Influences of Smoking and Aging on Allergic Airway Inflammation in Asthma

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ABSTRACT
Asthma is a heterogeneous disease with varying phenotypes and numerous risk factors. This condition results from complex interactions between genetic and environmental factors, and active smoking is one of these risk factors. The effects of aging should also be taken into account in these interactions. From an epidemiological standpoint, smokers and/or elderly patients with asthma are not small part in the total population with asthma. Furthermore, both smoking and aging are important risk factors for severe asthma. This review discusses the potential effects of smoking and aging on healthy subjects and patients with asthma, particularly from the perspective of inflammatory changes. First we show evidence that smokers and the elderly have increased neutrophil counts in their airways, which may have impacts on their clinical characteristics of elderly smokers with asthma. Secondly, on the basis of our recent findings on the interactions between smoking and aging in patients with asthma, we propose that IgE/eosinophilic inflammation should not be underestimated in elderly smokers with asthma, particularly those who are atopic. This review may expand our understanding of the effects of smoking and aging on asthma with a new perspective of an old issue.

KEY WORDS
aging, asthma, eosinophils, inflamm-aging, neutrophils, smoking

INTRODUCTION
It is well known that current smokers with asthma have poorer disease control and show an excessive declines in forced expiratory volume in 1 second (FEV1) compared with never-smokers with asthma. Smoking may also impair therapeutic responses to inhaled and oral corticosteroids in patients with asthma. Currently, mechanisms underlying the characteristics of smokers with asthma are mostly explained by the induction of neutrophilic airway inflammation and goblet cell hyperplasia, whereas studies on the relationship between smoking and eosinophilic inflammation in patients with asthma have yielded inconsistent findings. Nonetheless, considering that smoking is related to higher serum IgE levels in the general population and that relatively young patients with asthma are enrolled in studies on smokers with asthma, the involvement of IgE/eosinophilic inflammation in smokers with asthma should be re-evaluated in all age groups.

In the first part of this review, we summarize the evidence for the effects of smoking on asthma. We also address the impact of ex-smoking on the pathophysiology in this condition. Next, we review evidence for the effects of aging on asthma and offer insights into the interactions between smoking and age on IgE/eosinophilic inflammation in patients with asthma.

ASTHMA AND SMOKING
EPIDEMIOLOGY
The prevalence of smokers among adult patients with asthma is similar to that among the general popula-
tion. In Western countries, 17%-35% patients with asthma are current smokers, while 22%-43% patients are ex-smokers. In a survey of Japanese adult patients with asthma (n = 400) in 2005, Adachi et al. reported that the percentage of current smokers with asthma was 20.5%, which was similar to the general population (21.7% in 2009 according to the Ministry of Health, Labour and Welfare, Japan), while that of ex-smokers with asthma was 24.5%. In another recent study in Japan, Ikeue et al. reported that the percentage of current smokers among patients with asthma who visited emergency department due to asthma attack was 34.8%. In our university hospital, 15.0% patients with asthma were current smokers while approximately 21.2% were ex-smokers as shown later.

Passive smoking is a risk factor for the development of asthma. Studies on maternal smoking during pregnancy, parental smoking during childhood, and passive smoking in the workplace consistently demonstrate that passive smoking is related to the onset of asthma and respiratory symptoms. Recent studies, including a population-based incident case-control study, show that active smoking also causes asthma in adulthood, although it did not increase the risk of asthma onset in earlier studies. Furthermore, in a recent longitudinal study, allergic patients who smoked were more prone to newly develop asthma of greater severity. Ex-smoking may also be a risk factor for the development of asthma, as Piipari et al. showed that ex-smoking increased the risk of asthma by 1.34 in males and 2.38 in females. However, some analyses do not support this risk of asthma onset among ex-smokers.

CLINICAL CHARACTERISTICS

Current smokers with asthma consistently have more severe symptoms and poorer asthma control than never-smokers with asthma, while ex-smokers have poorer asthma control than never-smokers. Current smoking or passive smoking increases admission rates and decreases quality of life in patients with asthma. The mortality rate is higher among current smokers with asthma than among never-smokers with asthma. Current smokers are less likely to manage their asthma compared with never-smokers, possibly because they lack knowledge about asthma, use inhaled corticosteroids less frequently, and attend asthma education programs less frequently compared with never-smokers.

DECLINES IN LUNG FUNCTION

The combination of current smoking and asthma has a synergistic effect on the decline in FEV1. In a 15-year follow-up of adults with asthma in the Copenhagen City Heart Study, among male patients with asthma aged 40-59 years, current smokers and never- or ex-smokers showed an average annual decline in FEV1 of 58 ml and 33 ml, respectively.

THERAPEUTIC RESPONSES IN SMOKING ASTHMACTICS

Current smokers with asthma, and to a lesser extent, ex-smokers with asthma are resistant to oral corticosteroids, although their airways show reversibility to an inhaled short-acting β2 agonist. Similarly, current smokers with asthma are insensitive to inhaled corticosteroid treatment, particularly when low doses of inhaled corticosteroids are used. Several mechanisms for corticosteroid resistance in smokers with asthma have been suggested: (a) increased production of tumor necrosis factor-α with overexpression of glucocorticoid receptor β, (b) increased production of interleukin (IL)-4, (c) overexpression of nuclear factor-κB, (d) decreased histone deacetylase activity, and (e) increased numbers of neutrophils and CD8+ T cells.

In contrast to treatment with corticosteroids, current smokers with asthma may benefit from treatment with a leukotriene receptor antagonist. After 8 weeks of treatment with a leukotriene-receptor antagonist, morning peak flow increased to a greater extent in current smokers with asthma than in never-smokers with asthma. Smoking-induced increases in urinary excretion of leukotriene E4 in patients with asthma may have been involved in this effect.

IMMUNE SYSTEM AND AIRWAY INFLAMMATORY CELL PHENOTYPES

Cigarette smoking alters immune inflammatory responses in many ways. Smoking compromises host defense by suppressing innate immune responses in conjunction with impairing mucociliary clearance and epithelial junction. Meanwhile, cigarette smoke activates resident cells toward a pro-inflammatory status and recruits inflammatory cells into the airways. The effects of smoking on innate immunity in asthmatic airways have not been completely determined. However, it is interesting to note that among several Toll-like receptors (TLRs) that play key roles in innate immune responses, the expression of TLR2, which recognizes gram-positive bacteria, fungi, and rhinovirus capsids, may be altered in smokers with asthma compared to never-smokers with asthma.

In never-smokers with asthma, the mRNA expression of TLR2 in sputum, but not of TLR3 or TLR4, was increased compared to healthy subjects. TLR2 expression was higher during acute asthma attacks, particularly viral-induced asthma attacks, than in the stable condition. Of note, for patients with fatal asthma who smoked, the expression of TLR2, but not of TLR3 or TLR4, in the small airways was lower than that in never-smokers with fatal asthma. This was consistent with findings of decreased TLR2 expression in smokers. Although the subsequent changes in adaptive immune responses have not been shown, suppression of TLR2 may be involved in the altered immune system in smokers with asthma.
During the development of adaptive immune-inflammatory responses, dendritic cells play an important role in the differentiation of Th-1- and Th-2-type inflammation. Dendritic cells that develop under nicotine exposure have a defect in their Th-1-promoting capacity and promote Th-2 responses, which are augmented in a Th-2-biased environment. Indeed, smoking skews the immune inflammatory responses toward Th-2-type inflammation in healthy subjects. Serum immunoglobulin E (IgE) levels and blood eosinophil counts of current smokers are higher than those of never-smokers in the general population.

Cigarette smoking significantly alters the types of airway inflammatory cells. Recent studies on Th-17-type inflammation show that IL-17 levels are increased in the airways of smokers. Because IL-17 recruits and activates neutrophils, systemic and local increases in neutrophil counts in healthy current smokers may be partly induced by Th-17-type inflammation concomitant with the release of the neutrophil chemoattractant IL-8 from epithelial cells. At the same time, airway inflammation in smokers cannot be simply described as neutrophilic inflammation. Among individuals without asthma, in addition to neutrophils, the number of eosinophils that infiltrate the small airway submucosa is greater in current smokers than never-smokers and the number of neutrophils is correlated with the numbers of eosinophils.

Several studies that investigated bronchial biopsies and induced sputum samples from patients with asthma showed that smoking induced neutrophilic airway inflammation but did not aggravate eosinophilic inflammation. However, these studies excluded elderly asthma patients, presumably to exclude the comorbidity of chronic obstructive pulmonary disease (COPD). This is an important point for analysis, although it should be interpreted carefully as addressed below. In addition, Tsukioka et al. reported that total IgE levels and specific IgE levels against mites, cedar pollen, and Candida were higher in current smokers with asthma than never-smokers. In murine models of allergy, tobacco smoke exposure induces bronchial hyper-reactivity, eosinophilia, and Th2-type inflammation, which supports that IgE eosinophilic inflammation can be induced in current smokers with asthma.

Fractional exhaled nitric oxide (FeNO) is considered to be a useful biomarker of eosinophilic airway inflammation. However, cigarette smoke is known to consume NO that is produced in the airways via reactions with superoxide anion and/or peroxidase-dependent mechanisms. Therefore, FeNO levels in current smokers are low and are not considered to be a reliable marker of eosinophilic airway inflammation. Studies on FeNO levels in ex-smokers have yielded inconsistent findings.

**SMOKING CESSATION**

Needless to say, smoking cessation is the best treatment for smokers with asthma. In a cross-sectional study, the number of goblet cells and mucus-positive epithelium were increased in current smokers with asthma compared with never-smokers with asthma, however, there was no increase in ex-smokers. FEV1 in smokers with asthma improved within a week after smoking cessation, and this improvement increased further for an additional six weeks. Patients who had quit smoking for at least a year exhibited restored responses to an oral corticosteroid, with increased morning PEF values. In addition, sputum neutrophil counts were decreased within six weeks of smoking cessation. Therefore, many characteristics of smokers with asthma may be normalized after smoking cessation. However, the question remains if smoking cessation completely reverses airway inflammation or immune system responses. Indeed, the levels of several sputum mediators were not decreased after smoking cessation. In addition, the adjuvant effects of cigarette smoking on allergic subjects cannot be neglected because these effects may cause persistent eosinophilic inflammation after smoking cessation. Recall challenge with ovalbumin one month after the last concurrent exposure to smoking can result in ovalbumin-induced antigen-specific memory and significantly augments eosinophilic inflammation in mice models.

**ASTHMA IN THE ELDERLY**

**CLINICAL CHARACTERISTICS**

Asthma may emerge at any age and at a similar rate in all adults (approximately 5%-10%). Enhanced longevity in the general population may result in increased numbers of elderly patients with asthma. The term elderly adult is usually defined on the basis of chronological, biological, or sociocultural perspectives, and the cutoff age defining an individual as elderly ranges from 60 to 65 years.

Elderly patients with asthma have high rates of hospitalization and mortality, possibly because of comorbidities, underdiagnosis, and inadequate treatment. Although elderly patients with asthma are typically characterized as nonatopic, atopy is not uncommon among these patients according to some reports. Indeed, elderly patients with asthma are sensitized to allergens at a higher rate compared with age-matched controls (30% vs. 26%, respectively).

**DECLINES IN LUNG FUNCTION**

Lung function is at its maximum around 20 to 25 years of age and begins to decline thereafter, with annual declines of 25-30 ml in FEV1. Of note, the estimated rate of decline is not linear with age and can be greater in the elderly. The putative mechanisms for impairment of lung function in the elderly are a loss...
Nagasaki T et al.

Table 1  Inflammation in smoking asthmatics

<table>
<thead>
<tr>
<th>Authors, Published year</th>
<th>Subjects, Smoking status, Condition of treatment</th>
<th>Pack-years</th>
<th>Age, Mean (range) or mean ± SD</th>
<th>Samples</th>
<th>Effects of smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boulet LP, 2006</td>
<td>22 current smokers</td>
<td>14.0 ± 7.6</td>
<td>21.0 ± 10.6</td>
<td>Induced sputum</td>
<td>Neutrophil counts ↑</td>
</tr>
<tr>
<td></td>
<td>27 never-smokers</td>
<td>0 ± 0</td>
<td>36.3 ± 8.9</td>
<td></td>
<td>Eosinophil counts →</td>
</tr>
<tr>
<td></td>
<td>No use of ICS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chalmers GW, 2001</td>
<td>31 current smokers</td>
<td>21.0 ± 16.6</td>
<td>36.3 ± 10.6</td>
<td>Induced sputum</td>
<td>Neutrophils ↑</td>
</tr>
<tr>
<td></td>
<td>36 never-smokers</td>
<td>0 ± 0</td>
<td>36.0 ± 8.9</td>
<td>(both counts and proportions)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No use of ICS</td>
<td></td>
<td></td>
<td>Eosinophils ↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(both counts and proportions)</td>
<td></td>
</tr>
<tr>
<td>St-Laurent J, 2008</td>
<td>12 current smokers</td>
<td>16.7 ± 2.2</td>
<td>32.7 ± 2.3</td>
<td>Bronchial biopsies</td>
<td>Neutrophil elastase, IFN-γ, and IL-8 ↑</td>
</tr>
<tr>
<td></td>
<td>12 never-smokers</td>
<td>0 ± 0</td>
<td>25.8 ± 2.3</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>No use of ICS</td>
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<td></td>
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<tr>
<td>Broekema M, 2009</td>
<td>35 current smokers</td>
<td>3 (0-64)</td>
<td>50 (21-64)</td>
<td>Bronchial biopsies and induced sputum</td>
<td>Neutrophils → in biopsies (current and ex)</td>
</tr>
<tr>
<td></td>
<td>46 ex-smokers</td>
<td>15 (0.4-47)</td>
<td>52 (25-68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>66 never-smokers</td>
<td>0 (0-0)</td>
<td>47 (19-71)</td>
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<tr>
<td></td>
<td>44% used ICS</td>
<td></td>
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<tr>
<td>Sunyer J, 2003</td>
<td>301 current smokers</td>
<td>16.7 ± 2.2</td>
<td>32.7 ± 2.3</td>
<td>Bronchial biopsies and induced sputum</td>
<td>Neutrophils → in biopsies (current and ex)</td>
</tr>
<tr>
<td></td>
<td>406 ex-smokers, 713 never-smokers</td>
<td>0 ± 0</td>
<td>25.8 ± 2.3</td>
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<tr>
<td>Nagasaki T, 2013</td>
<td>46 current smokers</td>
<td>30 ± 19</td>
<td>47 ± 13</td>
<td>Blood</td>
<td>Neutrophil counts ↑</td>
</tr>
<tr>
<td></td>
<td>65 ex-smokers</td>
<td>27 ± 22</td>
<td>61 ± 15</td>
<td></td>
<td>Eosinophil counts ↑</td>
</tr>
<tr>
<td></td>
<td>196 never-smokers</td>
<td>0 ± 0</td>
<td>49 ± 20</td>
<td></td>
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<tr>
<td></td>
<td>No use of ICS</td>
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of chest wall compliance and decreased supporting tissue, such as elastic fibers, around alveolar ducts, which may result in enlarged air spaces and increased air trapping. Elderly patients with asthma have an accelerated rate of decline in FEV1 compared with healthy elderly individuals; furthermore, they may have pronounced impairments in the small airways. Pentosidine may be involved in one of the mechanisms underlying the accelerated decline in the lung function of elderly patients with asthma, although further studies are warranted.

**IMMUNE SYSTEM AND AIRWAY INFLAMMATORY CELL PHENOTYPES**

Immunosenescence or inflam-aging is defined as changes in innate and adaptive immune responses associated with increasing age. Age-related changes in immune function may alter susceptibilities to antigens, infection, malignancy, and autoimmunity. In the general population, serum total and allergen-specific IgE levels decrease with increasing age. Bronchoalveolar lavage fluid (BALF) samples from elderly healthy never-smokers without allergies showed increased airway neutrophils and CD4+ T cells when compared with samples from younger populations. The effects of increasing age on the balance between Th-1 and Th-2 cytokines can be altered by many factors.

There are few reports regarding age-related processes in the immune system, including airway inflammatory cell types, of patients with asthma. One study suggested that elderly patients with asthma had significantly increased percentages of sputum neutrophils. In addition, increased airway neutrophils in elderly patients with asthma corresponds to increased levels of sputum neutrophil-related mediators such as matrix metalloproteinase 9, neutrophil elastase, and IL-8, but not leukotriene B4. With regard to eosinophils, an earlier study showed that eosinophil activity was decreased in elderly patients with asthma. Antigen-sensitized and antigen-challenged aged mice showed higher numbers of eosinophils in BALF than younger mice.

Although FeNO reflects eosinophilic airway infla-
mation, some caution may be necessary when interpreting FeNO levels in the elderly. Gelb et al. showed that FeNO and alveolar NO levels increased with increasing age in healthy subjects who had never smoked.\textsuperscript{89} They speculated that the increase in NO levels in the elderly could be because of a decrease in capillary blood volume and decreased NO diffusion. Nonetheless, associations between higher FeNO levels and increasing age have not yet been established.\textsuperscript{62,65,89}

**SMOKING AND IMMUNOSENESCENCE IN PATIENTS WITH ASTHMA**

As described above, several clinical studies on asthma found that neutrophilic inflammation may be the main characteristic of current smokers with asthma.\textsuperscript{7-9} However, relatively young patients, mostly in their thirties,\textsuperscript{7-9} were examined in these studies, probably to avoid patients with COPD as a comorbidity (Table 1). Mitsunobu et al. showed that ex-smoking increased the rate of atopic predisposition among elderly patients with asthma.\textsuperscript{90} Therefore, the effects of smoking on eosinophilic inflammation in patients with asthma, including the elderly, remain undetermined.

To determine the effects of smoking on IgE/eosinophilic inflammation in elderly patients with asthma, we performed a cross-sectional analysis of the associations of serum IgE levels, blood eosinophil counts, and FeNO levels with smoking and age in steroid-naïve patients with asthma (n = 307).\textsuperscript{91} Current smokers were excluded when analyzing factors that contributed to FeNO. We found that serum IgE levels, blood eosinophil counts, and FeNO levels consistently decreased with increasing age in never-smokers (Table 2). When patients were stratified on the basis of age, IgE levels and blood eosinophil counts were higher in current smokers, followed by ex-smokers and never-smokers only in the elderly group (≥64 years of age).\textsuperscript{70} In addition, ex-smokers exhibited higher FeNO levels compared with never-smokers in the elderly group (Fig. 3). For younger patients (<64 years of age), significant differences with regard to smoking status were only ob-

<table>
<thead>
<tr>
<th>Table 2 Relationships between IgE/eosinophilic inflammation and age in never-smokers</th>
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<tr>
<td><strong>Correlation coefficient</strong></td>
</tr>
<tr>
<td>IgE, IU/mL</td>
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<tr>
<td>Blood eosinophil counts, /L</td>
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<tr>
<td>FeNO, ppb</td>
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</tbody>
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\textsuperscript{1} Log-transformed. FeNO, fractional exhaled nitric oxide. From reference 91.

**Fig. 1** Relationships between log-transformed serum IgE levels and smoking status when data were separately analyzed in the elderly patients (≥64 yr) (p = 0.003 using the Kruskal-Wallis test) and younger patients (<64 yr) with asthma. *Using the Wilcoxon rank-sum test. From reference 91.
Fig. 2  Relationships between log-transformed blood eosinophil counts and smoking status when data were separately analyzed in the elderly patients \( (p = 0.005 \text{ using the Kruskal-Wallis test}) \) and younger patients with asthma \( (p = 0.050 \text{ using the Kruskal-Wallis test}) \). *Using the Wilcoxon rank-sum test.

Fig. 3  Relationships between log-transformed fractional exhaled nitric oxide (FeNO) levels and smoking status when data were separately analyzed in the elderly and younger patients with asthma. *Using the Wilcoxon rank-sum test.
served for blood eosinophil counts ($p = 0.05$). Association analyses using continuous variables yielded similar findings, which were more pronounced when analyses were confined to atopic patients with asthma (data not shown).$^{91}$ These findings may suggest that current and ex-smoking attenuates age-related decreases in IgE levels and maintain eosinophilic inflammation, particularly in atopic asthmatics. One possible mechanism underlying persistent eosinophilic inflammation in current and ex-smokers is thymic stromal lymphopoietin (TSLP), a pro-allergic cytokine induced by smoking, viral infection, and/or allergen exposure, which promotes Th2-skewed immune responses. We found that sputum TSLP levels ($n = 139$) were weakly but positively correlated with smoking pack-years ($p = 0.29; p = 0.0005$) and the percentage of sputum eosinophils ($p = 0.17; p = 0.048$). Moreover, sputum TSLP levels in current smokers ($14.1 \pm 17.7 \text{ pg/mL}; p = 0.008$) and ex-smokers ($14.4 \pm 22.6 \text{ pg/mL}; p = 0.016$) were significantly higher than those in never-smokers ($6.4 \pm 15.7 \text{ pg/mL}$).$^{91}$ TSLP may be involved in persistent eosinophilic inflammation in current and ex-smokers.

**CONCLUSIONS**

Asthma is a common disease, even among smokers and elderly individuals. Smokers or older patients with asthma may have altered baseline airway inflammation with increased neutrophilic inflammation compared with never-smokers or younger patients. However, it is possible that current smoking and ex-smoking attenuates age-related decreases in IgE levels and maintains eosinophilic inflammation, particularly in atopic patients with asthma. Clinically, the presence of both neutrophilic and eosinophilic inflammation should be fully recognized during the management of elderly smokers with asthma. This review may expand our understanding on the effects of smoking and aging on asthma by offering insights into their interactions in patients with atopic asthma.

**ACKNOWLEDGEMENTS**

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**REFERENCES**

21. Plaschke P, Janson C, Normann E, Bjornsson E, Ellbjar S, Jarvholm B. Onset and remission of allergic rhinitis and asthma and the relationship with atopic sensitization
34. Lazarus SC, Chinchilli VM, Rollings NJ et al. Smoking affects response to inhaled corticosteroids or leukotriene receptor antagonists in asthma. Am J Respir Crit Care Med 2007;175:783-90.


