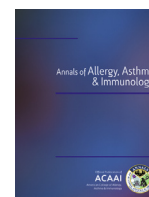




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Neutrophil-like low-density granulocytes are elevated in patients with moderate to severe persistent asthma

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ABSTRACT

Background: Elevations in neutrophil-like low-density granulocytes (LDGs) are observed in association with disease severity in some autoimmune and other disorders. This study evaluated whether a similar association with disease severity is observed in asthma.

Objective: To determine LDG levels in peripheral blood mononuclear cells of subjects with intermittent or mild persistent asthma, subjects with moderate persistent or severe persistent (SP) asthma, and control subjects without a history of allergy or asthma.

Methods: A brief medical history and physical examination, spirometry, and measurement of fraction of exhaled nitric oxide were performed. The LDGs were quantified by polychromatic flow cytometry.

Results: The LDGs displaying the same phenotype as those described previously for LDGs in other diseases were significantly elevated in peripheral blood mononuclear cells of subjects with moderate persistent or SP asthma. The LDGs comprised up to 39% of peripheral blood mononuclear cells, with elevated LDG levels most prevalent in subjects with SP asthma. The highest LDG levels were observed in 4 subjects with SP asthma. Fraction of exhaled nitric oxide levels and body mass were significantly increased in subjects with low LDG levels compared with control subjects, whereas fraction of exhaled nitric oxide levels and body mass were not elevated in subjects with moderate persistent or SP asthma and high LDG levels compared with control subjects.

Conclusion: These findings identify a previously unrecognized association between LDG levels and asthma severity. Identification of the factor(s) responsible for the increased LDG levels in moderate persistent or SP asthma may provide a serum biomarker to aid in the identification of neutrophil-associated phenotypes of severe asthma.

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Introduction

Neutrophils have been implicated in the pathophysiology of multiple asthma phenotypes, including severe and steroid-resistant asthma.^{1,2} Indeed, the presence or absence of neutrophils in patient sputum can be used to distinguish among severe asthma phenotypes.³ However, whether circulating neutrophils provide any insight into asthma severity or phenotypes is largely unknown.

A distinct population of neutrophil-like cells termed *low-density granulocytes* (LDGs) is present in increased numbers in the peripheral blood mononuclear cell (PBMC) fraction of patients with systemic lupus erythematosus (SLE)^{4–6} and other systemic autoimmune diseases^{6,7} and in patients infected with human immunodeficiency virus (HIV).⁸ The LDGs have the same CD15⁺CD16⁺CD66b⁺CD11b⁺CD33^{lo}–CD14^{lo} phenotype as neutrophils,^{5,8} but differ from

neutrophils in having increased expression of CD11b and CD66b⁵ characteristic of activated neutrophils^{9,10} and increased expression of CD15 (Lewis X).⁸ The LDGs, which also include a subset of cells with an immature granulocyte phenotype,^{4,5} display a range of proinflammatory activities,^{5,11} including increased levels of tumor necrosis factor- α and interferon- α production,⁵ and an increased propensity for neutrophil extracellular trap formation.¹¹ The LDGs constitute up to approximately 50% of PBMCs in patients with SLE^{4,5} and, importantly, larger percentages of LDG are observed in association with periods of disease activity or severity in patients with SLE⁵ and those with HIV.⁸

The present study was performed to determine whether LDG levels are elevated in PBMCs of patients with asthma and specifically in PBMCs of patients with moderate or severe persistent disease.

Methods

Subjects

Subjects were recruited from outpatients in the Section of Allergy and Clinical Immunology at Rush University Medical Center (Chicago, Illinois) and from personnel within Rush University

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Medical Center. The institutional review board of Rush University Medical Center approved the study protocol, and each study subject provided written informed consent. Subjects with asthma had a history of physician-diagnosed asthma and all but 3 with asthma had a confirmed immunoglobulin E (IgE)-mediated sensitivity to at least 1 aeroallergen. Asthma severity was classified as intermittent (I), mild persistent (MiP), moderate persistent (MoP), or severe persistent (SP) disease according to National Heart, Lung, and Blood Institute guidelines.¹² Exclusion factors for entry into the study were treatment with Xolair (omalizumab; Genentech, San Francisco, California; Novartis, East Hanover, New Jersey) within the preceding year, a respiratory infection within the previous month, starting or revising an allergen immunotherapy regimen within the past month, a history of alcohol or drug abuse, or being pregnant. Control subjects had a negative history for atopic disease and asthma. All subjects were nonsmokers, although 2 control subjects and 4 subjects with asthma reported a history of smoking.

Clinical Assessment

All subjects underwent a brief medical history and physical examination. Subjects with asthma did not withhold medications before the study visit. Forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), and FEV₁/FVC ratio were measured for all subjects using a KoKo Spirometer (nSpire Health, Longmont, Colorado); the highest value of 3 separate measurements was recorded. Fraction of exhaled nitric oxide (FE_{NO}) was measured using the Niox Mino (Niox, Morrisville, North Carolina), and total serum IgE levels were measured by enzyme-linked immunosorbent assay (Alpha Diagnostics International, San Antonio, Texas). Body mass index was calculated using the National Heart, Lung, and Blood Institute BMI Calculator (<http://www.nhlbi.nih.gov/guidelines/obesity/BMI/bmicalc.htm>). All subjects with asthma also completed the Asthma Control Test (ACT; Quality Metric, Inc, Lincoln, Rhode Island).

Cell Isolation

Blood was drawn by venipuncture and, with few exceptions, during the same study visit in which the clinical assessments were performed. The PBMC and normal-density granulocyte fractions were isolated as described previously.¹³ Briefly, venous blood was diluted 1:1 with sterile saline (Baxter, Deerfield, Illinois) containing 4 mmol/L of ethylenediaminetetraacetic acid and was centrifuged on a 3-mL cushion of Lymphocyte Separation Medium (Lonza, Walkersville, Maryland) in a 15-mL polystyrene conical centrifuge tube for 30 minutes at 300g (4°C) with the brake off. The PBMCs were carefully collected by aspiration from the plasma–lymphocyte separation medium interface, diluted 1:3 with sterile saline, and centrifuged for 10 minutes at 250g (4°C). The PBMCs were washed once in phosphate buffered saline. Normal-density granulocytes (neutrophils and eosinophils) were isolated from the granulocyte–erythrocyte pellet by 3 cycles of brief hypotonic lysis as described previously.¹³ The PBMCs and granulocytes were suspended in RPMI-1640 for cell counting and then analyzed immediately by flow cytometry. Cytospin preparations of the normal-density granulocytes were prepared and treated with Wright-Giemsa stain to quantify the percentage of eosinophils, which was determined by counting at least 200 cells.

Flow Cytometry

The PBMCs or granulocytes (10⁶ cells each) were transferred to micro-centrifuge tubes that had been pre-rinsed with 1% bovine serum albumin. The cells were washed once in 1 mL of phosphate buffered saline, 0.25% bovine serum albumin, and 1 mmol/L of ethylenediaminetetraacetic acid (PBE) and then suspended in 75 μ L of PBE supplemented with 0.05% human IgG (Gammagard; Baxter). The cells were incubated for 45 minutes on ice without or with the following mixture of fluorescent-labeled mouse antihuman

monoclonal antibodies: phycoerythrin-conjugated anti-CD15 (IgM); allophycocyanin-H7-conjugated anti-CD16 (IgG1); fluorescein isothiocyanate-conjugated anti-CD66b (IgM); Alexa Fluor 700-conjugated anti-CD11b (IgG1) (Molecular Probes, Eugene, Oregon); allophycocyanin-conjugated anti-CD33 (IgG1); and Pacific Blue-conjugated anti-CD14 (IgG2a). All monoclonal antibodies were used at concentrations recommended by the manufacturer (BD Biosciences, San Jose, California). The cells were washed once in 1 mL of cold PBE after incubation and suspended in 300 μ L of 1% formalin. Samples were acquired (100,000 events) using an LSRII flow cytometer (BD Biosciences) and analyzed using FloJo 8.8.7 software (TreeStar, Inc, Ashland, Oregon). Positive and negative quadrants were established at the start of the study using fluorescence minus-1 controls.¹⁴

Statistical Analysis

Unless indicated otherwise, results are expressed as mean $\pm$ SEM. Statistical analysis was performed using GraphPad Prism 5.0a (GraphPad, San Diego, California). The statistical significance of differences between group means was analyzed by 1-way analysis of variance, and significant differences between the asthma groups and the control group were analyzed post hoc using the Dunnett multiple comparison test. A *P* value less than .05 was considered significant.

Results

Characteristics of Study Subjects

The study population was comprised of 20 subjects with I (*n* = 9) or MiP (*n* = 11) asthma, 20 subjects with MoP (*n* = 10) or SP (*n* = 10) asthma, and 20 control subjects with no history of allergy or asthma (Table 1). Approximately three fourths of subjects with asthma were women, and approximately one half of subjects with MoP/SP asthma and control subjects were African American. Subjects with MoP/SP asthma had significantly lower predicted FEV₁ and FEV₁/FVC values compared with controls (*P* < .001) and had a significantly lower ACT score compared with subjects with I/MiP asthma (*P* < .001). Other characteristics are presented in Table 1. In results not shown, subjects with I/MiP asthma were being treated with a short-acting β -agonist alone or some combination of a short-acting β -agonist and a leukotriene modifier or an anticholinergic agent. Subjects with MoP/SP asthma were being treated with a short-acting β -agonist rescue drug and a combination of a long-acting

Table 1
Demographics of study subjects^a

Characteristic	Groups		
	Control	I or MiP asthma	MoP or SP asthma
Subjects (per class)	20	20 (9/11)	20 (10/10)
Men/women	6/14	5/15	6/14
Age (y)	41 $\pm$ 12	33 $\pm$ 9	43 $\pm$ 12
Race or ethnicity			
White	7	12	5
African American	9	5	11
Hispanic	1	0	2
Asian or Pacific Islander	3	3	2
FEV ₁ % predicted	101 $\pm$ 19.8	90 $\pm$ 12.8	74 $\pm$ 21 ^c
FEV ₁ /FVC	0.82 $\pm$ 0.01	0.79 $\pm$ 0.07	0.72 $\pm$ 0.11 ^c
Asthma Control Test		22.3 $\pm$ 2.5	17.8 $\pm$ 0.42 ^d
IgE (IU/mL)	32 $\pm$ 43	116 $\pm$ 108 ^b	70 $\pm$ 42

Abbreviations: FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; I, intermittent; MiP, mild persistent; MoP, moderate persistent; SP, severe persistent.

^aData are presented as number or mean $\pm$ SD.

^b*P* < .01 compared with control.

^c*P* < .001 compared with control.

^d*P* < .001 compared with I or MiP asthma by unpaired *t* test.

β -agonist and an inhaled corticosteroid. One subject with SP asthma also was being treated every other day with an oral corticosteroid, and 1 subject with MoP asthma had been treated with a 1-week course of an oral corticosteroid 2 weeks before the study visit.

LDG Levels in PBMCs

The LDGs were identified in PBMCs as $CD16^+CD15^+CD66b^+CD33^{lo/-}CD14^{lo}CD11b^+$ granulocytes (Fig 1A, B) that shared the same phenotype as normal-density neutrophils ($CD16^+$ granulocytes; Fig 1A, C). The PBMCs of subjects with MoP/SP asthma contained significantly elevated percentages of LDGs ($7.4 \pm 10.8\%$, mean $\pm$ SEM) compared with PBMCs of control subjects ($1.3 \pm 1.1\%$, $P < .01$; Fig 2A). The percentages of LDGs in the PBMCs of subjects with I/MiP asthma ($2.1 \pm 2.2\%$) did not differ significantly from control values (Fig 2A). The percentages of LDGs in the PBMCs of subjects with MoP/SP asthma ranged up to 39% and were paralleled by increases in the absolute numbers of LDGs in the PBMC samples (Fig 2B). The ratio of the number of LDGs in the PBMCs to 10^3 neutrophils isolated in the granulocyte fraction was likewise

significantly elevated in subjects with MoP/SP asthma (Fig 2C). No significant differences were noted in the numbers of PBMCs or neutrophils isolated from the 3 study groups (eTable 1). Hypodense eosinophils ($CD16^-CD15^{dim}CD66b^+CD33^{lo/-}CD14^{lo}CD11b^+$ granulocytes)¹⁵ constituted fewer than 0.1% of cells in the PBMCs of subjects with asthma (results not shown).

Prevalence of Elevated LDG Levels

An elevated LDG level was defined as a percentage greater than 2 SD above the mean percentage of LDGs in the PBMCs of control subjects (Fig 2A). The 13 subjects with increased levels of LDG included male and female white and African-American patients and 1 Hispanic male patient (eTable 2). Increased percentages of LDGs occurred in 45% of subjects (9 of 20) with MoP/SP asthma vs only 20% of subjects (4 of 20) with I/MiP asthma. Analysis of the prevalence of elevated LDG levels in the control subjects and in the 4 subgroups of subjects with asthma showed a statistically significant trend ($P < .01$ by χ^2 test), with the highest prevalence (60%) of elevated LDG levels occurring in the 10 subjects with SP asthma. The largest percentages

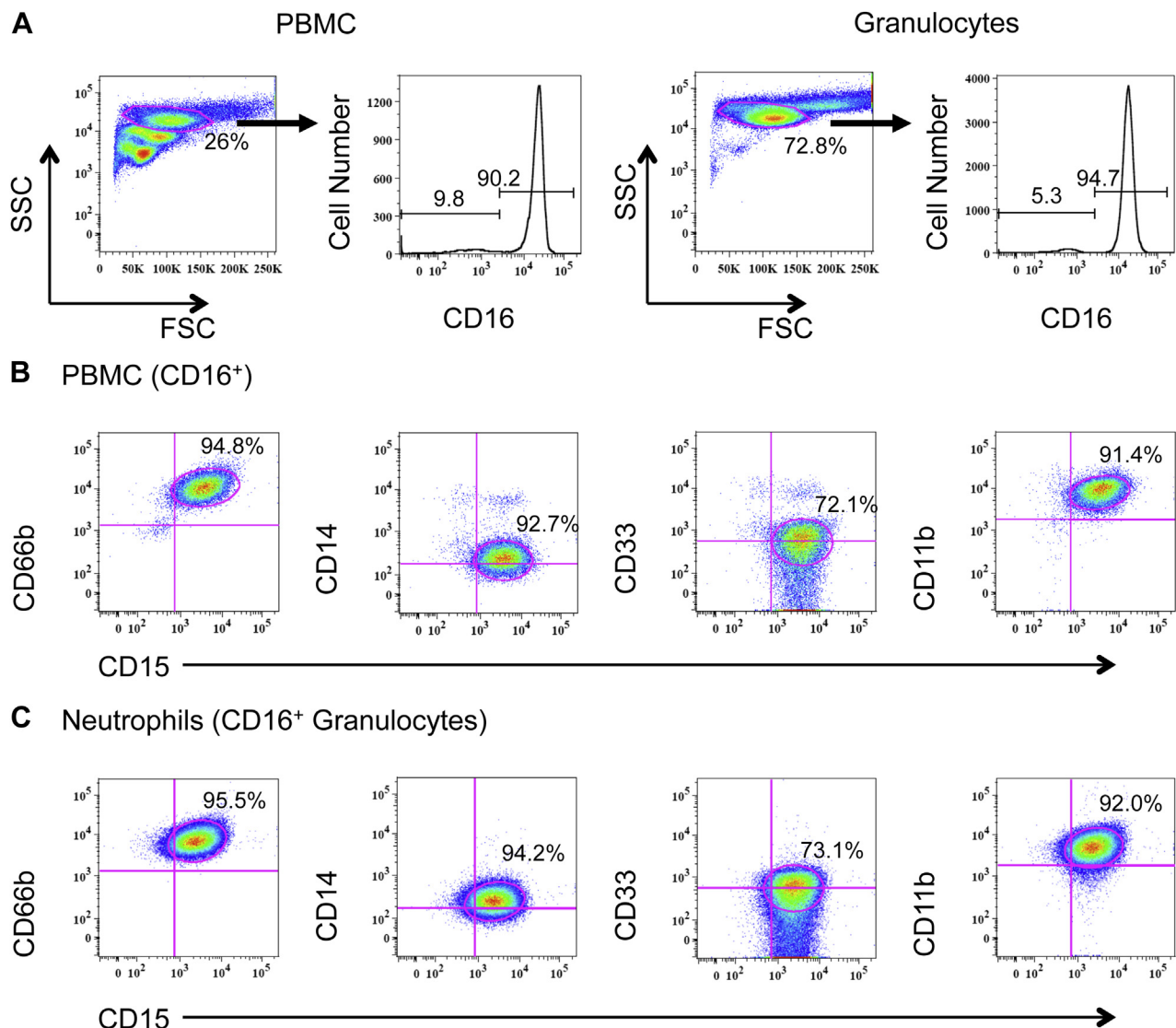


Figure 1. Flow cytometric analysis of low-density granulocytes. (A) A gate was set for peripheral blood mononuclear cells (PBMCs) having a forward scatter (FSC) and a side scatter (SSC) light profile identical to a single-cell population of normal-density granulocytes, and cells within the granulocyte gates were analyzed for CD16 expression. The $CD16^+$ granulocytes in the (B) PBMCs and (C) neutrophils ($CD16^+$ normal-density granulocytes) were analyzed for CD15, CD66b, CD33, and CD14 expression. The relative percentage of each cell population is displayed. Results are for a single subject with asthma.

of LDGs (Fig 2A) and the largest numbers (Fig 2B) of LDGs also were observed in PBMCs of 4 subjects with SP asthma. The highest ratios of LDGs to neutrophils were likewise observed in 3 subjects with SP asthma (Fig 2C). Of the 4 subjects with asthma who had reported a history of smoking, the level of LDGs was elevated (4.4%) in 1 subject (2.5 pack-years), whereas the mean LDG level for the other 3 subjects (1–5 pack-years) was $1.5 \pm 0.2\%$.

LDG Phenotype

Previous results have shown that LDGs in patients with SLE and those with HIV display increased levels of CD11b, CD66b, and CD15 expression compared with normal-density neutrophils^{5,8} and that LDGs include increased numbers of cells with an immature granulocyte morphology.^{4,5} Therefore, expression levels of surface CD molecules by LDGs and neutrophils (eFig 1) were compared to further confirm the identity of LDGs in the present study. The LDGs in subjects with asthma and in the control subjects expressed at least 2-fold higher levels of CD15 and significantly higher levels of CD11b and CD66b compared with expression levels by neutrophils (Fig 3). No differences were observed in the levels of CD33 or CD14 expression by the LDGs and neutrophils. Although the characterization and numbers of immature granulocytes were not addressed in the present study, band cells and mature neutrophils in the PBMCs of subjects with elevated LDG levels (eFig 2) were observed.

Association between Elevated LDG Levels and Clinical Measurements

Potential clinical distinctions for subjects with MoP/SP asthma and an elevated LDG level were evaluated. The 9 subjects with MoP/SP asthma and elevated LDG levels, like the 11 subjects with MoP/SP asthma and low LDG levels, had significantly lower FEV₁ and FEV₁/FVC values compared with the control subjects (Fig 4). The ACT scores for the 11 subjects with MoP/SP asthma and low LDG levels and the 9 subjects with MoP/SP asthma and elevated LDG levels were 17.7 ± 1.4 and 17.9 ± 1.2 , respectively. Subjects with MoP/SP asthma and low LDG levels exhibited statistically significant increases in the levels of FE_{NO}, percentages of eosinophils in the normal-density granulocyte fraction, and body mass index compared with control subjects. In contrast, subjects with MoP/SP asthma and elevated LDG levels did not differ from control subjects in FE_{NO} levels, eosinophil percentages, or body mass index. The total IgE levels in subjects with MoP/SP and high or low LDG levels were similar, albeit not statistically increased, compared with IgE levels of the control subjects (Fig 4).

Discussion

These results demonstrate that LDG levels are significantly elevated in the PBMCs of subjects with MoP/SP asthma. The highest levels of LDGs and the highest prevalence of elevated LDG levels were observed in subjects with SP asthma. As such, these findings are similar to the increased levels of LDG and the increased frequency of elevated LDG levels that have been observed in association with disease activity in patients with SLE^{4,5} and those with HIV.⁸ Although the sample number in this study is relatively small, the results indicate that increased levels of LDGs are not restricted to a specific sex, race, or ethnicity. Elevated percentages of LDG were observed in the PBMCs of white, African American, and Hispanic subjects and in male and female subjects. No relation between LDG levels and a history of smoking was noted for the 4 subjects with asthma who had smoked previously.

The LDGs described in the present study are very similar, if not identical, to the LDGs described previously in patients with SLE⁵ and those with HIV.⁸ The LDGs shared the same CD16⁺CD15⁺CD66b⁺CD33^{lo/-}CD14^{lo}CD11b⁺ phenotype as neutrophils but expressed increased levels of CD11b and CD66b. The most distinguishing phenotypic feature of LDGs in the present study is the 2-fold increase in the level of CD15 (Lewis X). Whether the increase in CD15 expression, which also was observed in LDGs from patients with HIV,⁸ reflects increased CD15 synthesis¹⁶ or cleavage of surface Sialyl Lewis X (CD15s) by activated sialidase¹⁷ is unknown at this point. However, it is known that neutrophils contain sialidases that become expressed on the neutrophil surface after activation.¹⁸ As such, the increased expression of CD15 by LDGs also may reflect an activated or primed neutrophil phenotype for the LDG. Also, it is noteworthy that the expression levels of CD11b, CD66b, and CD15 remained essentially constant for the LDGs of all subjects, thus indicating that only LDG levels and not the LDG phenotype changed as a function of asthma severity.

Although increased CD11b and CD66b expression has been associated with neutrophil activation,^{9,10} LDGs in patients with SLE also have been found to contain cells with myelocyte-like or band-shaped nuclei characteristic of immature granulocytes in addition to cells with multilobed nuclei characteristic of mature neutrophils.^{4,5} Indeed, immature granulocytes accounted for up to 40% of LDGs in 1 SLE study.⁵ In addition, microarray analysis has shown increased expression of selected azurophilic granule proteins in LDGs, consistent with an immature phenotype.^{4,11} Although not quantified in the present study, increased numbers of band cells were observed in addition to mature neutrophils in the PBMCs of

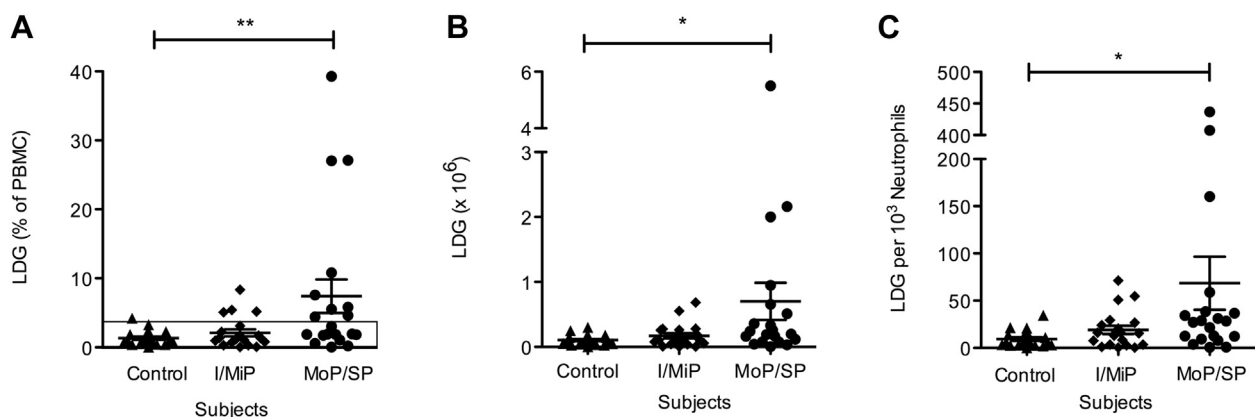


Figure 2. Levels of low-density granulocytes (LDGs). (A) Percentages and (B) numbers of LDGs in peripheral blood mononuclear cells (PBMCs) and (C) the number of LDGs in the granulocyte fraction of control subjects, subjects with intermittent (I) or mild persistent (MiP) asthma, or subjects with moderate persistent (MoP) or severe persistent (SP) asthma are shown. Each symbol denotes a single subject, and the mean $\pm$ SEM for each study population is shown. (A) The box identifies values less than 2 SD above the mean value for control subjects. * $P < .05$, ** $P < .01$ compared with control.

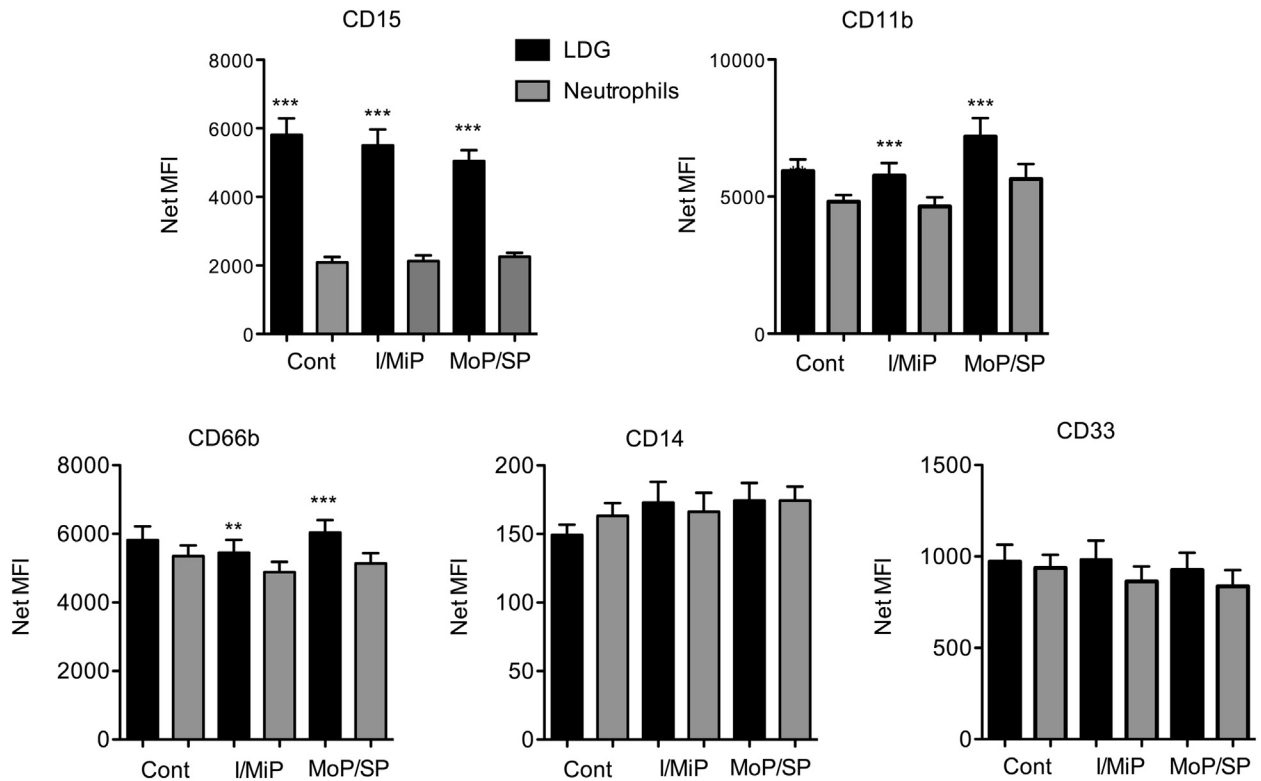


Figure 3. Expression levels of selected CD surface molecules. Expression levels for CD16⁺ low-density granulocytes (LDGs) and neutrophils (CD16⁺ normal-density granulocytes) are presented as the net median fluorescence intensity (MFI) after subtraction of the MFI for unstained cells. ** $P < .01$, *** $P < .001$. I, intermittent asthma; MiP, mild persistent asthma; MoP, moderate persistent asthma; SP, severe persistent asthma.

subjects with asthma and elevated levels of LDG. Despite having characteristics of activated neutrophils and immature granulocytes, LDGs appear to be a mostly uniform population of cells, because the expression of individual CD molecules conformed to a single peak.

Clinically, subjects with MoP/SP asthma and high levels of LDG were indistinguishable based on pulmonary function and asthma control from those with MoP/SP and low LDG levels. The FEV₁ and FEV₁/FVC values and ACT scores were comparable in the presence or

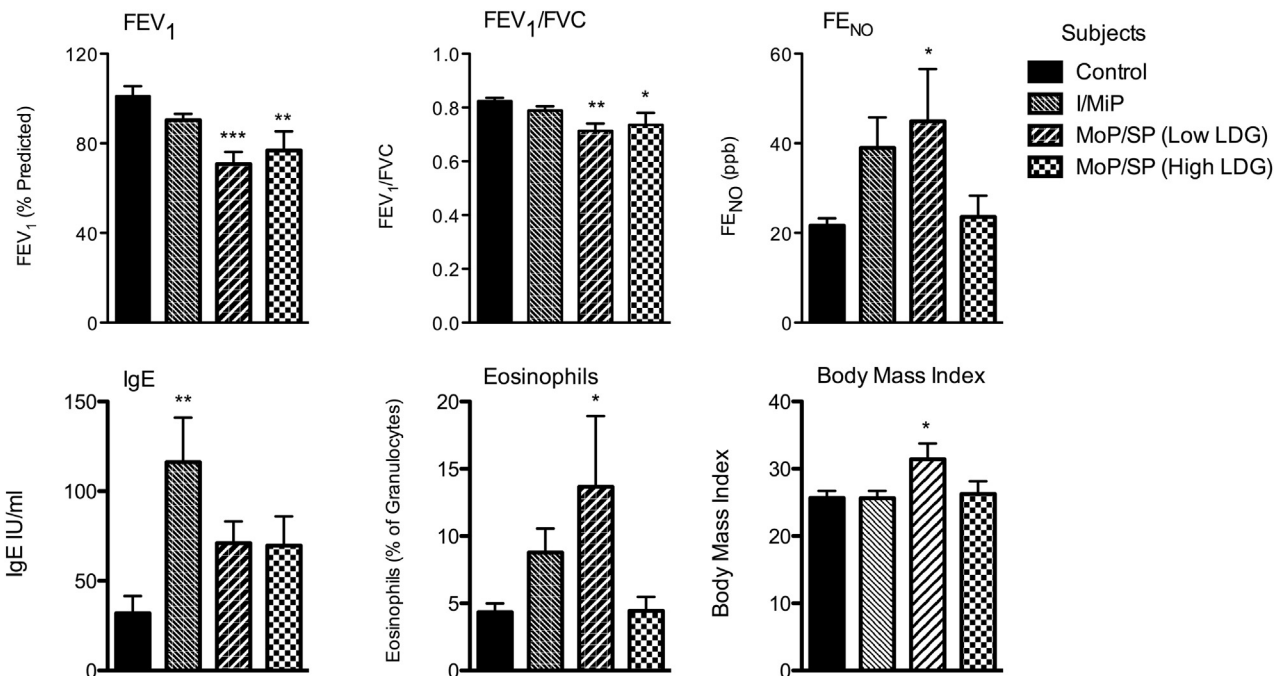


Figure 4. Clinical features associated with elevated low-density granulocyte (LDG) levels. Results are shown for 20 control subjects, 20 with intermittent (I) or mild persistent (MiP) asthma, 11 with moderate persistent (MoP) or severe persistent (SP) asthma and a low LDG level, and 9 with MoP/SP asthma and an elevated LDG level. * $P < .05$, ** $P < .01$, *** $P < .001$ compared with control subjects.

absence of elevated LDG levels. In contrast, a notable difference in FE_{NO} levels was observed in association with high LDG levels. Although FE_{NO} levels were significantly elevated in subjects with MoP/SP asthma and low LDG levels compared with controls, FE_{NO} levels for subjects with MoP/SP asthma and elevated LDG levels did not differ from control subjects. Elevated levels of FE_{NO} are used clinically to identify eosinophilic asthma and the eosinophilic form of severe asthma.^{3,19} Thus, although confirmation must await parallel measurement of sputum neutrophil levels, the low FE_{NO} levels in subjects with elevated LDG levels suggest that elevated LDG levels are associated with the neutrophilic²⁰ and, possibly, the mixed phenotype of severe asthma, which has been associated with lesser increases in FE_{NO} levels.³ Of note, eosinophil levels, as a percentage of normal-density granulocytes, also were not elevated in subjects with MoP/SP asthma and elevated LDG levels compared with control subjects. This finding, which must be confirmed by direct measurement of blood eosinophil counts, would be consistent with a non-T-helper cell type 2 or non-eosinophilic asthma. The body mass index of subjects with MoP/SP asthma and elevated LDG levels was within normal limits (<http://www.nhlbi.nih.gov/guidelines/obesity/BMI/bmicalc.htm>), thus indicating that elevated LDG levels are not specifically associated with the obese asthma phenotype.² Because elevated FE_{NO} values also are predictors for responsiveness to inhaled steroid therapy,²¹ it is noteworthy that oral corticosteroids did not lower LDG levels in patients with SLE and that, in the present study, one of the highest levels of LDG (29%) was present in a subject with SP asthma who was being treated every other day with an oral corticosteroid.

Importantly, the present results identify a previously unrecognized association between LDG levels and asthma severity. Whether the elevated levels and multiple inflammatory activities^{5,11} of LDGs contribute to asthma severity, including as a potential source of sputum neutrophils,³ remains to be determined. However, these initial results do point to the possibility that elevated levels of an LDG-related mediator could serve as a serum biomarker for neutrophil-associated asthma phenotypes in a manner analogous to that of periostin and eosinophilic (T-helper cell type 2) asthma phenotypes.²² Molecules, such as granulocyte colony-stimulating factor, that stimulate neutrophil mobilization from the bone marrow⁹ represent 1 potential class of candidates to consider in this context. However, the finding that LDG levels in the PBMC fraction increased in the absence of a parallel increase in neutrophil levels in the granulocyte fraction appears to exclude, at least preliminarily, a link between increased LDG levels and increased mobilization of neutrophils from the bone marrow. Alternatively, LDGs display an activated neutrophil phenotype,^{5,8} and microarray analysis has identified increased expression of several molecules, including defensins, matrix metalloproteinase-8, and elastase, in LDGs.^{4,11} Together, these findings suggest the possibility that serum levels of a molecule that is selectively enriched in LDGs may parallel LDG levels and thus serve as a plausible biomarker candidate.

In summary, although the link between LDG levels and disease severity is not specific to asthma,^{4–6,8} it is proposed that further investigation of the link between LDG levels and asthma severity could lead to a clinically useful serum biomarker for the identification of severe asthma, in particular neutrophil-associated phenotypes and possibly steroid-resistant asthma.

Acknowledgments

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Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2014.08.024>.

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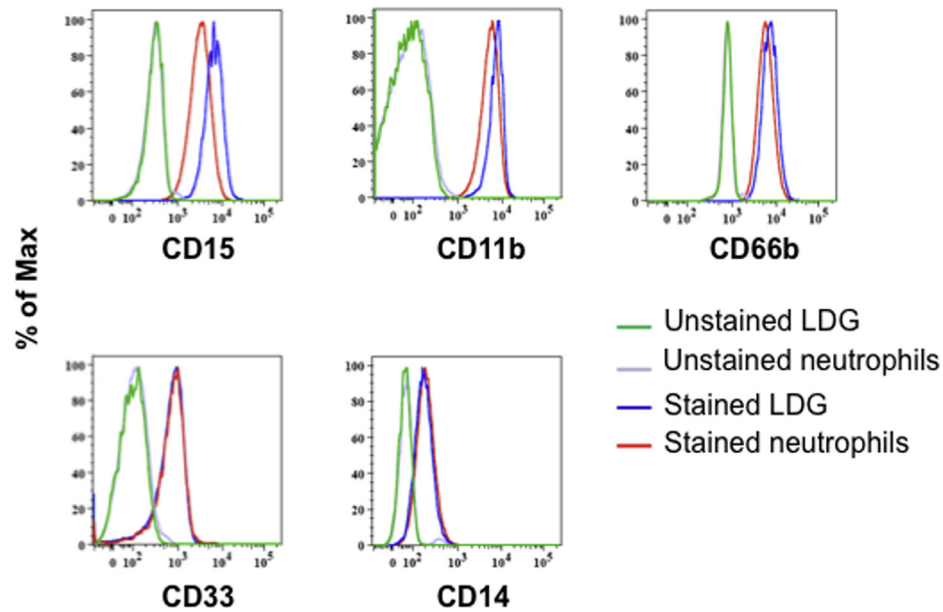


Figure e1. Representative expression of selected CD surface molecules. Histograms for surface marker expression by CD16⁺ low-density granulocytes (LDGs) and neutrophils (CD16⁺ normal-density granulocytes) are shown. All results are from a single subject with asthma.

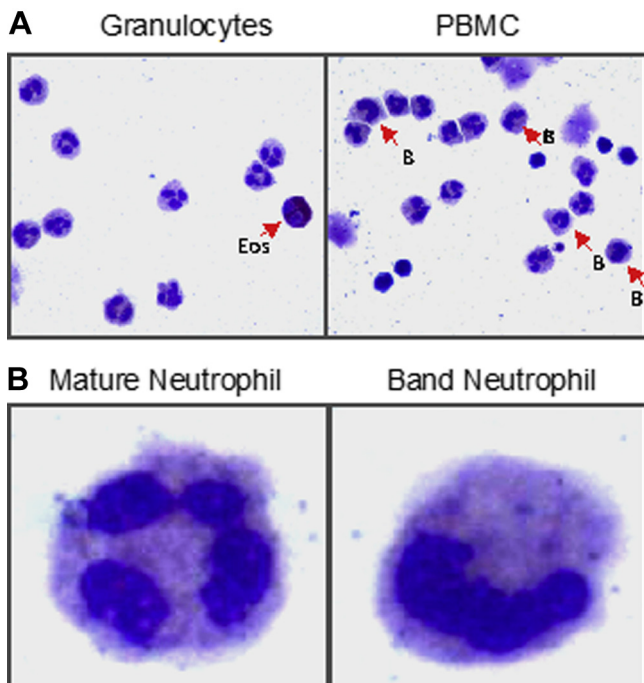


Figure e2. Cytospins of peripheral blood mononuclear cells (PBMCs). (A) Wright-Giemsa-stained cytopsin preparations of granulocyte and PBMC fractions from a subject with asthma and a high level of low-density granulocytes are shown at 40 $\times$ magnification. (B) Two cells in the PBMC fraction in A are shown at 100 $\times$ (oil-immersion) magnification to illustrate the morphologies of the mature neutrophil and band neutrophil morphologies of low-density granulocytes. B, band cell; Eos, eosinophil.

eTable 1Numbers of PBMCs and neutrophils isolated from study subjects^a

	Study group		
	Control	I or MiP asthma	MoP or SP asthma
PBMCs $\times 10^6$	7.02 $\pm$ 0.62	7.38 $\pm$ 0.43	8.86 $\pm$ 0.70
Neutrophils $\times 10^6$	13.54 $\pm$ 1.94	10.94 $\pm$ 1.09	12.78 $\pm$ 1.62

Abbreviations: I, intermittent; MiP, mild persistent; MoP, moderate persistent; PBMCs, peripheral blood mononuclear cells; SP, severe persistent.

^aResults are presented as mean $\pm$ SEM.

eTable 2

Age, sex, and race or ethnicity of subjects with asthma and a high LDG level

Subject	Asthma classification	LDGs, %	Age (y)	Sex	Race or ethnicity
1	MoP	4.4	44	F	white
2	SP	39.3	26	F	African American
3	SP	5.5	54	F	African American
4	MoP	5.8	52	M	Hispanic
5	SP	4.6	51	F	African American
6	MiP	5.2	24	M	African American
7	MiP	5.4	29	F	African American
8	SP	27.1	51	M	white
9	SP	10.8	39	F	white
10	I	8.3	45	F	white
11	SP	27.0	49	F	African American
12	MoP	7.6	36	M	white
13	I	5.1	28	M	white

Abbreviations: F, female; I, intermittent; LDGs, low-density granulocytes; M, male; MiP, mild persistent; MoP, moderate persistent; SP, severe persistent.