Effect of aging on sputum inflammation and asthma control

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Background: Aged asthmatic patients experience increased morbidity and mortality. Knowledge of the aging effect on airway inflammation and asthma control is limited.

Objective: We sought to compare airway inflammation and its relationship to asthma control in aged versus younger patients and determine whether differences are asthma specific or caused by "inflamm-aging."

Methods: We performed a prospective study of aged (>60 years) and younger (21-40 years) inner-city patients with asthma. After a run-in period to control for inhaled corticosteroid use, induced sputum was collected. Age-matched nonasthmatic control subjects were included to measure age-related inflammatory changes.

Results: Aged (mean age, 67.9 ± 5.1 years; n = 35) compared with younger (mean age, 30.8 ± 5.9 years; n = 37) asthmatic patients had significantly worse asthma control and lower FEV_1. Aged asthmatic patients had higher sputum neutrophil (30.5×10^4/mL and 23.1%) and eosinophil (7.0×10^4/mL and 3.8%) numbers and percentages compared with younger patients (neutrophils, 13.0×10^4/mL [$P < .01$] and 6.9% [$P < .01$]; eosinophils, 2.0×10^4/mL [$P < .01$] and 1.2% [$P < .01$]). Aged asthmatic patients had higher sputum IL-6 ($P < .01$) and IL-8 ($P = .01$) levels. No significant inflammatory differences between aged and younger control subjects were observed. In aged asthmatic patients increased sputum IL-6 and macrophage inflammatory protein 3α/CCL20 levels were significantly associated with decreased asthma control and increased sputum neutrophil numbers and IL-1β, IL-6, and macrophage inflammatory protein 3α/CCL20 levels were associated with hospitalization.

Conclusions: The inflammatory patterns of aged versus younger asthmatic patients are associated with increased sputum neutrophil and eosinophil values and cytokine levels related to neutrophil recruitment. Differences in airway inflammation can contribute to diminished asthma control in the aged. Further understanding of asthma pathophysiology in aged patients is needed to improve management of this vulnerable population. (J Allergy Clin Immunol 2017;139:1808-18.)

Key words: *Asthma, sputum, inflammation, sensitization, inner city*

Asthma is reported in 4% to 13% of persons older than 60 years.[1-5] Not only is asthma in the aged more prevalent than previously considered but also morbidity and mortality in this group are high, with 50% to 66% of all asthma-related deaths occurring in adults older than 65 years.[2,6-9] Although coexisting lung disease,[10] comorbidities,[11,12] and undertreatment[2,8] contribute to worse outcomes, the effect of aging on asthma-related airway inflammation and its regulation is also a likely major factor in heightened morbidity. Because of increases in age of the worldwide population, the number of persons older than 60 years is projected to grow to 72 million by the year 2030,[13] and with this increase, so will the unmet needs of aged patients with asthma.

The limited data available on airway inflammation in aged adults with asthma suggest there are increased airway neutrophil number values.[14,15] However, these studies have not considered the potential effects of "inflamm-aging" (ie, low-grade basal systemic inflammation, such as increased IL-1β, IL-6, and TNF-α levels) that are often observed with aging[16] nor have they been conducted in inner-city subjects, a population with a higher asthma morbidity.[17,18] Additionally, analyses have not correlated airway inflammation with clinical features of disease or appropriately controlled for inhaled corticosteroid (ICS) use, a factor that might influence airway cellularity.[19] Establishing the characteristics of airway inflammation in aged asthmatic patients is an important step toward a greater understanding of the high morbidity and mortality rates in this population and to begin to formulate more effective treatments.

To address these unanswered questions, we recruited aged and younger asthmatic patients from an inner-city population, defined their clinical characteristics, and obtained sputum samples to measure patterns of airway inflammation. Furthermore, control populations of age-matched subjects without asthma were included to more fully assess the age-independent effects of asthma. Thus our study design allowed us to evaluate 3 aspects of aging and airway inflammation: (1) the effect of aging on asthma (ie, aged vs younger patients with asthma), (2) the effect of asthma on aging (ie, aged patients with asthma vs aged control subjects), and (3) the effect of aging itself (ie, aged vs younger control subjects).

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Abbreviations used
ACT: Asthma Control Test
BMI: Body mass index
DTT: Dithiothreitol
Foxp3: Forkhead box p3
ICS: Inhaled corticosteroid
MFI: Median fluorescence intensity
Mini-AQLQ: Mini-Asthma Quality of Life Questionnaire
MIP: Macrophage inflammatory protein
OR: Odds ratio
Treg: Regulatory T

METHODS

Patient population

Potential study participants were adults receiving medical care at a tertiary-care center in New York City and were eligible if they were between the ages of 21 and 40 years or 60 years or older, spoke English, and had mild, moderate, or severe persistent asthma, as defined according to the National Heart, Lung, and Blood Institute's Expert Panel on Asthma.[20] Subjects were classified with asthma based on the presence of current episodic respiratory symptoms in the preceding 12 months, a doctor's diagnosis of asthma, and evidence of past or present reversible airflow obstruction. Subjects were excluded if they had a physician's diagnosis of chronic obstructive lung disease or emphysema, restrictive lung disease, or other chronic respiratory illness; had a smoking history of 10 or more pack-years; had dementia; received immunosuppressive medications (excluding corticosteroids) in the past 12 months; or were pregnant or lactating. To limit confounding because of comorbidities, we excluded subjects who did not meet the eligibility criteria for immunogerontological studies, as defined in the SENIEUR protocol.[21] To adjust for potential age-related effects on airway inflammation, we recruited age-matched control subjects who met the same inclusion and exclusion criteria but did not have a history of past or current asthma.

The study protocol was approved by the institutional review board, and written informed consent was obtained from the participants before enrollment. Additional information, including subject recruitment, is provided in the Methods section in this article's Online Repository at www.jacionline.org.

Baseline assessment

Data were collected on sociodemographic characteristics, past asthma history, asthma morbidity over the 12 months before study enrollment, current asthma medications, comorbidities, and smoking history. Baseline level of asthma control was assessed with the Asthma Control Test (ACT),[22] and asthma-related quality of life was assessed with the Mini-Asthma Quality of Life Questionnaire (Mini-AQLQ).[23,24] Lung function was performed in asthmatic patients and control subjects according to the standards specified by consensus guidelines.[25] Additional information is provided in the Methods section in this article's Online Repository.

Run-in period

Asthmatic patients entered a 2-week run-in period to measure ICS adherence and adjust for potential differences in sputum inflammatory cell counts secondary to corticosteroid use. During this time, ICS adherence was determined either by attaching a DOSER inhalations tracker (MediTrack, Hudson, Mass) to the inhaler or by recording the counter numbers on the patients' ICS at the run-in visit and again at sputum collection.

Sputum induction visit

Sputum induction and processing are described in the Methods section in this article's Online Repository at www.jacionline.org.

Multiplex for detection of inflammatory cytokine proteins

Sputum supernatants were assayed for a panel of T_H17 cytokines, eotaxin-1, TGF-β, and IL-8, as described in this article's Online Repository at www.jacionline.org.

Measurement of sputum regulatory T cells

To assess whether aging alters regulation of airway inflammation, we measured regulatory T (Treg) cells from the sputum using flow cytometry, as described in the Methods section in this article's Online Repository. Treg cells were reported as the percentage of $CD3^+CD4^+$ cells expressing forkhead box P3 (Foxp3) and being $CD127^{low}$.[26]

Statistical analysis

Comparisons between groups are presented by using numbers (percentages), means (SDs), or medians (interquartile ranges) for skewed distributions. Comparison of cell counts, cytokine levels, baseline clinical characteristics, atopic sensitization, and past and present clinical features of asthma among aged versus younger asthmatic patients and aged versus younger control subjects was done by using χ^2 tests, *t* tests, or Wilcoxon tests, as appropriate. We also conducted multiple regression analyses comparing sputum cell counts and cytokine levels among aged versus younger asthmatic patients and control subjects after controlling for medication adherence, ICS dose, years with asthma, atopy, race, sex, body mass index (BMI), and pack-years of smoking.

We used linear or logistic regression (as appropriate) analyses to assess the adjusted relationship between sputum inflammatory markers (categorized as "low" vs "high" based on whether the level was less than or greater than the median) and asthma control and hospitalizations for asthma in the past 12 months among aged versus younger asthmatic patients. The model included an interaction term between age group and each marker to test whether specific cell types or cytokines have a differential effect on asthma control and odds ratios (ORs) for asthma hospitalizations according to age. Additional information is provided in the Methods section in this article's Online Repository.

RESULTS

Baseline characteristics of participants

The study sample consisted of 112 participants, including 35 aged and 37 younger asthmatic patients and 18 aged and 22 younger control subjects. Baseline characteristics of study participants according to age and asthma status are shown in Table I. Consistent with the epidemiology of an inner-city population, the cohort consisted predominantly of racial and ethnic minority groups. There were no significant differences in the distribution of sex, income, or education among aged versus younger asthmatic patients ($P > .05$ for all comparisons). Aged asthmatic patients were more likely to be non-Hispanic ($P = .05$) and insured by Medicare and Medicaid/Medicare ($P < .01$). Although the percentages of aged asthmatic patients who were allergen sensitized was high (74.3%), it was significantly lower than for younger patients (100%, $P < .01$). The distribution of allergens to which the aged and younger asthmatic patients were sensitized is shown in Table II. A higher comorbidity index score was seen in aged asthmatic patients ($P < .01$). There were no significant differences in the distribution of food allergies, eczema, rhinitis, or gastroesophageal reflux disease among aged versus younger asthmatic patients ($P > .05$ for all comparisons). There were no significant differences in the percentages of never smokers in aged compared with younger asthmatic patients (60.0% vs 78.4%, respectively; $P = .15$). The