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ORIGINAL ARTICLE

Plasma Resistin Levels in Asthmatics as a Marker of Disease State

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Background. Resistin is a protein produced by adipocytes and circulating macrophages that has been found to be associated with inflammatory states. **Objective.** To determine the levels of resistin in relation to asthma disease state and severity, we investigated a cohort of adult patients with asthma. **Methods.** A cohort of moderate to severe persistent asthma patients and control patients were recruited and underwent fasting labs to evaluate levels of serum glucose, C-reactive protein (CRP), and resistin. **Results.** No significant differences were found between the control and asthma group with respect to serum CRP at 0.78 ± 0.60 mg/dL and 0.48 ± 0.60 mg/dL, ($p < 0.36$) or glucose at 92.2 ± 11.9 mg/dL and 89.5 ± 7.2 mg/dL, ($p < 0.084$), respectively (mean $\pm$ SD). However, plasma resistin levels were found to be significantly elevated in asthma patients, 186 ng/mL (95%CI 169–202) compared with control patients 121 ng/mL (95%CI 90.4–151), ($p < 0.005$). **Conclusions.** Patients with asthma were found to have higher levels of resistin, and resistin levels were increased with disease severity in the asthma cohort.

Keywords resistin, asthma, CRP, diabetes, FIZZ

INTRODUCTION

Asthma is a chronic disease characterized by reversible airway obstruction, airway hyper-responsiveness, and airway inflammation that currently affects nearly 21 million Americans (1). Despite advances in diagnosis and treatment, the underlying disease process is not well understood, and control of the disease lags far behind established guidelines leading to nearly 13 million office visits and 1.7 million emergency room visits annually (1–3). Pulmonary function testing with either methacholine challenge or bronchodilator response has been the mainstay of diagnosing airway hyper-responsiveness for many years, but these tests may not be the most accurate assessment of disease state or severity (4). Other noninvasive measurements of airway inflammation including measurement of exhaled nitric oxide (FENO), sputum eosinophils, or eosinophilic cationic protein (ECP) have aided in our ability to determine asthma severity and response to treatment (5–9). However, these tests also have various limitations in clinical practice due to sputum sample processing and analysis and the fact that foods and medications may alter the results of FENO testing (10, 11). Ideally, the measurement of a plasma marker that could be correlated with levels of airway inflammation would aid greatly in the identification and treatment of asthmatics.

Resistin is part of the FIZZ (found in inflammatory zones) family of genes and was first characterized in murine models. The FIZZ family of genes also includes RELM- α , predominantly found in adipose, heart, and lung; RELM- β , predominantly found in the gastrointestinal tract; and resistin, predominantly found in macrophages and adipocytes (12, 13). Resistin has been extensively studied as a potential

link between type 2 diabetes and obesity in both mice and humans (12, 14–19). Although no link between type 2 diabetes and resistin has been established in humans, there has been a growing body of literature to support its role as an inflammatory cytokine (20–26). Specifically, studies have shown that resistin levels are associated with elevated C-reactive protein (CRP) in patients with adult-onset diabetes but show no correlation with obesity or insulin resistance (12). The fact that resistin is predominantly found in the adipocytes in mice as opposed to the macrophage in humans may account for the difference between a primarily metabolic role to a primarily inflammatory role (13). Since the macrophage is the major source of resistin in humans, resistin can be readily measured in human plasma with a commercially available enzyme-linked immunoassay (12, 19, 27). While resistin has not yet been investigated in asthmatic patients, RELM- α and RELM- β have been associated with airway inflammation and allergen-induced asthma (21, 28).

To further elucidate the possible link between airway inflammation associated with asthma and plasma resistin levels, we identified a cohort of previously diagnosed moderate to severe persistent asthmatics and measured serum resistin, CRP, and glucose levels in comparison to matched control patients.

METHODS

Study Design and Subjects

Subjects between 18 and 65 years of age who were previously diagnosed with moderate to severe persistent asthma as defined by the National Asthma Education and Prevention Program (NAEPP) were enrolled from the Allergy and Immunology clinic at Wilford Hall Medical Center. Subjects on inhaled corticosteroids or other controller medications were allowed in the study, but subjects on oral corticosteroids any time over the previous 6 months were excluded. Subjects from the asthma cohort were given a questionnaire to quantify their current disease state based on symptom frequency as

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TABLE 1.—Asthma symptom questionnaire.

Asthma group	Symptoms	Nighttime symptoms
Group 1	≤2×/week	≤2×/month
Group 2	>2× week but < once daily	>2×/month
Group 3	Daily symptoms Daily albuterol use Exacerbations ≥2×/week	>once weekly
Group 4	Continual symptoms Frequent exacerbations	Frequent symptoms

defined by wheezing, dyspnea, cough, and chest tightness regardless of their initial diagnostic class. These subjects were then stratified into four groups corresponding to the symptom criteria used by the NAEPP (Table 1). Spirometry was not performed on subjects during the time of the study. Since the role of resistin in inflammation has not been well described, all subjects with either diabetes or a known systemic inflammatory disorder were excluded from this study. Subjects from the control cohort were excluded if they carried a diagnosis of any chronic respiratory disease. All studies were carried out with approval of the local institutional review board, and subjects provided informed consent.

Blood Collection and Analysis

For the collection of blood, samples were obtained after a 12-hour fast for the determination of blood glucose, CRP, and resistin. Measurement of blood glucose and CRP were performed using routine laboratory procedures. Measurement of plasma resistin was accomplished using the Resistin (51–108)-NH₂ (Human) (JKC373) EIA Kit from Phoenix Pharmaceuticals (Belmont, CA) and a Bio-Tek Synergy HT plate reader (Winooski, VT) equipped with KC4 analysis software at 450 nm with a standard curve in the range of 0.05 to 1,000 ng/mL. Briefly, blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes. Plasma was collected after centrifugation, and aprotinin was added to each sample before storage at –70°C. Samples were stored for no longer than 30 days before final analysis.

Statistical Analysis

Statistical analysis of age, glucose, CRP, and resistin levels between control and asthma cohorts was accomplished with either a Student's *t* test or Mann-Whitney test, as appropriate. Comparison of dichotomous variables between control and asthma cohorts was accomplished with the χ^2 test.

RESULTS

Study Population

A total of 22 subjects were enrolled in the asthma cohort that completed the questionnaire and laboratory studies, and a total of 22 subjects were enrolled in the control cohort. One patient from the asthma cohort and control cohort were excluded owing to current use of statins. Baseline characteristics at the time of the study are provided in Table 2. There were no significant differences among the control and asthma cohorts with respect to age, gender, or serum glucose. Body mass index (BMI) and hemoglobin A_{1c} were not

TABLE 2.—Baseline characteristics.

	Asthma group (n = 21)	Control group (n = 21)	Significance
Male	38.1%	47.6%	<i>p</i> < 0.533*
Female	61.9%	52.4%	
Age (years)	38 ± 10	41 ± 11	<i>p</i> < 0.437**
Glucose (mg/dL)	89.5 ± 7.2	92.2 ± 11.9	<i>p</i> < 0.084**

*Chi square.

**Students *t* test.

Data recorded as mean ± standard deviation.

measured at the time of the study. Among the subjects in the asthma cohort, 95.2% were using inhaled corticosteroids (ICS), 76.2% were using salmeterol, and 100% were using albuterol. None of the subjects in the asthma cohort were using salmeterol without the use of inhaled corticosteroids. The dose of ICS ranged from 100 µg to 500 µg of fluticasone with most patients (54%) on low-dose ICS. There was no correlation between ICS dose and degree of symptoms at the time of the study. Despite the similarities in asthma treatment, there was a significant diversity in the frequency and severity of asthma symptoms with over 40% of subjects classified inadequately controlled at the time enrollment into the study based on NAEPP guidelines.

Inflammatory Markers

Comparison of serum CRP levels in the asthma and control cohorts showed no significant difference, 0.48 ± 0.22 mg/dL (mean ± SD) and 0.78 ± 0.60 mg/dL respectively, *p* < 0.363. Notably, 81% of the asthma cohort and 62% of the control cohort had no measurable CRP levels. However, this was not a significant difference between the two cohorts as determined by the χ^2 test, *p* < 0.141. Resistin levels were found to be significantly elevated in the asthma group as compared to the control group, 186 ng/mL (95%CI 169–202) and 121 ng/mL (95%CI 90.4–151), respectively, *p* < 0.005. Owing to the non-parametric variance in resistin levels found in the control group versus the asthma group, we analyzed the data using the Mann-Whitney test (reported as median and interquartile range), which verified the significant difference between the asthma and control groups, 190 ng/mL (interquartile range of 36 ng/mL) and 125 ng/mL (interquartile range of 115 ng/mL), respectively, *p* < 0.004 (Figure 1). Lastly, the asthmatic subjects were separated by symptoms into mild intermittent (group 1) and mild persistent through severe (groups 2–4), which revealed a trend towards elevated plasma resistin as the frequency and severity of asthma symptoms increased (Figure 2).

DISCUSSION

We documented that plasma resistin levels were significantly higher in patients with a diagnosis of moderate to severe persistent asthma than in control patients without evidence of pulmonary or systemic inflammatory disease. Resistin levels were elevated despite the majority of patients receiving appropriate therapy for their asthma (29, 30). In addition, elevations in plasma resistin were independent of fasting serum CRP or glucose indicating an etiology separate from the previously characterized association with diabetic

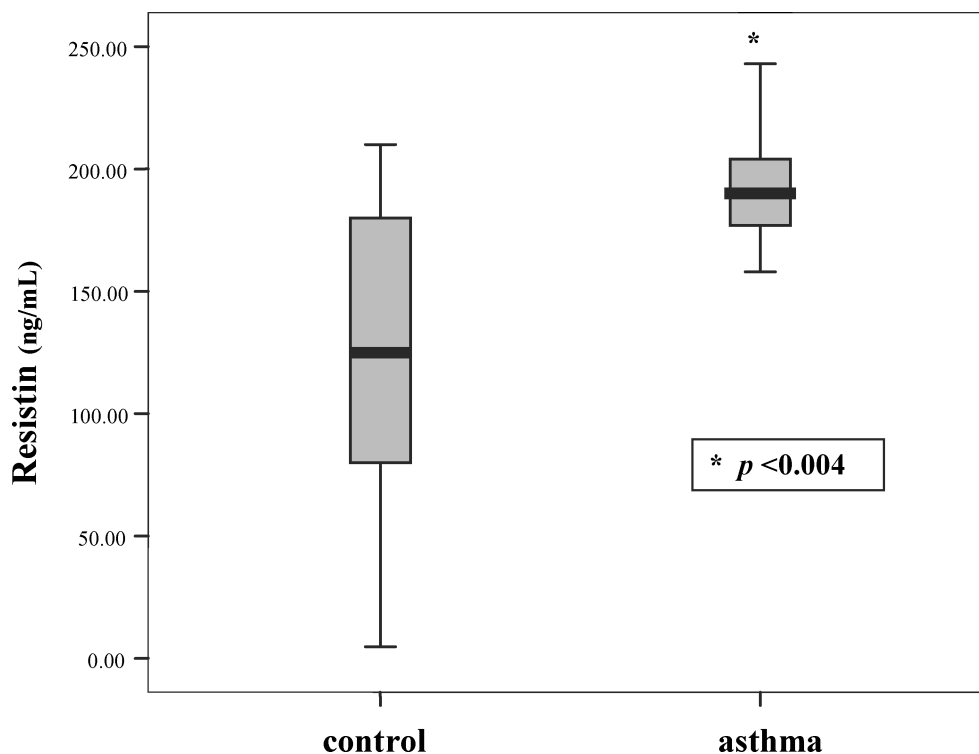


FIGURE 1.—Plasma resistin levels.

This figure is a box and stem plot of plasma resistin levels (ng/mL) in control versus asthma patients. The figure represents median values (indicated by solid line) and interquartile range (indicated by box). Asthma patients were found to have significantly elevated resistin levels ($p < 0.004$) utilizing the Mann-Whitney test.

inflammation. Plasma resistin was seen to be consistently elevated in patients with increased frequency of symptoms. Taken together, these data suggest that plasma resistin may be associated with the airway inflammation seen in asthma and that plasma levels may reflect disease state as a function of symptom frequency and severity despite appropriate medical therapy.

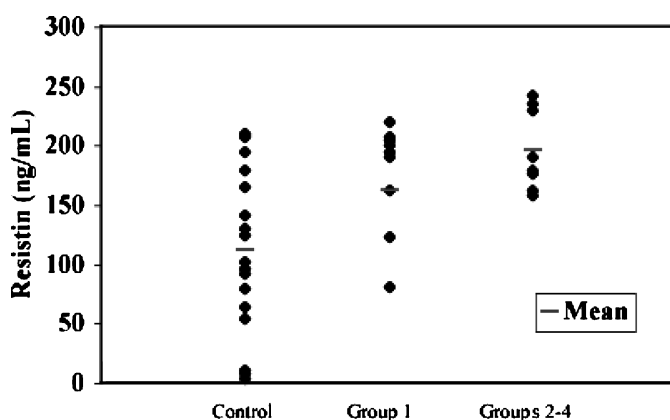


FIGURE 2.—Plasma resistin levels by symptom frequency.

This figure is a scatter plot comparing the range and mean plasma resistin levels (ng/mL) by symptom frequency as determined by the initial asthma questionnaire. Each diamond represents a single data point, and the red line indicates the mean in each group.

Resistin is a relatively new protein that has been extensively studied as a potential link between obesity and diabetes in murine models. Although studies have demonstrated a metabolic role for resistin murine models, this metabolic link has not been identified in human studies (15, 19). However, both murine and human studies have demonstrated an association between resistin and inflammatory states (25). This relation to inflammation is more pronounced in human models where the predominant producer of resistin is the macrophage (27). Kawanami et al. demonstrated that resistin can induce vascular inflammation by increasing expression of vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule (ICAM-1), and long pentraxin 3 on vascular endothelium (22). Macrophage exposure to resistin has been shown to induce expression of tumor necrosis factor alpha (TNF- α) and interleukin 12 (IL-12), which suggests that resistin is more than simply a marker of inflammation (25). Additionally, there have been studies indicating a time-dependent increase in resistin mRNA in response to lipopolysaccharide (LPS) exposure (23, 24). The growing body of literature certainly suggests that resistin may have an active role as a cytokine in various inflammatory pathways.

Apart from an association with vascular inflammatory markers and chronic inflammation in diabetics, there have been no studies looking at the role of resistin in asthma. However, the related FIZZ gene products, RELM- α and RELM- β , have been linked to airway inflammation. Expression of RELM- α has been shown to be elevated in response to pulmonary inflammation (21). In addition, RELM- β expression

was increased in mouse lung tissue after induction of asthma with ovalbumin (OVA) and *Aspergillus fumigatus* (28). This study also found that IL-4 and IL-13 could induce Relm- β expression via a signal-transducer-and-activator-of-transcription (STAT) 6 dependent mechanism, indicating a link between the FIZZ gene family and allergic airway inflammation. Continued research of alternate markers of airway inflammation has greatly increased our understanding of asthma.

The concept of measuring a serum marker for asthma is not a novel one, and a mechanistic pathway consisting of activated alveolar macrophages initiating a cytokine cascade to induce systemic changes is conceivable. Previous studies have shown common serum inflammatory markers, such as amyloid-A and fibrinogen, to be elevated in the setting of asthma (31). Consistent with our findings, CRP was not shown to be elevated in association with asthma in this same study. Levels in C3a have been shown to be elevated in the setting of an acute asthma exacerbation, and anaphylatoxins in general have mechanistic links to asthma in their ability to cause smooth muscle contraction and mucus hypersecretion and to recruit other inflammatory cells (32–34). Resistin may represent a new inflammatory marker associated with asthma. Based on our studies, it is unclear whether resistin is simply a marker for the presence of severity of disease or if resistin is actually playing an etiologic role in airway inflammation. Given the fact that resistin is both responsive to lipopolysaccharide (LPS) and can induce a cytokine response in macrophages lends evidence to support a role as an important inflammatory cytokine.

Several limitations in our study must be considered. All the patients in our asthma group carried a previous diagnosis of moderate to severe persistent asthma, and nearly all of these patients were on ICS, in addition to salmeterol, which have been shown to significantly reduce markers of airway inflammation (4, 5, 7). Therefore, these medications may be affecting the degree of measurable systemic inflammatory markers. Although we showed a trend towards increased plasma resistin in association with symptom severity and frequency, the numbers of patients in group 3 (moderate persistent) and group 4 (severe) were small, and our study was not powered sufficiently to determine any statistical significance for this type of subgroup analysis. Additionally, the presence or absence of atopy was not recorded among control or asthma groups. Atopy is a known risk factor in the development of asthma and would certainly lend more information about the host factors in relation to levels of resistin. Further studies to include pulmonary function test results correlated with plasma resistin levels over time in conjunction with asthma questionnaire results would provide additional information into the role of resistin in asthma. The high degree of variability seen in the control population could potentially be attributed to other unrecognized inflammatory conditions or may simply represent a wide range of normal values for resistin similar to other biomarkers, such as B-type natriuretic peptide.

Overall, our study demonstrates a statistically significant elevation in plasma resistin associated with the presence of asthma independent of serum glucose or CRP levels. The ability to assess and monitor airway inflammation associated with asthma could conceivably improve the treatment of this

disease. The search for a better marker to predict treatment response and track disease state is essential and must continue.

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REFERENCES

1. FastStats A to Z: asthma. Available at: <http://www.cdc.gov/nchs/fastats/asthma.htm>. Accessed 15 Jan 2006.
2. Asthma in America Survey, Executive Summary. Available at: http://www.asthmainamerica.com/aaa_index.html. Accessed 15 Jan 2006.
3. Asthma in America Survey, Executive Summary. Available at: <http://www.asthmainamerica.com/slides/slide20.htm>. Accessed 15 Jan 2006.
4. Leuppi JD, Salome CM, Jenkins CR, Anderson SD, Xuan W, Marks GB, Koskela H, Brannan JD, Freed R, Andersson M, Chan HK, Woolcock AJ. Predictive markers of asthma exacerbation during stepwise dose reduction of inhaled corticosteroids. *Am J Respir Crit Care Med* 2001; 163:406–412.
5. Deykin A, Lazarus SC, Fahy JV, Wechsler ME, Boushey HA, Chinchilli VM, et al. Sputum eosinophil counts predict asthma control after discontinuation of inhaled corticosteroids. *J Allergy Clin Immunol* 2005; 115:720–727.
6. Bousquet J, Chaney P, Lacoste JY, Barneon G, Ghavanian N, Enander I, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323:1033–1039.
7. Jatakanon A, Lim S, Barnes PJ. Changes in sputum eosinophils predict loss of asthma control. *Am J Respir Crit Care Med* 2000; 161:64–72.
8. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998; 53:91–95.
9. Jeffrey PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma. An ultrastructural, quantitative study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989; 140:1745–1753.
10. Saraiva-Romanholo BM, Barnabe V, Carvalho AL, Martins MA, Saldiva PH, Nunes Mdo P. Comparison of three methods for differential cell count in induced sputum. *Chest* 2003; 124:1060–1066.
11. Vints AM, Oostveen E, Eekhout G, Smolders M, De Backer WA. Time-dependent effect of nitrate-rich meals on exhaled nitric oxide in healthy subjects. *Chest* 2005; 128:2465–2470.
12. McTernan PG, Fisher FM, Valsamakis G, Chetty R, Harte A, McTernan CL, et al. Resistin and type 2 diabetes: regulation of resistin expression by insulin and rosiglitazone and the effects of recombinant resistin on lipid and glucose metabolism in human differentiated adipocytes. *J Clin Endocrinol Metab* 2003; 88:6098–6106.
13. Steppan CM, Brown EJ, Wright CM, Baht S, Banerjee RR, Dai Cy, et al. A family of tissue-specific resistin-like molecules. *Proc Natl Acad Sci USA* 2001; 98:502–506.
14. Hartman HB, Hu X, Tyler KX, Dalal CK, Lazar MA. Mechanisms regulating adipocyte expression of resistin. *J Biol Chem* 2002; 277:19754–19761.
15. Lee JH, Chan JL, Yiannakouris N, Kontogianni M, Estrada E, Seip R, et al. Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: Cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. *J Clin Endocrinol Metab* 2003; 88:4848–4856.
16. Nagaev I, Smith U. Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. *Biochem Biophys Res Commun* 2001; 285:561–564.
17. Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, et al. Resistin is expressed in human macrophages and directly regulated by PPAR γ activators. *Biochem Biophys Res Commun* 2003; 300:472–476.

18. Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, et al. Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-[gamma] action in humans. *Diabetes* 2001; 50:2199–2202.
19. Youn BS, Yu KY, Park HJ, Lee NS, Min SS, Youn YM, et al. Plasma resistin concentrations measured by enzyme-linked immunosorbent assay using a newly developed monoclonal antibody are elevated in individuals with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2004; 89:150–156.
20. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, et al. A central role for JNK in obesity and insulin resistance. *Nature* 2002; 420:333–336.
21. Holcomb IN, Kabakoff RC, Chan B, Baker TW, Gurney A, Henzel W, et al. FIZZ1, a novel cysteine-rich secreted protein associated with pulmonary inflammation, defines a new gene family. *EMBO J* 2000; 19:4046–4055.
22. Kawanami D, Maemura K, Takeda N, Harada T, Nojiri T, Imai Y, et al. Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine-endothelial cell interactions. *Biochem Biophys Res Commun* 2004; 314:415–419.
23. Lu SC, Scieh WY, Chen CY, Hsu SC, Chen HL. Lipopolysaccharide increases resistin gene expression *in vivo* and *in vitro*. *FEBS Lett* 2002; 530:158–162.
24. Rajala MW, Lin Y, Ranalletta M. Cell type-specific expression and coregulation of murine molecule- α in adipose tissue. *Mol Endocrinol* 2002; 16:1920–1930.
25. Silswal N, Singh AK, Aruna B, Mukhopadhyay S, Ghosh S, Ehtesham NZ. Human resistin stimulates the pro-inflammatory cytokines TNF- α and IL-12 in macrophages by NF- κ B-dependent pathway. *Biochem Biophys Res Commun* 2005; 334:1092–1101.
26. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikk β . *Science* 2001; 293:1673–1677.
27. McTernan PG, McTernan CL, Chetty R, Jenner K, Fisher FM, Lauer MN, et al. Increased resistin gene and protein expression in human abdominal adipose tissue. *J Clin Endocrinol Metab* 2002; 87:2407–2410.
28. DeBrosse CW, Zimmermann N, King NE, Pope SM, Fulkerson PC, Mishra A, et al. C2 induction of resistin-like molecule beta (relm-beta) by respiratory allergen, IL-4, IL-13 and stat6 in experimental asthma. *J Allergy Clin Immunol* 2003; 111:901.
29. Guidelines for the diagnosis and management of asthma. National Heart, Lung, and Blood Institute. National Asthma Education Program. Expert Panel Report 2. Bethesda (MD): National Institutes of Health; 1997, NIH publication 97-4051.
30. NHLBI/WHO Workshop Report. Global strategy for asthma management and prevention. Global initiative for asthma. Bethesda (MD): National Institutes of Health; 1995. NIH publication 95-3659.
31. Jousilahti P, Salomaa V, Hakala K, Rasi V, Vahtera E, Palosuo T. The association of sensitive systemic inflammatory markers with bronchial asthma. *Ann Allergy Asthma Immunol* 2002; 89:381–385.
32. Humbles AA, Lu B, Nilsson CA, Lilly C, Israel E, Fujiwara Y, et al. A role for the C3a anaphylatoxin receptor in the effector phase of asthma. *Nature* 2000; 406:998–1001.
33. Krug N, Tschernig T, Erpenbeck VJ, Hohlfeld JM, Kohl J. Complement factors C3a and C5a are increased in bronchoalveolar lavage fluid after segmental allergen provocation in subjects with asthma. *Am J Respir Crit Care Med* 2001; 164:1841–1843.
34. Nakano Y, Morita S, Kawamoto A, Suda T, Chida K, Nakamura H. Elevated complement C3a in plasma from patients with severe acute asthma. *J Allergy Clin Immunol* 2003; 112:525–530.