preparation. Side light scatter reflects cell density/granularity and forward light scatter reflects cell size (41). Using these techniques, discrete populations of lymphocytes, monocytes, neutrophils, macrophages, and eosinophils were observed. Fluorescein (FITC)- and phycoerythrin-conjugated nonspecific antibodies of the same isotype as the receptor antibodies were used as controls to establish background fluorescence and nonspecific antibody binding. The mean fluorescence intensity (MFI) of the cells stained with control antibody was subtracted from the mean fluorescence of the cells stained with receptor antibodies to provide a measure of receptor-specific fluorescence.

Immunofluorescence staining. Aliquots of 100 μ l (100,000 cells/tube) or 200 μ l (200,000 cells/tube) of sputum cell suspension (1×10^6 cells/ml) were stained with 10 or 20 μ l, respectively, of saturating concentrations of monoclonal antibodies (Immunotech, Coulter, France) for 60 min in the dark at 4°C. After they were stained, the cells were washed with 2 ml of cold Hanks' balanced salt solution (HBSS) and centrifuged for 5 min at 1,000 rpm at 4°C, and supernatants were decanted and blotted. The cells were then resuspended in cold HBSS (250 μ l) and fixed with paraformaldehyde (250

μ l, 0.5%) for a final volume of 500 μ l. The cells were then stored at 4°C in the dark until analyzed on the flow cytometer within 24 h of staining. The following monoclonal antibodies were used: CD11b to recognize the complement receptor (CR3), CD64 to recognize the Fc γ RI receptor, and CD45 to recognize all white cells. Measurement of surface marker expression was done using a Becton Dickinson FACSORT flow cytometer. Analysis of surface marker expression was done using the Cell Quest software (Becton Dickinson), which provided a calculation of MFI for the gated populations.

Phagocytosis. *Saccharomyces cerevisiae* zymosan A Bio-Particles (Molecular Probes, Eugene, OR) conjugated to FITC were opsonized with opsonizing reagent (IgG) for 45 min at 37°C and then washed with RPMI 1640 medium two times before the particle concentration was adjusted to 2×10^6 /ml. 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 h at 37°C in the presence of human serum (20 μ l) before tubes were placed on ice. Next, 200 μ l of 2% paraformaldehyde were added to each tube, and the tubes were stored at 4°C in the dark until analyzed by flow cytometry (FACSORT) within 24 h of particle exposure. Particle uptake was identified on histogram analysis as a uniform rightward shift into the M2 region (MFI) from the control (non-particle-exposed) M1 region. Phagocytosis was determined by measuring the MFI of the cells in the particle-exposed region (M2) less the MFI of the cells in the control region (M1). A uniform shift in all sputum phagocyte populations was routinely observed when cells were exposed to particles such that at least 93% of the cells shifted (with particle exposure) into M2 compared with M1 (no particle exposure) on all occasions.

Statistical analyses. Statistically significant differences between subject groups were assessed by one-way ANOVA. Parametric statistics were applied on log-transformed PC20 data for tests of differences between means and for correlations. Simple linear regression analyses were performed to analyze associations between variables, and Pearson's correlation coefficients (*R*) were determined from these analyses. A value of *P* < 0.05 was considered statistically significant.

RESULTS

Characterization of subjects, their lung function, and sputum differentials. Subject characteristics including baseline lung function and nonspecific airway responsiveness are shown in Table 1. All the asthmatics examined had mild, well-controlled disease, i.e., inhaled bronchodilator was used as needed, no inhaled corticosteroids were taken within the previous 6 mo, and a normal range spirometry was present. Sputum eosinophil counts are shown in Table 2. Asthmatic subjects with 5% or greater sputum eosinophils were classified as eosinophilic (AEo+; *n* = 9), and asthmatic subjects with <5% sputum eosinophils were classified

Table 2. Total and differential cell counts

Subject	Total Cells/mg Sputum	Viability, %	Sputum, %PMN	Sputum, %MAC	Sputum, %EOS	Sputum, %LYM	Blood, %EOS
Control	5,128 $\pm$ 640.0	77 $\pm$ 2.0	37 $\pm$ 3.0	56 $\pm$ 3.0	1 $\pm$ 0.3	2 $\pm$ 0.4	2 $\pm$ 0.3
AEo+	4,780 $\pm$ 1,193.0	76 $\pm$ 3.0	18 $\pm$ 4.0*	47 $\pm$ 3.0†	32 $\pm$ 3.0	2 $\pm$ 0.6	4.2 $\pm$ 0.7‡
AEo-	3,081 $\pm$ 563.0	79 $\pm$ 2.0	31 $\pm$ 5.0	62 $\pm$ 5.0	2 $\pm$ 0.4	3 $\pm$ 0.6	1.9 $\pm$ 0.3

Values are means $\pm$ SE. PMN, polymorphonuclear neutrophils; MAC, macrophages; EOS, eosinophils; LYM, lymphocytes. **P* < 0.05, significantly different from control. †*P* < 0.05, significantly different from AEo-. ‡*P* < 0.004, significantly different from AEo-.

as noneosinophilic (AEo-; $n = 11$). The arbitrary 5% cutoff for designating eosinophilia was based on prior reports describing sputum eosinophilia in asthmatics (33, 18, 22, 46, 47). Asthmatics demonstrated lower baseline lung function (%predicted FVC, %predicted FEV₁) and higher nonspecific airway responsiveness (PC_{20,FEV1}) than healthy controls, and AEo+ subjects demonstrated lower baseline lung function, higher nonspecific airway responsiveness, and greater peripheral blood eosinophils than AEo- subjects. The AEo- subjects demonstrated significantly greater nonspecific airway responsiveness than healthy controls (PC_{20,FEV1} = 2.3 vs. 11.9 mg/ml, $P < 0.05$), verifying their mild asthma status. Significant inverse relationships were observed (data not shown) between the percentage of sputum eosinophils and the percentage of predicted FEV₁ ($R = -0.5$, $P < 0.001$), FVC ($R = -0.5$, $P < 0.001$) and PC_{20,FEV1} ($R = -0.5$, $P < 0.002$) for all asthmatics.

Sputum differential and total cell counts (TCC) and cell viability are shown in Table 2. All subjects were able to successfully produce an acceptable sputum sample. The samples contained <40% squamous cells and had >60% cell viability based on trypan blue exclusion staining. No difference in TCC was observed between the AEo+ and AEo- subjects, and there was no significant difference in the weight of the selected sputum portion (in mg) between the AEo+ and AEo- subjects (940 ± 260 vs. $1,069 \pm 269$ mg, $P < 0.2$).

Flow cytometric analysis of induced sputum samples. The flow cytometer was used to quantify the expression of CD11b, CD14, CD16, CD64, and HLA-DR on BM. The identification of the BM population was easily achieved by the light-scatter properties (forward scatter and side scatter) of the population with no overlap with neutrophils or monocytes. Figure 1 shows the

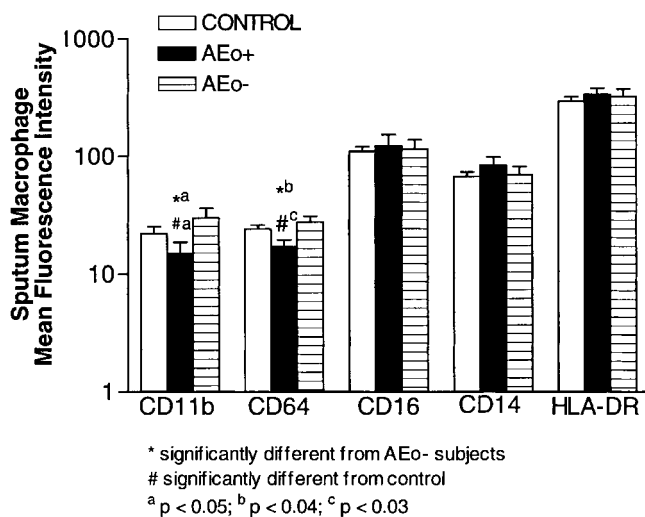


Fig. 1. Expression of surface markers expressed as mean fluorescence intensity on sputum macrophages in healthy controls, eosinophilic asthmatics (AEo+), and noneosinophilic asthmatics (AEo-). Compared with controls and AEo- subjects, AEo+ subjects showed decreased expression of CD11b (CR3) and CD64 (FcR γ I). No differences were observed in CD16 (FcR γ III), CD14 (lipopolysaccharide receptor), and human leukocyte antigen-DR (major histocompatibility complex class II).

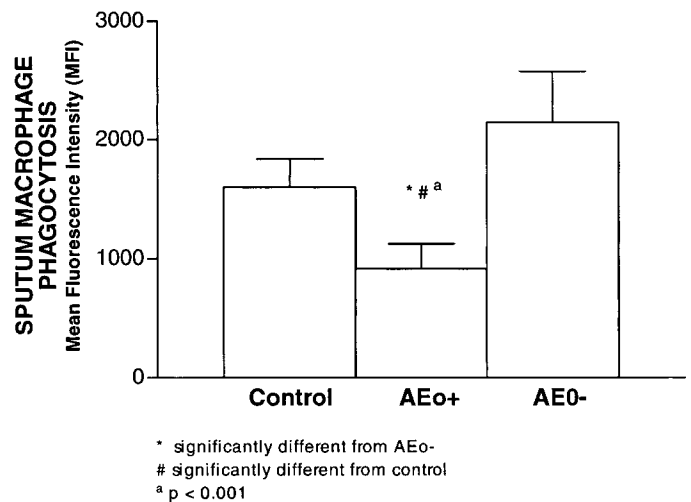


Fig. 2. Sputum macrophage phagocytosis of IgG opsonized yeast (in the presence of human serum) expressed as mean fluorescence intensity (MFI) in healthy controls, eosinophilic asthmatics (AEo+) and noneosinophilic asthmatics (AEo-). Compared with healthy controls and AEo- subjects, AEo+ subjects demonstrated decreased phagocytosis. *Significantly different from AEo- subjects; #significantly different from control; $P < 0.01$.

results of surface marker expression of CD11b, CD64, CD16, CD14, and HLA-DR on BMs in healthy and asthmatic AEo+ and AEo- subjects. If all the mild asthmatics were analyzed as one group, there was no difference between healthy and asthmatic subjects for any of the markers. When subdivided into AEo+ and AEo- asthmatics, however, Fc γ RI (CD64, $P < 0.04$) and CR3 (CD11b, $P < 0.05$) expression (MFI) were significantly decreased in AEo+ subjects compared with the AEo- subjects and controls (CD64, $P < 0.03$; CD11b, $P < 0.05$). Expression of CD16, CD14, and HLA-DR on BMs was not altered in subjects with eosinophilic inflammation.

Sputum macrophage phagocytosis of IgG-opsonized yeast particles. No difference was observed in the percentage of BM-phagocytosing particles between asthmatics and controls or between asthmatic subgroups. More than 94% of BMs took up particles in all subjects.

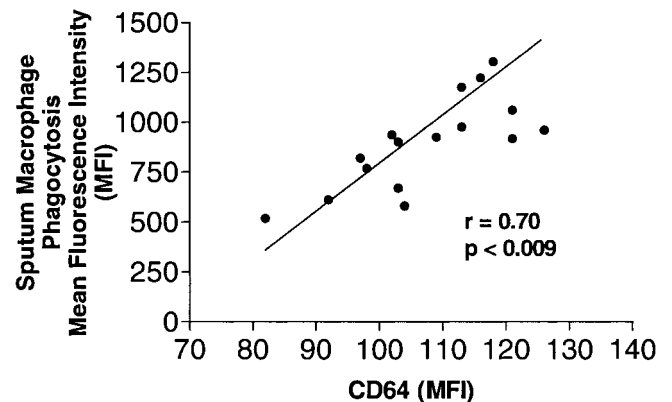


Fig. 3. Linear regression analysis of sputum macrophage phagocytosis (expressed as mean fluorescence intensity) and CD64 (FcR γ I) expression on sputum macrophages in all asthmatics. A significant ($P = 0.009$) positive linear correlation ($R = 0.7$) was observed between phagocytosis and CD64 expression.

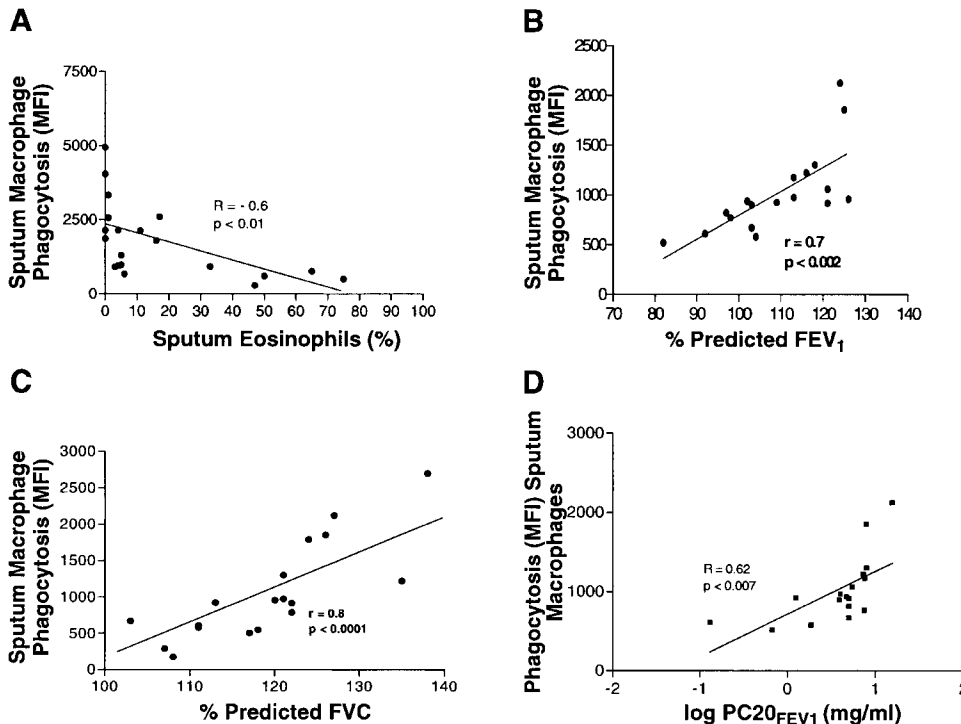


Fig. 4. A: Linear regression analysis of sputum macrophage phagocytosis (expressed as mean fluorescence intensity) and sputum eosinophils (%) in all asthmatics. A significant ($P = 0.01$) negative correlation ($R = -0.6$) was observed between phagocytosis and percent of sputum eosinophils. B and C: linear regression analyses of sputum macrophage phagocytosis (MFI) and %predicted forced expiratory volume in 1 s (FEV_1 ; B) and forced vital capacity (FVC; C) in all asthmatics. Significant positive linear correlations were observed between sputum macrophage phagocytosis and %predicted FEV_1 ($R = 0.7$, $P < 0.002$) and FVC ($R = 0.8$, $P < 0.0001$). D: linear regression analysis of sputum macrophage phagocytosis (MFI) and nonspecific airway responsiveness [provocative concentration that produces a 20% fall in FEV_1 (PC_{20,FEV_1})] in all asthmatics. A significant ($P < 0.007$) positive correlation ($R = 0.6$) was observed between sputum macrophage phagocytosis and PC_{20,FEV_1} .

When the number of particles taken up by the BM was measured as MFI of the BM population, AEO+ subjects showed significantly decreased phagocytosis compared with the AEO- subjects ($P < 0.001$) and controls ($P < 0.001$) (Fig. 2). BM phagocytosis was significantly related to the expression of $Fc\gamma RI$ (CD64; $R = 0.7$, $P < 0.01$; Fig. 3).

Association between sputum macrophage phagocytosis and markers of asthma severity. Sputum macrophage phagocytosis appeared to be predictive of disease severity. Significant correlations were observed between sputum macrophage phagocytosis and markers of disease severity, such as percentage of eosinophils ($R = -0.6$, $P < 0.01$; Fig. 4A), percentage of predicted FEV_1 ($R = 0.7$, $P < 0.002$; Fig. 4B), percentage of predicted FVC ($R = 0.8$, $P < 0.0001$; Fig. 4C), and PC_{20,FEV_1} ($R = 0.6$, $P < 0.007$), when all asthmatics were included.

Fluid-phase components. The subjects with eosinophilia (AEO+) demonstrated significantly higher levels of ECP compared with the AEO- subjects ($P < 0.05$) and healthy controls ($P < 0.001$), and ECP levels were significantly related to the percentage of eosinophils in all asthmatic subjects ($R = 0.7$, $P < 0.001$; data not shown) (Table 3). Cytokines IL-5 and IL-8 were detect-

able in airway fluid but did not differ between AEO+ and AEO- subjects. IL-4 was not detectable in the same samples.

DISCUSSION

This study has analyzed the phenotype and function of BMs obtained from induced sputum from mild asthmatic and healthy subjects. Based on their composition of airway leukocytes, the asthmatic subjects could be separated into those with airway eosinophilia as defined by the presence of $>5\%$ sputum eosinophils (mean = 32%) and those with noneosinophilic sputum (mean = 2%). The assessment of asthma severity by nonspecific bronchial responsiveness, baseline pulmonary function, and ECP levels supported the notion that increased levels of eosinophilic inflammation is associated with increased airway responsiveness and therefore disease severity (22). Accordingly, the AEO+ subject group was hyperreactive compared with the AEO- subject group. Our results showed that BMs from AEO+ subjects have decreased phagocytic function and decreased expression of $Fc\gamma RI$ (CD64) and CR3 (CD11b) compared with AEO- subjects; however, as one group, the mild asthmatics did not differ from

Table 3. Fluid-phase components in sputum

Subject	IL-4	IL-5	IL-6	IL-8	IL-10	ECP	IFN- γ
Control	ND	51 $\pm$ 4.0	204 $\pm$ 42.0	1,915 $\pm$ 1,587.0	167 $\pm$ 12.0	284 $\pm$ 73.0	98 $\pm$ 10.0
AEO+	ND	50 $\pm$ 7.0	258 $\pm$ 90.0	774 $\pm$ 761.0	204 $\pm$ 42.0	893 $\pm$ 85.0*	97 $\pm$ 11.0†
AEO-	ND	39 $\pm$ 5.0	603 $\pm$ 293.0	2,171 $\pm$ 1,743.0	180 $\pm$ 27.0	539 $\pm$ 127.0	65 $\pm$ 7.0‡

Values are means $\pm$ SE in pg/ml. IL, interleukin; IFN- γ , interferon- γ ; ECP, eosinophil cationic protein. ND, not detectable. *Significantly different from control ($P < 0.001$) and from AEO- ($P < 0.05$). †Significantly different from AEO- ($P < 0.01$). ‡Significantly different from control ($P < 0.05$).

normal subjects. This is the first study to demonstrate that macrophages obtained from a site proximal to asthma pathology are phenotypically and functionally altered. Earlier observations on peripheral blood phagocytes from subjects with asthma and chronic bronchitis have suggested that phagocytic dysfunction may also be present in the circulation of asthmatic individuals (26–28).

Impaired phagocytosis by BMs was found to be associated with cellular and spirometric markers of asthma severity. Relatively strong positive correlations were observed between sputum macrophage phagocytosis and percent predicted FEV₁ ($R = 0.7$), FVC ($R = 0.8$) and PC_{20,FEV1} ($R = 0.8$), and an inverse correlation was observed between sputum macrophage phagocytosis and sputum eosinophils ($R = -0.6$). Although it is unlikely that eosinophils directly inhibit macrophage phagocytosis or that phagocytosis directly impacts lung volumes, these correlations do not likely represent an epiphenomenon, but rather the pathogenesis of asthma severity may be related to the ability of the airways to mount a basic host defense response.

We hypothesized that changes in BM phenotype and function were the result of the cellular environment in asthmatic airways. T cells in allergic asthma preferentially produce Th2-type cytokines including IL-4 and IL-5, and cytokine-producing cells can be identified in the mucosa of asthmatic individuals and in bronchoalveolar lavage fluid following allergen provocation (9, 31, 42, 44). In vitro experiments have shown that small concentrations of IL-4 (10 pg/ml) can significantly downregulate the expression of CD64 and inhibit FcγR-mediated phagocytosis by human mononuclear phagocytes (42). Unfortunately, the attempts to measure IL-4 in the sputum supernatant were unsuccessful because most measurements were below the lower limit of detection of the immunoassay (35 pg/ml). Other investigators have also reported difficulty in recovering IL-4 in sputum supernatant (23). Even spike experiments with exogenous purified IL-4 have yielded poor results, suggesting that the sputum contains components interfering with IL-4 immunoreactivity. On the other hand, both IL-4 and IL-5 mRNAs have been detected in the sputum cells of asthmatic subjects, and the number of cells expressing these cytokines was significantly higher in asthmatics than in controls (31). In the present study, immunoreactive IL-5, IL-8, and IFN-γ could be detected in the sputum samples of most of the subjects, but no difference was found in the levels of cytokines between normal subjects and asthmatics or, with the exception of IFN-γ, between the AEo+ and AEo- groups. While induced sputum is a good technique that gives reproducible and valid results on inflammatory cells and most soluble markers of inflammation (IL-8, ECP) (13, 17, 31a, 34, 50), it may not recover cytokines (IL-4, IL-5) that are known to be synthesized and secreted by cells predominantly found in the airway wall, i.e., lymphocytes (14, 25, 54).

The data showed that, along with decreased FcγRI expression, BMs from AEo+ subjects also demonstrated significantly decreased CR3 (CD11b) expression. Since these two receptors interact in the phago-

cytic process (4, 48, 52), the decrease in uptake of opsonized yeast may be the result of decreased expression of both these receptors. What then may be the significance of decreased BM phagocytic function in asthmatic individuals? They are not known to have increased fungal or bacterial airway infections. However, many microbes, both opportunistic pathogenic and nonpathogenic organisms, are frequently found in asthmatic airways where their presence may lead to increased asthma severity (7). The presence of protease-producing fungi has been shown to lead to asthma symptoms, and histamine release induced by bacteria exacerbates the disease (21, 29). *Aspergillus fumigatus* is an opportunistic pathogen to which asthmatic subjects are particularly susceptible (21). Furthermore, decreased microbial clearance due to impaired bronchial macrophage phagocytosis may require the involvement of neutrophil inflammation to resist subsequent infection. This process may, in turn, exacerbate asthma. Interestingly, some of studies have implicated bacterial infections as causative in the development of asthma severity (30).

In conclusion, this study has demonstrated that, in a population of mild asthmatics, eosinophil inflammation, airway function, and macrophage function and phenotype are interrelated. How these markers of asthma severity link to and affect each other is still an open question.

This study has been reviewed by the National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, and mention of trade names and commercial products does not constitute endorsement or recommendation for use.

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