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ORIGINAL ARTICLE **Asthma and Rhinitis**

Smoking attenuates the age-related decrease in IgE levels and maintains eosinophilic inflammation

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Summary

Background Epidemiological studies have shown that smoking increases the propensity for atopy and asthma. However, the effects of smoking on atopy and eosinophilic inflammation in asthmatics, including the elderly, remain unknown.

Objective To determine the effects of smoking on serum immunoglobulin E (IgE) levels and eosinophilic inflammation in asthmatics of all ages.

Methods The associations of serum IgE levels, blood eosinophil counts and fractional exhaled nitric oxide (FeNO) levels with smoking and age in steroid-naive asthmatics were cross-sectionally assessed (n = 307). Levels of sputum eosinophil and thymic stromal lymphopoietin (TSLP) that promotes Th2 inflammation were also analysed. Current smokers were excluded when analysing contributing factors of FeNO.

Results Levels of serum IgE, blood eosinophil and FeNO decreased with increasing age in never-smokers, whereas decrease in serum IgE levels with increasing age was not observed in current smokers. In addition, current smoking was associated with higher blood eosinophil counts. In atopic asthmatics, age-related declines in serum IgE levels were less steep in ex-smokers than in never-smokers, and atopic ex-smokers with asthma showed higher blood eosinophil counts and higher FeNO irrespective of age. Lastly, sputum TSLP levels were associated with sputum eosinophil proportions and pack-years. Current and ex-smokers had higher TSLP levels than never-smokers.

Conclusions and Clinical Relevance In steroid-naive asthmatics, smoking may attenuate the age-related decrease in IgE levels and maintain eosinophilic inflammation, in which TSLP may be involved.

Keywords asthma, ex-smoking, immunosenescence, nitric oxide, thymic stromal lymphopoietin

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Introduction

Asthma is a chronic inflammatory condition characterized by an infiltration of eosinophils, Th2 lymphocytes and mast cells within the airways. The degree of eosinophilic inflammation is closely related to the clinical features of asthma, including its symptoms, disease severity, reversible airflow limitation and airway hyperresponsiveness [1]. Identifying factors that may initiate or augment Th2/eosinophilic inflammation is important in the management of asthma. *In vitro* [2] and animal [3] studies suggest that active exposure to cigarette smoke may be one such factor. However, the effects of smoking on IgE/eosinophilic inflammation in patients with asthma remain unclear.

It is well known that asthmatic smokers have more severe asthma-related symptoms [4] and show an accelerated decline in forced expiratory volume in 1 s (FEV_1) compared with asthmatics that do not smoke. Recent studies on asthmatic smokers have shown that neutrophilic inflammation may be a key feature of smoker asthma [5–7] and eosinophilic inflammation is not aggravated by smoking [8, 9]. In these studies, relatively young patients, mostly in their thirties

[5–8], or asthmatic smokers with relatively few pack-years [9] were examined. Conversely, in general population, serum immunoglobulin E (IgE) levels [10–12] and blood eosinophil counts [12–14] of smokers are higher than those of non-smokers. Therefore, the effects of smoking on serum IgE levels and eosinophilic inflammation in patients with asthma, not limited only to young patients, need to be further elucidated.

Immunosenescence or inflamm-ageing, which refers to the ageing of the immune system, is a recent concept [15]. For instance, serum total IgE levels are now known to decrease with increasing age [10, 15, 16]. On the basis of the concept of immunosenescence and the possible contribution of smoking to the elevation of serum IgE levels and blood eosinophil counts in general population, we hypothesized that serum IgE levels and eosinophilic inflammation decreases with increasing age in never-smokers with asthma, whereas such decreases are not present in asthmatics that currently smoke or have previously smoked. The main aim of our study was to determine the effects of smoking and age on serum IgE levels and eosinophilic inflammation in patients with asthma of all ages. The contribution of thymic stromal lymphopoietin (TSLP), which is induced by smoking and leads to dendritic cells favouring and maintaining a Th2 immunity [17], was also examined.

Material and methods

For full details see Supporting Information.

Subjects

This study was a cross-sectional study on adult patients with asthma that were newly referred to the Asthma Clinic of Kyoto University Hospital between June 2006 and October 2011. Asthma was newly diagnosed according to the American Thoracic Society criteria [18]. The diagnosis of asthma was made independent from this study and the presence of atopy, levels of serum IgE and blood eosinophil counts were not considered at the time smokers were assigned to this study. Patients were not treated with a steroid or leukotriene antagonist, and demonstrated normal chest radiographic findings. Ex-smokers were defined as those who had stopped smoking for at least 1 year. Patients with asthma who had smoked less than 5 pack-years were excluded. Smoking status and the presence of allergic rhinitis and childhood asthma were evaluated via a self-reported questionnaire. The study protocol was approved by the Ethics Committee of Kyoto University (approval number E1225), and written informed consent was obtained from all subjects.

Measurements

Patients underwent a work-up, including a physical examination, blood tests, chest radiographs, fractional exhaled nitric oxide (FeNO) concentration measurements, pulmonary function tests and sputum induction.

Total and specific serum IgE antibody titres were measured via radioimmunosorbent testing (Pharmacia Diagnostics, Uppsala, Sweden). Patients were considered atopic when one or more specific IgE antibodies against common inhaled allergens were positive. FeNO at a constant exhalation flow rate of 50 mL/s, as an alternative marker of eosinophilic airway inflammation [19], was measured with a chemiluminescence analyser (NOA 280, Sievers, Boulder, CO, USA) [20], according to the current guidelines [21].

Pre-bronchodilator forced vital capacity (FVC), FEV_1, and mid-forced expiratory $flow_{25-75\%}$ ($FEF_{25-75\%}$) were tested using a ChestGraph HI-701 spirometer (Chest MI Corp, Tokyo, Japan), according to the guidelines of the American Thoracic Society [22].

Sputum induction and processing were performed according to the slightly modified methodology of Pin et al. [23]. Cell differentials were determined by counting at least 400 non-squamous cells on each sputum slide. Sputum supernatants were stored at −20°C for later use. TSLP concentrations in sputum supernatants were measured with an enzyme-linked immunosorbent assay kit (R&D Systems, Inc., Minneapolis, MN, USA), according to the manufacturer's instructions. A spike-back analysis using exogenous TSLP resulted in greater than 70% recovery.

Statistical analysis

Statistical analyses were performed with JMP system version 8 (SAS Institute Inc., Cary, NC, USA). Data are expressed as means ± standard deviation. Serum IgE levels, blood cell counts or proportions, and FeNO levels were log-transformed to achieve normal distributions. Two or more groups were compared using the Wilcoxon rank-sum test, Kruskal–Wallis test, analysis of variance (ANOVA) or χ^2 test, where appropriate. The Spearman or Pearson correlation coefficients were used to analyse the relationships among data, where appropriate. Stepwise multivariate regression analysis was performed to determine variables predictive of serum IgE levels, blood eosinophil counts and FeNO levels, including gender, age, smoking status, atopic status and second order interactions between explanatory variables. Analysis of covariance (ANCOVA) was used to analyse associations of serum IgE levels, blood eosinophil counts and FeNO levels with smoking status and age. Post hoc analyses with the Bonferroni correction for ANOVA and ANCOVA were conducted using StatView software 5.0

(SAS Institute Inc.). Current smokers were excluded when analysing the contributing factors to FeNO as FeNO levels in current smokers are low and are not considered a reliable marker of eosinophilic airway inflammation [19]. A *P*-value of < 0.05 was considered significant.

Results

Subject characteristics based on smoking status

The clinical characteristics of the study subjects are presented in Table 1 ($n = 307$). More males had ever smoked than females. Ex-smokers with asthma were the oldest, whereas current smokers were the most likely to be atopic and showed the highest blood neutrophil counts of all groups. In unadjusted analyses, log-transformed serum IgE levels and blood eosinophil counts were the highest in current smokers, followed by ex-smokers, and the lowest in never-smokers. Ex-smokers had lower $FEF_{25-75\%}$ than never-smokers. Sixteen patients that were either current or ex-smokers had FEV_1/FVC less than 70%.

Associations between clinical indices and serum IgE levels, blood eosinophil counts or FeNO levels

The correlations between various clinical indices and total serum IgE levels, blood eosinophil counts or FeNO levels are shown in Tables 2, 3 and 4 respectively. The presence of atopy, male gender, higher sputum eosinophil proportions and narrower airway calibre were associated with higher serum IgE levels, blood eosinophil counts and FeNO levels. Current smoking and pack-years were also related to higher serum IgE levels and blood eosinophil counts. Younger age was associated with higher serum IgE levels.

Multivariate analysis revealed that higher IgE levels were associated with atopy, male gender, age, a negative interaction between age and atopy, and a positive interaction between age and current smoking (Table 2). Higher blood eosinophil counts were also associated with higher serum IgE levels and current smoking (Table 3). Similarly, higher serum FeNO levels were associated with higher IgE levels, male gender and a negative interaction between age and serum IgE levels (Table 4). In atopic patients, higher FeNO levels were

Table 1. Patient characteristics

	Current smokers ($n = 46$)	Ex-smokers ($n = 65$)	Never-smokers ($n = 196$)	*P*-value*
Gender (F/M)	17/29	20/45	141/55	< 0.0001
Age, year	47 ± 13	61 ± 15†	49 ± 20	< 0.0001**
(range)	(24–74)	(26–98)	(16–95)	
Atopy,%	87	58	75	0.003
Allergic rhinitis,%	45	40	43	0.97
Childhood asthma,%	19	26	22	0.81
Disease duration, year	2.0 ± 5.8	3.0 ± 8.4	2.7 ± 6.1	0.43‡
Pack-years	30 ± 19	27 ± 22	0 ± 0	–
Serum IgE, IU/mL	822 ± 1819§	670 ± 2043	569 ± 1740¶	0.009**
WBC, cells/L	8,400 ± 2484††	6,372 ± 1652	6,130 ± 1706	<.0001**
Eosinophils,%	5.2 ± 4.1	6.2 ± 5.2	4.7 ± 4.3‡‡	0.048**
Eosinophils, cells/L	416 ± 317	414 ± 416	302 ± 327§§	0.002**
Neutrophils,%	56.5 ± 11.1	55.3 ± 8.6	56.6 ± 10.3	0.95**
Neutrophil, cells/L	4,793 ± 2012¶¶	3,535 ± 1139	3,501 ± 1266	< 0.0001**
FeNO, p.p.b.	55 ± 56	70 ± 73	57 ± 60	–
FEV_1 (% predicted)	91.3 ± 17.4	92.5 ± 23.1	94.2 ± 22.1	0.55‡
FEV_1/FVC,%	78.5 ± 13.7	79.5 ± 17.9	79.9 ± 13.2	0.36‡
$FEF_{25-75\%}$ (% predicted)	67.0 ± 26.2	58.8 ± 28.1***	72.8 ± 30.9	0.004‡
Induced sputum	$n = 23$	$n = 32$	$n = 101$	0.09‡
Eosinophils,%	17.0 ± 18.4	13.8 ± 21.3	11.5 ± 20.0	
Neutrophils,%	55.8 ± 25.5	63.6 ± 24.5	63.3 ± 23.6	0.45‡

Results are presented as means ± SD. Childhood asthma were all remitting/relapsing. FeNO; fractional concentration of exhaled nitric oxide.
*P-values were for comparisons among the three groups of current smokers, ex-smokers and never-smokers; †$P < 0.0001$ vs. current smokers or never-smokers by the Bonferroni correction; ‡by the Kruskal–Wallis test; §$P = 0.046$ vs. ex-smokers, $P = 0.002$ vs. never-smokers; ¶missing in one patient; **by ANOVA, after data were log-transformed except for age; ††$P < 0.0001$ vs. ex-smokers or never-smokers; ‡‡$P = 0.012$ vs. ex-smokers; §§$P = 0.003$ vs. current smokers, $P = 0.009$ vs. ex-smokers; ¶¶$P < 0.001$ vs. ex-smokers or never-smokers; *$^{**}P = 0.001$ vs. never-smokers by the Wilcoxon rank-sum test.

Table 2. Univariate and multivariate analyses for the relationships between log-transformed serum IgE and various clinical indices

	Univariate analysis		Multiple regression	
	Correlation coefficient	*P*-value	Estimate (SE)	*P*-value
Gender	0.27	< 0.0001	0.35 (0.07)	< 0.0001
Age, year	−0.24	< 0.0001	0.009 (0.004)	0.033
Smoking	0.17	0.004	–	–
Pack-years	0.13	0.028		
Atopy	0.50	< 0.0001	0.86 (0.09)	< 0.0001
Disease duration, year	0.02	0.78		
FEV_1 (% predicted)	−0.24	< 0.0001		
FEV_1/FVC,%	−0.14	0.014		
$FEF_{25-75\%}$ (% predicted)	−0.22	0.0002		
Sputum eosinophils,%	0.35	< 0.0001		
Interaction between age and atopy			−0.013(0.005)	0.004
Interaction between age and current smoking			0.008 (0.004)	0.031

Gender (male = 1, female = 0), Smoking (current smoking = 2, ex-smoking = 1, never-smoking = 0), Atopy (atopy = 1, non-atopy = 0). Gender, age, smoking status, atopic status and second order interactions between explanatory variables were included for the multivariate regression analysis.

Table 3. Univariate and multivariate analyses for the relationships between log-transformed blood eosinophil counts and various clinical indices

	Univariate analysis		Multiple regression	
	Correlation coefficient	*P*-value	Estimate (SE)	*P*-value
Gender	0.20	0.0005	–	–
Age, year	−0.10	0.08	–	–
Smoking	0.20	0.0003	0.07 (0.02)	0.005
Pack-years	0.17	0.003		
Atopy	0.22	0.0001	–	–
Disease duration, year	−0.05	0.41		
*Serum IgE, IU/mL	0.39	< 0.0001	0.22 (0.03)	< 0.0001
FEV_1 (% predicted)	−0.21	0.0002		
FEV_1/FVC,%	−0.19	0.001		
$FEF_{25-75\%}$ (% predicted)	−0.27	< 0.0001		
Sputum eosinophils,%	0.61	< 0.0001		

*log-transformed.
Gender (male = 1, female = 0), Smoking (current smoking = 2, ex-smoking = 1, never-smoking = 0), Atopy (atopy = 1, non-atopy = 0). Gender, age, smoking status (current smoking vs. ex- and never-smoking), atopic status, serum IgE levels and second order interactions between explanatory variables were included for the multivariate regression analysis.

Table 4. Univariate and multivariate analyses for the relationships between log-transformed fractional exhaled nitric oxide levels and various clinical indices of never- and ex-smokers

	Univariate analysis		Multiple regression	
	Correlation coefficient	*P*-value	Estimate (SE)	*P*-value
Gender	0.18	0.003	0.09 (0.05)	0.045
Age, year	−0.09	0.16	–	–
Smoking	0.09	0.16	–	–
Pack-years	0.07	0.26		
Atopy	0.27	< 0.0001	–	–
Disease duration, year	0.03	0.61		
*Serum IgE, IU/mL	0.40	< 0.0001	0.27 (0.07)	0.0003
FEV_1 (% predicted)	−0.26	< 0.0001		
FEV_1/FVC,%	−0.13	0.044		
$FEF_{25-75\%}$ (% predicted)	−0.26	< 0.0001		
Sputum eosinophils,%	0.68	<.0001		
Interaction between age and *serum IgE			−0.004 (0.002)	0.009

*log-transformed.
Gender (male = 1, female = 0), Smoking (ex-smoking = 1, never-smoking = 0), Atopy (atopy = 1, non-atopy = 0).
Current smokers were excluded from the analysis.
Gender, age, smoking status, atopic status, serum IgE levels and second order interactions between explanatory variables were included for the multivariate regression analysis.

associated with higher serum IgE levels ($P < 0.0001$) and ex-smoking ($P = 0.0498$), as well as a negative interaction between age and serum IgE levels ($P = 0.035$).

Associations of serum IgE levels, blood eosinophil counts, and FeNO levels with age and smoking status

In never-smokers, serum IgE levels ($r = -0.31$, $P < 0.0001$), blood eosinophil counts ($r = -0.16$, $P = 0.028$) and FeNO levels ($r = -0.14$, $P = 0.05$) were all weakly but significantly associated with age.

We also stratified patients according to their age. In elderly asthmatics ($\geq$ 64 years of age) [24], log-transformed serum IgE levels ($P = 0.003$, Kruskal–Wallis test) and blood eosinophil counts ($P = 0.005$, Kruskal–Wallis test) were the highest in current smokers ($n = 7$), followed by ex-smokers ($n = 33$), and were the lowest in never-smokers ($n = 50$). FeNO levels were significantly higher in ex-smokers than in never-smokers ($P = 0.014$, Wilcoxon rank-sum test); however, in younger asthmatics (< 64 years of age), significant differences with regard to smoking status were only observed in blood eosinophil counts ($P = 0.050$, Kruskal–Wallis test) (Figs. S1, S2 and S3).

To determine the associations of serum IgE levels, blood eosinophil counts and FeNO levels with age and smoking status more clearly, in particular with ex-smoking, ANCOVA was then performed in atopic and non-atopic patients separately. In atopic patients (n = 224), there was a significant positive interaction between age and smoking status (current and ex-smoking) in relation to higher serum IgE levels (P for interaction = 0.044) (Fig. 1). In addition, ex-smokers had significantly higher blood eosinophil counts (P = 0.002) (Fig. 2) and a trend towards higher FeNO levels (P = 0.08) than never-smokers without any interactions between age and smoking status. In non-atopic patients, there were no significant associations between age and serum IgE levels, blood eosinophil counts and FeNO levels across smoking status.

Relationships between sputum TSLP levels and various clinical indices

Sputum TSLP levels were measured in 139 patients, and were found to be positively correlated with pack-years and smoking status, and negatively correlated with FEV_1/FVC (Table 5). There was also a weak association between sputum TSLP levels and sputum eosinophil proportions, which was primarily exhibited by the atopic patients (ρ = 0.23, P = 0.024, n = 100).

Sputum TSLP levels in current smokers (14.1 ± 17.7 pg/mL, P = 0.008) and ex-smokers (14.4 ± 22.6 pg/mL, P = 0.016) were significantly higher than those of never-smokers (6.4 ± 15.7 pg/mL) (P = 0.008, Kruskal–Wallis test).

Discussion

To the best of our knowledge, this is the first study that comprehensively demonstrates the associations of serum IgE levels and eosinophilic inflammation with smoking and age in newly diagnosed steroid-naïve asthmatic patients. Serum IgE levels, blood eosinophil counts and FeNO levels consistently decreased with increasing age in never-smokers, whereas decreases in serum IgE levels with increasing age were not observed in current smokers. In addition, current smoking and higher IgE levels were independent risk factors for higher blood eosinophil counts. When analysis was confined to atopic patients with asthma, higher FeNO levels were associated with ex-smoking, and there was a positive interaction between age and current or ex-smoking in relation to higher IgE levels. Moreover, blood eosinophil counts in atopic ex-smokers were significantly higher than those of atopic never-smokers irrespective of age. Lastly, current smokers and

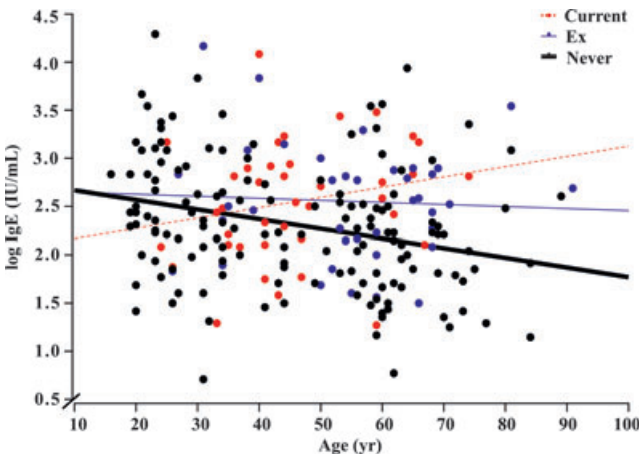

Fig. 1. Associations of log-transformed serum immunoglobulin E levels with age and smoking status in patients with atopic asthma.

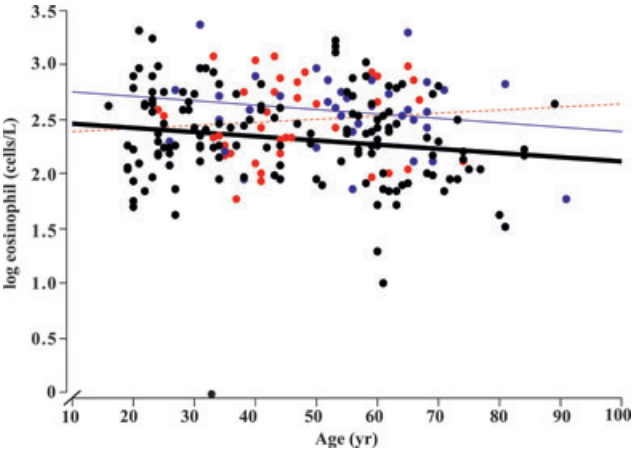

Fig. 2. Associations of log-transformed blood eosinophil counts with age and smoking status in patients with atopic asthma.

Table 5. Univariate analyses for the relationships between sputum TSLP levels and various clinical indices

	Correlation coefficient	*P*-value
Gender	0.09	0.28
Age, year	0.15	0.08
Smoking	0.27	0.001
Pack-years	0.29	0.0005
Atopy	−0.04	0.67
Disease duration, year	0.07	0.42
*Serum IgE, IU/mL	0.14	0.10
*Blood Eosinophil counts, cells/L	0.12	0.18
FEV_1 (% predicted)	−0.04	0.65
FEV_1/FVC,%	−0.26	0.002
$FEF_{25-75\%}$ (% predicted)	−0.13	0.12
Sputum eosinophils,%	0.17	0.048
*,†FeNO, p.p.b.	0.08	0.39

*log-transformed, †current smokers were excluded from the analysis.
TSLP, thymic stromal lymphopoietin; FeNO, fractional concentration of exhaled nitric oxide.
Gender (male = 1, female = 0), Smoking (current smoking = 2, ex-smoking = 1, never-smoking = 0), Atopy (atopy = 1, non-atopy = 0).

ex-smokers had significantly higher sputum TSLP levels than never-smokers. These findings may suggest that current and ex-smoking, particularly in atopic asthmatics, appeared to attenuate the age-related decrease in IgE levels and maintain eosinophilic inflammation, in which TSLP may be involved.

The associations between current smoking and propensity for atopy and asthma have been extensively examined in epidemiological studies. Most of these studies found that serum IgE levels [10–12] and blood eosinophil counts [12–14] in current smokers were higher than those in never-smokers, which is consistent with our findings in steroid-naïve asthmatic patients. Although the relationships between smoking and development of sensitization to a specific allergen are controversial [25], current smoking may act as an adjuvant for allergens [17, 26].

The present findings, specifically that higher IgE levels are associated with a negative interaction between age and atopy, agree with those of a community cohort study that reported decreases in serum IgE levels with increasing age only in the atopic population [10]. Thus, the augmenting effect of atopy on serum IgE levels was attenuated with increasing age, and the net levels of serum IgE decreased with increasing age in atopic patients, indicating the presence of immunosenescence. Furthermore, we were the first to find that there was a positive interaction between age and current smoking in relation to higher IgE levels; decreases in serum IgE levels with increasing age were not observed in current smokers. When analysis was confined to atopic patients, we found that there was a positive interaction between age and current or ex-smoking in relation to higher serum IgE levels. These results are in line with those of Mitsunobu and colleagues who found that ex-smoking increased atopic predisposition [27]. These findings suggest that current smoking and, to a lesser extent, ex-smoking may offset the decrease in serum IgE levels with increasing age and maintain elevated serum IgE levels in elderly asthmatics, particularly if they are atopic.

Higher serum IgE levels and current smoking were also found to be independently associated with higher blood eosinophil counts in asthmatics. Moreover, in atopic asthmatic patients, blood eosinophil counts in ex-smokers were significantly higher than in never-smokers irrespective of age, indicating a potential role of ex-smoking in higher blood eosinophil counts in atopic asthmatics. Only one study to date has examined the association between blood eosinophil proportions and smoking in asthma. In contrast to our study, Sunyer and colleagues reported that current smoking might attenuate eosinophilic inflammation in patients with asthma [8]. In their study, however, they examined relatively young patients in their thirties that had low average blood eosinophil proportions ranging from 2.58 to 2.91%. Consequently, their findings are not easily comparable to those of our study.

Consistent with the findings of blood eosinophil counts, ex-smoking was found to be independently associated with higher FeNO levels in atopic asthmatics, suggesting that ex-smoking may augment both systemic and airway eosinophilic inflammation in atopic asthmatics. In contrast to our study, two epidemiological studies have demonstrated that FeNO levels in ex-smokers are lower than those in never-smokers [28, 29]. However, in one of these studies [28], mean FeNO levels in never-smokers (22.8 p.p.b.) were less than half of our levels, and in the other study [29], the frequency of atopic subjects (46.4%) was lower than that in our study. Therefore, the discrepancies between our findings and those of the epidemiological studies may be related to the degree of basal inflammation in the airways or the atopic status in the studied populations. The mechanisms underlying the augmenting effects of ex-smoking on eosinophilic inflammation in atopic asthma remain unknown. However, we speculate that the effects of smoking on IgE/eosinophilic inflammation in atopic subjects may be sustained even after cessation of smoking, potentially via the induction of memory T-cells. A previous murine study demonstrated that recall challenge with ovalbumin 1 month after the last concurrent exposure to smoking and ovalbumin induced antigen-specific memory and significantly augmented eosinophilic inflammation [26].

Thymic stromal lymphopoietin, an important mediator in asthma [30] and pro-allergic reactions [31], is induced via smoking in airway epithelial cells [17, 31]. Although the sample size was limited, the elevation of sputum TSLP levels in ex-smokers and their association with sputum eosinophil proportions suggest that TSLP may have a role in increasing eosinophil counts and/or FeNO levels in ex-smokers, particularly if they are atopic. The mechanisms underlying the elevation of sputum TSLP levels in smokers remain unknown, but we speculate that epigenetic modification of epithelial cells might be involved.

Lastly, we do not deny the concrete evidence that cigarette smoke induces neutrophilic airway inflammation in asthma, and induces a phenotype similar to chronic obstructive pulmonary disease [5–7]. Indeed, blood neutrophil counts in current smokers with asthma were the highest of all three patient groups. However, there were no differences in the neutrophil proportions between current smokers and never-smokers in this study, which may be partly related to the age of our studied population. The average ages of current smokers and never-smokers in our study were 47 years and 49 years, respectively, whereas in earlier studies [5, 6] patients were in their thirties. In never-smoker

asthmatics, the proportions of sputum neutrophils were reportedly higher in older patients than in younger patients [32], which suggests that a sharp difference in the proportions of sputum neutrophils between current smokers and never-smokers would more likely be observed in a younger population. In addition, the proportion of sputum neutrophils in current smokers in our study may be superficially decreased because of the relative increase in the proportion of their sputum eosinophils. Taken together, the lack of a difference in the proportions of sputum neutrophils between current smokers and never-smokers in this study and some of the discrepancies between our findings and those of earlier clinical studies [8, 9] may be explained by the interactions between smoking status and ageing.

The limitations of this study are as follows: (1) This was not a cohort study, and patients from different generations may have different backgrounds, including environmental levels of allergens and/or chemical compounds, which in turn may have acted as additional adjuvants. In addition, whether the development of atopy was preceded by smoking or *vice versa* was unclear. (2) Smoking status was evaluated via a self-reported questionnaire. However, to minimize ambiguous cases, we excluded smokers with less than 5 pack-years. (3) Some of the older, smoking and ex-smoking patients with asthma may have been complicated with chronic obstructive pulmonary disease, as 16 (14%) of current or ex-smokers in this study had a FEV_1/FVC of less than 70%. However, we often encounter these patients in real-world clinical situations and do not think that the inclusion of these patients critically affected our conclusions. Despite these limitations, the strength of this study was that we evaluated steroid-naïve asthmatics of all ages and performed interaction analysis to find previously unknown associations.

In conclusion, we have demonstrated that serum IgE levels and eosinophilic inflammation decrease with increasing age in never-smokers, whereas current and ex-smoking, particularly in atopic patients, may have attenuated immunosenescence and maintain eosinophilic inflammation potentially via TSLP. We conclude that the presence of eosinophilic inflammation should be fully recognized in the management of elderly smokers, particularly if they are atopic.

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Conflicts of interest

The authors declare that they have no relevant conflicts of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Relationships between log-transformed serum IgE levels and smoking status when data were separately analysed in the elderly patients ($\geq$ 64 year) ($P = 0.003$ using the Kruskal–Wallis test) and younger patients (< 64 year) with asthma. *using the Wilcoxon rank-sum test.

Figure S2. Relationships between log-transformed blood eosinophil counts and smoking status when data were separately analysed in the elderly patients ($P = 0.005$ using the Kruskal–Wallis test) and younger patients with asthma ($P = 0.050$ using the Kruskal–Wallis test). *using the Wilcoxon rank-sum test.

Figure S3. Relationships between log-transformed fractional exhaled nitric oxide (FeNO) levels and smoking status when data were separately analysed in the elderly and younger patients with asthma. *using the Wilcoxon rank-sum test.

Data S1. Smoking attenuates the age-related decrease in IgE levels and maintains eosinophilic inflammation in patients with asthma.

Table S1. Multivariate regression analyses for predictors of log-transformed fractional exhaled nitric oxide levels in atopic and non-atopic patients.