Ann Allergy Asthma Immunol 119 (2017) 408–414

Contents lists available at ScienceDirect

Sensitization to *Staphylococcus aureus* enterotoxins in smokers with asthma

Tadao Nagasaki, MD, PhD [*]; Hisako Matsumoto, MD, PhD [*]; Tsuyoshi Oguma, MD, PhD [*]; Isao Ito, MD, PhD [*]; Hideki Inoue, MD, PhD [*]; Toshiyuki Iwata, MD, PhD [*]; Tomoko Tajiri, MD, PhD [*]; Yoshihiro Kanemitsu, MD, PhD [*,†]; Yumi Izuhara, MD, PhD [*]; Chie Morimoto, MD [*]; Yumi Ishiyama, MD [*]; Hironobu Sunadome, MD [*]; Akio Niimi, MD, PhD [*,†]; Toyohiro Hirai, MD, PhD [*]

[*] *Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan*
[†] *Department of Respiratory Medicine, Allergy and Clinical Immunology, Graduate School of Medical Science, Nagoya City University, Aichi, Japan*

ARTICLE INFO

Article history:
Received for publication April 30, 2017.
Received in revised form June 29, 2017.
Accepted for publication August 4, 2017.

ABSTRACT

Background: Sensitization to *Staphylococcus aureus* enterotoxins (SEs) augments eosinophilic inflammation in asthma. Recent epidemiologic studies demonstrate that sensitization to SEs is increased in healthy smokers; however, there is no evidence on the association between sensitization to SEs and eosinophilic inflammation in smokers with asthma.

Objective: To clarify the role of SEs on clinical indexes, including eosinophilic inflammation and lung function in smokers with asthma.

Methods: The frequency of atopic sensitization to SEs was examined in adult patients with asthma. In current or ex-smokers with asthma, the association of sensitization to SEs with eosinophilic inflammation, airflow limitation, or treatment steps was determined. Clinical indexes were examined at the first visit, and treatment steps were assessed 6 months after enrollment.

Results: Overall, 23 current smokers, 40 ex-smokers, and 118 never smokers with asthma were enrolled. The frequency of sensitization to SEs, but not to other aeroallergens, was significantly higher in current, ex-, and never smokers, in decreasing order. In current or ex-smokers with asthma, patients with sensitization to SEs exhibited higher serum levels of total and specific IgE to aeroallergens, higher blood eosinophil counts, greater airflow limitation, and more severe disease 6 months later than those without sensitization to SE. A longer smoking abstinence period was associated with serum specific IgE levels to SEs, and 3 years was the best cutoff of abstinence period to predict the absence of sensitization to SEs.

Conclusion: Sensitization to SEs is increased in smokers with asthma, and it may be a marker of eosinophilic inflammation and severe asthma in smokers with asthma.

Trial Registration: umin.ac.jp Identifier: UMIN000007818.

Introduction

Staphylococcus aureus is an opportunistic human pathogen and a commensal on human skin. It is frequently found in the nose. Accumulating evidence on *S aureus* enterotoxins (SEs) in allergic conditions suggests that the sensitization to SEs modifies the pathophysiologic findings of asthma and chronic rhinosinusitis with nasal polyposis.[1–3] SEs induce human dendritic cells to undergo a type 2 cell polarization by acting as superantigens[4] and promote type 2 or eosinophilic inflammation in both in vitro[4–7] and in vivo studies.[8,9] Huvenne et al[10] found that the concomitant application of ovalbumin and SE type B (SEB) facilitated sensitization to ovalbumin and eosinophilic airway inflammation. Furthermore, SEs induce the production of their specific IgE antibodies, and sensitization to SEs is associated with asthma severity[11,12] and type 2 or eosinophilic inflammation, particularly in late-onset asthma or seemingly intrinsic asthma, as revealed in human studies.[13,14] Epidemiologic studies from Europe and Korea revealed that sensitization to SEs was significantly associated with asthma in the

Reprints: Hisako Matsumoto, MD, PhD, Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan; E-mail: hmatsumo@kuhp.kyoto-u.ac.jp.

Disclosures: Authors have nothing to disclose.

Funding Sources: This study was partly supported by grant JP16K19450 from the Grants-in-Aid for Scientific Research (KAKENHI) program of the Japan Society of the Promotion of Science.

http://dx.doi.org/10.1016/j.anai.2017.08.001

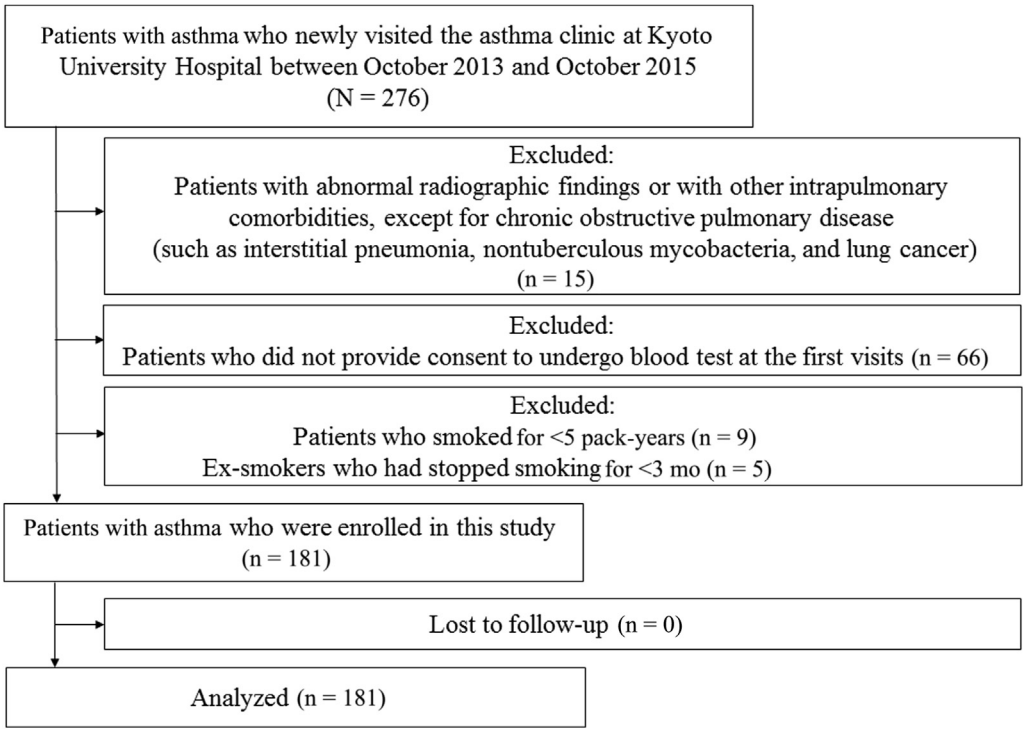

Figure 1. CONSORT flow diagram.

general population.[15,16] More importantly, current smoking was associated with the frequent sensitization to SEs in epidemiologic studies.[15,16]

It is well known that smoking increases airway inflammation, particularly neutrophilic inflammation, in patients with asthma.[17–19] Moreover, exposure to SEB alone induces neutrophilic inflammation in animal models[20]; however, when considering the frequent sensitization to SEs in smokers in the general population, it is plausible that sensitization to SEs is increased in smokers with asthma and may augment IgE or eosinophilic inflammation. This finding is supported by previous studies[21,22] demonstrating that not only neutrophilic but also IgE or eosinophilic inflammation is increased in the airways of smokers with asthma. Therefore, the effects of sensitization to SEs in smokers with asthma remain unknown. In this study, we aimed to clarify the role of sensitization to SEs in smokers with asthma by assessing the frequency of sensitization to SEs in current and ex-smokers with asthma and the effects of sensitization to SEs on inflammation and disease severity.

Methods

Adult patients with asthma who had never smoked or had smoked for more than 5 pack-years were enrolled at their first visit to the asthma clinic at Kyoto University Hospital between October 2013 and October 2015, irrespective of their treatment condition. Asthma was diagnosed according to the American Thoracic Society criteria.[23] Ex-smokers were determined as those who had stopped smoking for at least 3 months. Patients who had abnormal radiographic findings and other pulmonary diseases, except for chronic obstructive pulmonary disease (COPD), as comorbid conditions were excluded. The smoking status and presence of allergic rhinitis, atopic dermatitis, and sinusitis were based on self-reported questionnaires and medical history interview and/or examination performed by specialists. The study protocol was approved by the Ethics Committee of Kyoto University, and oral consent was obtained from all patients.

At their first visit, the patients underwent a workup examination that included questionnaires, a physical examination, blood tests, chest radiography, fractional exhaled nitric oxide measurement, and spirometry. Serum total and specific IgE levels against common aeroallergens, including mixed grass pollens (orchard grass, sweet vernal grass, Bermuda grass, timothy grass, and reeds), mixed molds (*Penicillium, Cladosporium, Aspergillus, Candida, Alternaria*, and *Helminthosporium*), weed, house dust mite, Japanese cedar pollen, cat dander, and dog dander, were measured using ImmunoCAP (Phadia, Tokyo, Japan). The patients were considered atopic when serum specific IgE levels to one or more aeroallergens were 0.35 UA/mL or higher. Sensitization to SEs (ImmunoCAP) was determined when specific IgE levels to SEs were 0.10 UA/mL or higher.[12] Fractional exhaled nitric oxide level was measured at a constant exhalation flow rate of 50 mL per second using a chemiluminescence analyzer (NOA 280, Sievers, Boulder, Colorado),[24] according to the current guidelines.[25] Prebronchodilator and postbronchodilator (inhalation of 200 μg of salbutamol), forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), and the forced expiratory flow between 25% and 75% ($FEF_{25\%-75\%}$) were measured using a ChestGraph HI-801 spirometer (Chest MI Corp, Tokyo, Japan), according to the guidelines of the American Thoracic Society.[26] The treatment steps were assessed according to the Global Initiative for Asthma (GINA) 2016 guidelines 6 months after enrollment. The sample size was determined based on the means (SDs) of the serum total IgE levels in patients with and without sensitization to SEs in our previous study.[14] Overall, 60 current or ex-smokers with asthma were estimated to be necessary to detect the differences in serum total IgE levels between patients with and without sensitization to SEs with a 1-sided α of .05 and a power of 0.80.

Statistical analyses were performed with JMP system, version 12 (SAS Institute Inc, Cary, North Carolina). Two or three groups were compared using the Wilcoxon rank sum test, Kruskal-Wallis test, χ^2 test, or Fisher exact test, as appropriate. The Spearman correlation coefficient was used to analyze the associations among the data. A

Table 1
Patient Characteristics[a]

Characteristic	Current smokers (n = 23)	Ex-smokers (n = 40)	Never smokers (n = 118)	*P* value[b]	*P* value[c]
Sex, F/M	11/12	12/28	93/25	<.001	<.001
Age at enrollment, y	58 (11) [31–75]	59 (14) [34–84]	52 (18) [14–87]	.16	.06
Age at asthma onset, y	43 (23) [1–74]	45 (24) [0–80]	41 (24) [0–86]	.53	.33
Disease duration, y	15 (19)	13 (19)	11 (15)	.66	.36
Duration after smoking cessation, y	NA	15.7 (12.7)	NA	NA	NA
Pack-years	35 (21)	27 (23)	NA	NA	NA
Body mass index[d]	23.8 (3.9)	24.5 (3.6)	23.4 (4.3)	.15	.08
Atopy, %	65	73	72	.79	.76
Allergic rhinitis, %	50	44	47	.92	.98
Atopic dermatitis, %	5	11	17	.30	.15
Sinusitis, %	20	9	10	.43	.65
Blood eosinophils, /μL	287 (190)	428 (470)	267 (289)	.08	.03
Blood neutrophil, /μL	4,671 (2,293)	4,220 (3,381)	4,030 (1,800)	.22	.57
Total serum IgE, IU/mL	200 (5–11,000)	125 (5–3,200)	120 (5–3,900)	.57	.34
Sensitization, %					
SEA	48	25	18	.007	.02
SEB	52	33	25	.04	.047
Both SEA and SEB	35	23	14	.04	.03
Either SEA or SEB	65	35	31	.006	.04
Mixed molds	22	10	14	.44	.86
House dust mite	35	43	50	.35	.18
Cat dander	13	18	15	.89	.91
Dog dander	17	25	19	.70	.66
Japanese cedar pollen	39	50	58	.23	.14
Mixed grass pollens	22	21	25	.84	.57
Weed	13	8	12	.71	.80
FeNO, ppb	41 (61)	60 (50)	44 (49)	.002	.13
FEV_1, % predicted	89.1 (23.7)	91.7 (25.4)	94.0 (23.2)	.66	.36
FEV_1/FVC, %	71.0 (11.1)	72.9 (10.9)	76.8 (11.2)	.007	.002
$FEF_{25\%-75\%}$, % predicted	57.7 (33.2)	60.1 (30.6)	69.9 (31.0)	.06	.02
Airway reversibility, %	3.8 (11.3)	7.3 (9.9)	4.5 (7.9)	.26	.40
Daily doses of ICS, μg[e]	534 (201)	468 (411)	488 (363)	.08	.91
GINA treatment step 1/2/3/4/5	0/0/6/17/0	1/8/11/15/5	5/12/28/65/8	.09	.83

Abbreviations: $FEF_{25\%-75\%}$, forced expiratory flow between 25% and 75%; FeNO, fractional concentration of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; NA, not applicable; SEA, *Staphylococcus aureus* type A; SEB, *Staphylococcus aureus* type B.
[a]Data are presented as mean (SD) or mean (SD) [range] except for pack-years and IgE.
[b]Three groups were compared.
[c]Current or ex-smokers vs never smokers.
[d]Calculated as weight in kilograms divided by square of height in meters.
[e]Equivalent to fluticasone propionate.

receiver operating characteristic (ROC) analysis was performed to determine the cutoff of the smoking abstinence period to predict the absence of sensitization to SEs. The data were expressed as means (SDs). $P < .05$ was considered significant.

Results

Overall, 181 patients with asthma were enrolled (Fig 1), 63 of whom were current or ex-smokers. Twenty-six patients had been enrolled in our previous study. The clinical characteristics of current smokers, ex-smokers, and never smokers with asthma are presented in Table 1. A higher proportion of current or ex-smokers were men and had higher blood eosinophil counts and lower FEV_1/FVC and $FEF_{25\%-75\%}$ than never smokers. The frequency of sensitization to SE type A (SEA) and/or SEB was significantly higher in the current smokers (65%), ex-smokers (35%), and never smokers (31 %), in decreasing order ($P = .006$) (Fig 1 and Fig 2). Particularly, current smoking was a risk factor for sensitization to SEA and/or SEB, with an odds ratio of 3.99 (95% CI, 1.52–11.2; $P = .005$) compared with never smoking and 3.05 (95% CI, 1.03–9.57; $P = .04$) compared with ex-smoking, after adjusting for sex, age at enrollment, and GINA treatment step 4 and 5 at 6 months after enrollment. Meanwhile, the frequencies of sensitization to other aeroallergens did not significantly differ among the 3 patient groups. The frequencies of comorbidities (atopic dermatitis, allergic rhinitis, and sinusitis) were not different among the 3 patient groups or between patients with and without sensitization to SEs.

Subsequently, our analysis was confined to current or ex-smokers. Sensitization to SEA and SEB was associated with higher serum total IgE levels and greater frequency of sensitization to other aeroallergens, including molds, house dust mite, dog dander, and weed (Table 2 and Table 3). These findings were also observed when current or ex-smokers with asthma were stratified according to the sensitization status to both SEA and SEB or either SEA or SEB (eTable 1 and eTable 2). Current or ex-smokers with sensitization to SEB exhibited higher blood eosinophil counts and greater airway reversibility than those without sensitization to SEB (Table 2). Lower FEV_1 and FEV_1/FVC at enrollment and higher frequencies of GINA treatment step 5 at 6 months after enrollment were significantly associated with sensitization to SEA (Table 3) and marginally with sensitization to SEB (Table 2). The specific IgE levels to SEA ($\rho = 0.33$, $P = .008$) and SEB ($\rho = 0.28$, $P = .03$) were weakly but significantly associated with GINA treatment steps 6 months after enrollment (Fig 2 and Fig 3). Characteristics of never smokers with asthma stratified according to the sensitization status to SEB and SEA (eTable 3) are also presented in the online supplement.

Finally, in the analysis of current or ex-smokers, a longer duration after smoking cessation was significantly associated with

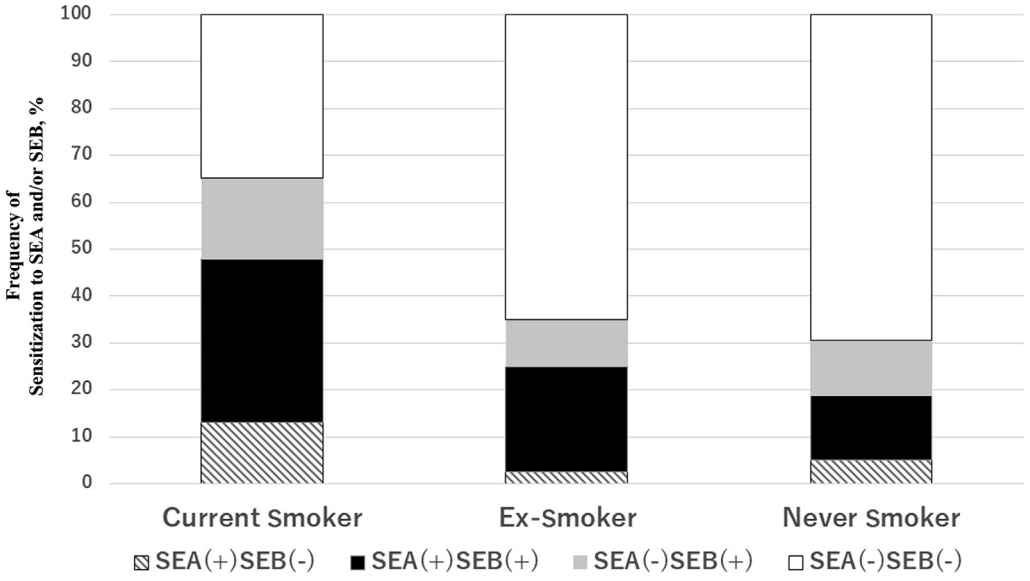

Figure 2. Frequencies of sensitization to *Staphylococcus aureus* enterotoxin A (SEA) and/or *S aureus* enterotoxin B (SEB) in current smokers, ex-smokers, and never smokers with asthma. Plus sign indicates positive; minus sign, negative.

lower specific IgE levels to SEA and marginally for specific IgE levels to SEB (Table 4). None of the other aeroallergen specific IgE levels were associated with the smoking abstinence period. An ROC curve analysis revealed that 3 years after smoking cessation was the best cutoff for predicting the absence of sensitization to SEA or SEB in current or ex-smokers with asthma, with a sensitivity of 0.71 and specificity of 0.66 (Fig 3 and Fig 4). The area under the ROC curve was 0.675.

Table 2
Patient Characteristics Stratified According to Sensitization to SEB in Current or Ex-smokers With Asthma[a]

	SEB(−) (n = 38)	SEB(+) (n = 25)	*P* value
Sex, F/M	15/23	8/17	.55
Age at asthma onset, y	43 (25) [0–80]	46 (21) [3–74]	.77
Age at enrollment, y	59 (12) [31–84]	58 (13) [34–77]	.93
Disease duration, y	16 (22)	12 (14)	.98
Current or ex-smokers, %	29/71	48/52	.12
Pack-years	20 [5.3–114]	23 [6.5–82.3]	.69
Duration after smoking cessation, y	11.3 (12.3)	7.9 (13.0)	.09
Allergic rhinitis, %	41	54	.31
Atopic dermatitis, %	6	13	.64
Blood eosinophils, cells/L	303 (281)	488 (511)	.03
Blood neutrophil, cells/L	4435 (3,564)	4307 (1,981)	.48
Total serum IgE, IU/mL	57 [5–600]	510 [76–11,000]	<.001
Sensitization, %			
Mixed molds	3	32	.002
House dust mite	29	56	.03
Cat dander	11	24	.15
Dog dander	8	44	.001
Japanese cedar pollen	37	60	.07
Mixed grass pollens	14	32	.08
Weed	0	24	.003
FEV_1, % predicted	95.3 (23.0)	83.9 (25.9)	.07
FEV_1/FVC, %	74.1 (9.7)	69.3 (12.2)	.09
$FEF_{25\%-75\%}$, % predicted	63.7 (31.5)	52.3 (30.5)	.15
Airway reversibility	3.8 (9.7)	9.4 (10.9)	.04
Daily doses of ICS, μg[b]	433 (332)	583 (361)	.03
GINA treatment step 5, %	3	16	.08

Abbreviations: $FEF_{25\%-75\%}$, forced expiratory flow between 25% and 75%; FeNO, fractional concentration of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; SEB, *Staphylococcus aureus* type B (plus sign, positive; minus sign, negative).
[a]Data are presented as mean (SD) or mean (SD) [range] except for pack-years and IgE.
[b]Equivalent to fluticasone propionate.

Table 3
Patient Characteristics Stratified According to Sensitization to SEA in Current or Ex-smokers With Asthma[a]

	SEA(−) (n = 42)	SEA(+) (n = 21)	*P* value
Sex, F/M	17/25	6/15	.35
Age at asthma onset, y	45 (25) [0–80]	43 (20) [3–72]	.63
Age at enrollment, y	59 (12) [31–84]	57 (13) [34–77]	.65
Disease duration, y	14.5 (21.1)	13.6 (14.6)	.55
Current or ex-smokers, %	29/71	52/48	.06
Pack-years	20 [5.3–114]	30 [6.8–70]	.38
Duration after smoking cessation, y	12.0 (12.7)	5.8 (11.5)	.01
Allergic rhinitis, %	47	45	.87
Atopic dermatitis, %	8	10	.79
Blood eosinophils, cells/L	338 (275)	455 (566)	.60
Blood neutrophil, cells/L	3,866 (1,297)	5,422 (4,804)	.17
Total serum IgE, IU/mL	83 [5–1,098]	510 [19–11,000]	<.001
Sensitization, %			
Mixed molds	5	35	.004
House dust mite	31	57	.045
Cat dander	14	19	.63
Dog dander	12	43	.005
Japanese cedar pollen	43	52	.47
Mixed grass pollens, %	14	35	.06
Weed	0	29	<.001
FEV_1, % predicted	97.4 (22.5)	77.4 (23.8)	.002
FEV_1/FVC, %	74.8 (9.5)	66.9 (12.0)	.006
$FEF_{25\%-75\%}$, % predicted	66.7 (30.7)	44.2 (27.7)	.005
Airway reversibility	4.6 (8.4)	9.0 (13.6)	.12
Daily doses of ICS, μg[b]	418 (282)	642 (423)	.02
GINA treatment step 5, %	2	19	.04

Abbreviations: $FEF_{25\%-75\%}$, forced expiratory flow between 25% and 75%; FeNO, fractional concentration of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; SEA, *Staphylococcus aureus* type A (plus sign, positive; minus sign, negative).
[a]Data are presented as mean (SD) or mean (SD) [range] except for pack-years and IgE.
[b]Equivalent to fluticasone propionate.

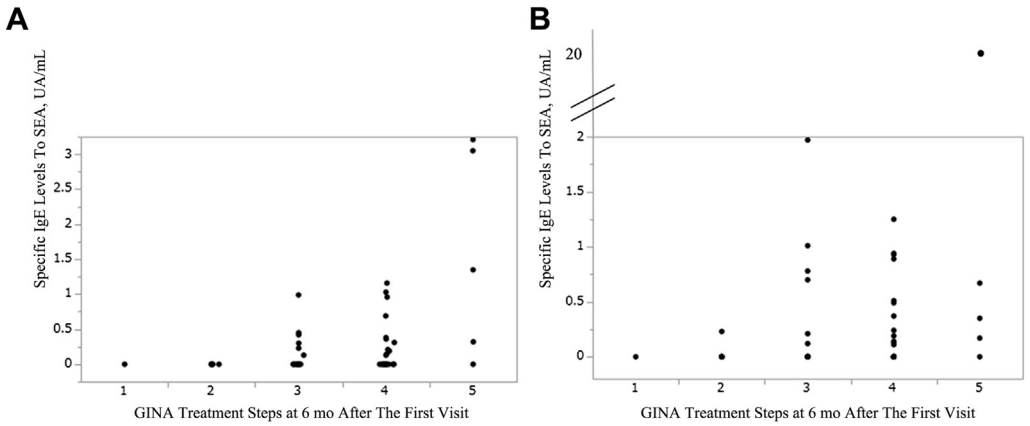

Figure 3. Association between specific IgE levels to a *Staphylococcus aureus* enterotoxin A (SEA) (A) or *S aureus* enterotoxin B (SEB) (B) and the Global Initiative for Asthma (GINA) treatment steps 6 months after study enrollment in current or ex-smokers with asthma.

Discussion

To the best of our knowledge, this is the first study to find that among patients with asthma, sensitization to SEs, but not other aeroallergens, was more common in current or ex-smokers than in never smokers. In addition, sensitization to SE augmented IgE or eosinophilic inflammation, airflow limitation, and treatment intensity in current or ex-smokers with asthma. Notably, sensitization to SEs decreased in association with a longer smoking abstinence period.

The overall frequency of sensitization to SEs in the present study was 48%, which was similar to that in patients with asthma in the GA2LEN study (41%)[15] and the results presented by Bachert et al (41%).[12] In this study, the frequency of sensitization to SEs in current or ex-smokers was significantly higher than that in never smokers with asthma; its frequency in current smokers with asthma increased to 65%, whereas the frequency of sensitization to other aeroallergens did not differ between current or ex-smokers and never smokers with asthma. These findings are consistent with findings of the general population in the GA2LEN study, which demonstrated that compared with never smokers, current smokers were more frequently sensitized to SEs, with an odds ratio of 2.02, but not to house dust mite or other tested aeroallergens.[15]

Although the mechanisms underlying the increased frequency of sensitization to SEs in current or ex-smokers with asthma remain unclear, 2 potential mechanisms should be discussed: (1) increased type 2 proinflammatory responses of epithelial cells to microorganisms in smokers with asthma, which may be represented by increased thymic stromal lymphopoietin levels in the airways,[22,27,28] and (2) increased colonization of *S aureus* in the airways. Cigarette smoke disrupts the bronchial epithelial barrier, leading to increased *S aureus* colonization on epithelial cells, which may serve as a reservoir of SEs by the formation of biofilms,[29] induces SEs penetration into subepithelial layers, and increases stimulation of T cells.[30] However, the influence of current smoking on *S aureus* nasal colonization in healthy individuals yielded conflicting results. Bogaert et al[31] found that active and passive smoking were risk factors for nasal colonization in children aged 1 to 19 years, whereas Andersen et al[32] found that current smoking reduced risk of *S aureus* nasal colonization in middle-aged and elderly twins. Therefore, the effects of smoking on *S aureus* colonization and responses to *S aureus* in the lower airways of patients with asthma should be clarified in future studies.

As expected, current or ex-smokers with sensitization to SEs were more frequently sensitized to other aeroallergens, and they had higher serum total IgE levels and eosinophil counts than those without sensitization to SEs. These data agree with the findings of various clinical studies[11–14,33] and mice models[9,10] of allergic

Table 4
Associations Between Specific IgE Levels and Duration After Smoking Cessation in Current or Ex-smokers With Asthma

Variable	Correlation coefficient	*P* value
SEA	−0.31	.01
SEB	−0.22	.08
Mixed molds	−0.04	.76
House dust mite	−0.02	.89
Cat dander	0.06	.64
Dog dander	0.02	.88
Japanese cedar pollen	−0.01	.94
Mixed grass pollens	−0.01	.94
Weed	−0.07	.57

Abbreviations: SEA, *Staphylococcus aureus* type A; SEB, *Staphylococcus aureus* type B.

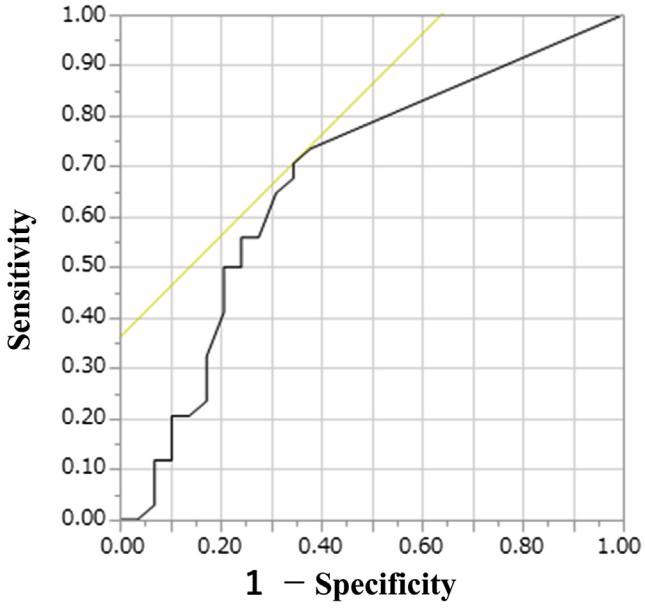

Figure 4. Receiver operating characteristic curve analysis of the period after smoking cessation for the absence of sensitization to *Staphylococcus aureus* enterotoxin A or *S aureus* enterotoxin B.

asthma, and they may be explained by its nature of superantigens.[3,10] Notably, current or ex-smokers with asthma who were sensitized to weed were all sensitized to SEs as well, but the mechanism underlying this sensitization remains unknown. Sensitization to SE was clinically associated with airflow limitation and GINA treatment step 5 at steady-state conditions in current or ex-smokers with asthma in the present study. Therefore, sensitization to SE may play an important role in smokers with asthma and could be a potential marker of type 2 or eosinophilic inflammation and severe disease in this population.

Although the effect of sensitization to SEA and SEB on clinical indexes exhibited similar trends in current or ex-smokers with asthma, higher blood eosinophil counts and greater airway reversibility were associated with sensitization to SEB. In contrast, airflow limitation, severe asthma, and a shorter smoking abstinence period were associated with sensitization to SEA alone. Although the specific mechanisms behind these associations remain unknown, similar results were observed in previous studies. SEB augmented type 2 or eosinophilic inflammation in animal models[9,10] and sensitization to SEA alone was associated with poor asthma control[14,33] in patients with asthma, not restricted to smokers with asthma. Vulnerable conditions with impaired barrier integrity may be necessary to establish sensitization to SEA, when considering that the rate of transcytosis of SEA was several-fold slower than that of SEB in a study using intestinal epithelial cells.[34] Therefore, sensitization to SEA, rather than SEB, would be a potential marker of severe disease in current or ex-smokers with asthma.

One of our important findings was that patients with a longer duration after smoking cessation had lower levels of SE specific IgE, particularly, SEA specific IgE. Furthermore, we observed that 3 years of smoking abstinence was the cutoff for predicting the absence of sensitization to SEs. These associations were not observed for other aeroallergens. It is possible to speculate that impaired airway barrier integrity and an overproduction of mucin that binds SA[35] may be normalized after smoking cessation, which reduces the burden of SEs. A small study recently found that smoking abstinence for 3 months did not alter the microbial diversity in the lower airways of patients with asthma.[36] Therefore, it may take several years to detect a change in the lung microbial communities and to be desensitized to SEs after smoking cessation. In fact, it took 2 years after smoking cessation to normalize $CD4^+$ cell counts[37] and goblet cell hyperplasia.[38]

Our study has several limitations. First, we did not provide microbiological data, including *S aureus* colonization in the lower airways. Second, smoking status and duration after smoking cessation were based on a self-reported questionnaire. Therefore, *S aureus* colonization and objective measures of smoking status, such as cotinine levels, should be evaluated in future studies. Prospective longitudinal studies that follow SE sensitization levels after smoking cessation are warranted. Third, we included current or ex-smokers with COPD as a comorbid condition of asthma. However, the inclusion of patients with asthma-COPD overlap may not critically detract from our conclusion because patients with COPD were sensitized to SEs with a frequency of 56%,[39] indicating the presence of a similar association between smoking and sensitization to SEs in patients with COPD. Further studies are necessary to investigate the role and specific mechanisms of sensitization to SEs in patients with asthma-COPD overlap.

In conclusion, smoking may facilitate sensitization to SEs in patients with asthma. Sensitization to SEs may be a key marker in smokers with asthma, indicating the presence of IgE or eosinophilic inflammation, airflow limitation, and severe disease.

Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.anai.2017.08.001.

References

[1] Bachert C, Zhang N, Holtappels G, et al. Presence of IL-5 protein and IgE antibodies to staphylococcal enterotoxins in nasal polyps is associated with comorbid asthma. *J Allergy Clin Immunol*. 2010;126:962–968.

[2] Bachert C, Zhang N. Chronic rhinosinusitis and asthma: novel understanding of the role of IgE 'above atopy'. *J Intern Med*. 2012;272:133–143.

[3] Huvenne W, Hellings PW, Bachert C. Role of staphylococcal superantigens in airway disease. *Int Arch Allergy Immunol*. 2013;161:304–314.

[4] Mandron M, Ariès M, Brehm RD, et al. Human dendritic cells conditioned with *Staphylococcus aureus* enterotoxin B promote TH2 cell polarization. *J Allergy Clin Immunol*. 2006;117:1141–1147.

[5] Neuber K, Steinrücke K, Ring J. Staphylococcal enterotoxin B affects in vitro IgE synthesis, interferon-γ, Interleukin-4 and Interleukin-5 production in atopic eczema. *Int Arch Allergy Immunol*. 1995;107:179–182.

[6] Patou J, Gevaert P, Van Zele T, Holtappels G, Van Cauwenberge P, Bachert C. *Staphylococcus aureus* enterotoxin B, protein A, and lipoteichoic acid stimulations in nasal polyps. *J Allergy Clin Immunol*. 2008;121:110–115.

[7] Zhang N, Holtappels G, Gevaert P, et al. Mucosal tissue polyclonal IgE is functional in response to allergen and SEB. *Allergy*. 2011;66:141–148.

[8] Herz U, Rückert R, Wollenhaupt K, et al. Airway exposure to bacterial superantigen (SEB) induces lymphocyte-dependent airway inflammation associated with increased airway responsiveness-a model for non-allergic asthma. *Eur J Immunol*. 1999;29:1021–1031.

[9] Hellings P, Hens G, Meyts I, et al. Aggravation of bronchial eosinophilia in mice by nasal and bronchial exposure to *Staphylococcus aureus* enterotoxin B. *Clin Exp Allergy*. 2006;36:1063–1071.

[10] Huvenne W, Callebaut I, Plantinga M, et al. *Staphylococcus aureus* enterotoxin B facilitates allergic sensitization in experimental asthma. *Clin Exp Allergy*. 2010;40:1079–1090.

[11] Bachert C, Gevaert P, Howarth P, Holtappels G, Van Cauwenberge P, Johansson S. IgE to Staphylococcus aureus enterotoxins in serum is related to severity of asthma. *J Allergy Clin Immunol*. 2003;111:1131–1132.

[12] Bachert C, Van Steen K, Zhang N, et al. Specific IgE against *Staphylococcus aureus* enterotoxins: an independent risk factor for asthma. *J Allergy Clin Immunol*. 2012;130:376–381.

[13] Song W, Sintobin I, Sohn K, et al. Staphylococcal enterotoxin IgE sensitization in late-onset severe eosinophilic asthma in the elderly. *Clin Exp Allergy*. 2015;46:411–421.

[14] Matsumoto H, Kanemitsu Y, Nagasaki T, et al. *Staphylococcus aureus* enterotoxin sensitization involvement and its association with the CysLTR1 variant in different asthma phenotypes. *Ann Allergy Asthma Immunol*. 2017;118:197–203.

[15] Tomassen P, Jarvis D, Newson R, et al. *Staphylococcus aureus* enterotoxin specific IgE is associated with asthma in the general population: a GA2LEN study. *Allergy*. 2013;68:1289–1297.

[16] Song W, Chang Y, Lim M, et al. Staphylococcal enterotoxin sensitization in a community-based population: a potential role in adult onset asthma. *Clin Exp Allergy*. 2014;44:553–562.

[17] Chalmers GW, MacLeod KJ, Thomson L, Little SA, McSharry C, Thomson NC. Smoking and airway inflammation in patients with mild asthma. *Chest*. 2001;120:1917–1922.

[18] Boulet LP, Lemiere C, Archambault F, Carrier G, Descary MC, Deschesnes F. Smoking and asthma: clinical and radiologic features, lung function, and airway inflammation. *Chest*. 2006;129:661–668.

[19] Nagasaki T, Matsumoto H. Influences of smoking and aging on allergic airway inflammation in asthma. *Allergol Int*. 2013;62:171–179.

[20] Huvenne W, Lanckacker EA, Krysko O, et al. Exacerbation of cigarette smoke-induced pulmonary inflammation by *Staphylococcus aureus* enterotoxin B in mice. *Respir Res*. 2011;12:69.

[21] Matsunaga K, Yanagisawa S, Hirano T, et al. Associated demographics of persistent exhaled nitric oxide elevation in treated asthmatics. *Clin Exp Allergy*. 2012;42:775–781.

[22] Nagasaki T, Matsumoto H, Nakaji H, et al. Smoking attenuates the age related decrease in IgE levels and maintains eosinophilic inflammation. *Clin Exp Allergy*. 2013;43:608–615.

[23] American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis*. 1987;136:225–244.

[24] Nagasaki T, Matsumoto H, Kanemitsu Y, et al. Using exhaled nitric oxide and serum periostin as a composite marker to identify severe/steroid-insensitive asthma. *Am J Respir Crit Care Med*. 2014;190:1449–1452.

[25] American Thoracic Society/European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med*. 2005;171:912–930.

[26] Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26:319–338.
[27] Horvath KM, Brighton LE, Zhang W, Carson JL, Jaspers I. Epithelial cells from smokers modify dendritic cell responses in the context of influenza infection. *Am J Respir Cell Mol Biol*. 2011;45:237–245.
[28] Nakamura Y, Miyata M, Ohba T, et al. Cigarette smoke extract induces thymic stromal lymphopoietin expression, leading to TH2-type immune responses and airway inflammation. *J Allergy Clin Immunol*. 2008;122:1208–1214.
[29] Kulkarni R, Antala S, Wang A, et al. Cigarette smoke increases Staphylococcus aureus biofilm formation via oxidative stress. *Infect Immun*. 2012;80:3804–3811.
[30] Gangl K, Reininger R, Bernhard D, et al. Cigarette smoke facilitates allergen penetration across respiratory epithelium. *Allergy*. 2009;64:398–405.
[31] Bogaert D, van Belkum A, Sluijter M, et al. Colonisation by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. *Lancet*. 2004;363:1871–1872.
[32] Andersen P, Larsen LA, Fowler V, Stegger M, Skov R, Christensen K. Risk factors for *Staphylococcus aureus* nasal colonization in Danish middle-aged and elderly twins. *Eur J Clin Microbiol Infect Dis*. 2013;32:1321–1326.
[33] Tanaka A, Suzuki S, Ohta S, et al. Association between specific IgE to *Staphylococcus aureus* enterotoxins A and B and asthma control. *Ann Allergy Asthma Immunol*. 2015;115:191–197.
[34] Hamad AR, Marrack P, Kappler JW. Transcytosis of staphylococcal superantigen toxins. *J Exp Med*. 1997;185:1447–1454.
[35] Trivier D, Houdret N, Courcol RJ, Lamblin G, Roussel P, Davril M. The binding of surface proteins from *Staphylococcus aureus* to human bronchial mucins. *Eur Respir J*. 1997;10:804–810.
[36] Munck C, Helby J, Westergaard CG, Porsbjerg C, Backer V, Hansen LH. Smoking cessation and the microbiome in induced sputum samples from cigarette smoking asthma patients. *PLoS One*. 2016;11:e0158622.
[37] Tollerud DJ, Clark JW, Brown LM, et al. The effects of cigarette smoking on T cell subsets. *Am Rev Respir Dis*. 1989;139:1446–1451.
[38] Wright JL, Lawson LM, Pare PD, Wiggs BJ, Kennedy S, Hogg JC. Morphology of peripheral airways in current smokers and ex-smokers. *Am Rev Respir Dis*. 1983;127:474–477.
[39] Rohde G, Gevaert P, Holtappels G, et al. Increased IgE-antibodies to *Staphylococcus aureus* enterotoxins in patients with COPD. *Respir Med*. 2004;98:858–864.

Supplementary Data

eTable 1
Patients Characteristics Stratified According to Sensitization to Both SEA and SEB in Current or Ex-smokers With Asthma[a]

Characteristic	SEA/SEB overlap(−) (n = 46)	SEA/SEB overlap(+) (n = 17)	*P* value
Sex, F/M	19/27	4/13	.19
Age at asthma onset, y	45 (24) [0–80]	42 (22) [3–72]	.44
Age at enrollment, y	59 (12) [31–84]	57 (14) [34–77]	.74
Disease duration, y	14 (20)	16 (15)	.22
Current or ex-smokers, %	15/31	8/9	.29
Pack-years	20 [5.3–114]	25 [6.8–70]	.58
Duration after smoking cessation, y	11.0 (12.6)	7.0 (12.5)	.09
Allergic rhinitis, %	44	53	.52
Atopic dermatitis, %	7	12	.58
Blood eosinophils, /L	319 (271)	533 (603)	.11
Blood neutrophil, /L	4,401 (3,258)	4,339 (2,330)	>.99
Total serum IgE, IU/mL	72 [5–1,098]	720 [76–11,000]	<.001
Sensitization, %			
Mixed molds	4	41	.001
House dust mite	28	71	.002
Cat dander	13	24	.31
Dog dander	11	53	<.001
Japanese cedar pollen	13	41	.02
Mixed grass pollens	22	38	.12
Weed	0	35	<.001
FEV_1, % predicted	94.8 (23.5)	79.9 (25.1)	.03
FEV_1/FVC, %	73.8 (9.7)	67.8 (13.1)	.06
$FEF_{25\%-75\%}$, % predicted	63.3 (31.4)	48.2 (29.3)	.08
Airway reversibility	3.6 (9.0)	12.6 (11.6)	.004
ICS daily maintenance dose, μg[b]	437 (307)	643 (416)	.04
GINA treatment step 5, %	6	13	.17

Abbreviations: $FEF_{25\%-75\%}$, forced expiratory flow between 25% and 75%; FeNO, fractional concentration of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; SEA, *Staphylococcus aureus* type A; SEB, *Staphylococcus aureus* type B (plus sign, positive; minus sign, negative).
[a]Data are presented as mean (SD) or mean (SD) [range] except for pack-years and IgE.
[b]Equivalent to fluticasone propionate.

eTable 2
Patients Characteristics Stratified According to Sensitization to Either SEA or SEB in Current or Ex-smokers With Asthma[a]

Characteristic	SEA(−)/SEB(−) (n = 34)	SEA(+) or SEB (+) (n = 29)	*P* value
Sex, F/M	13/21	10/19	.76
Age at asthma onset, y	42 (26) [0–80]	47 (20) [3–74]	.60
Age at enrollment, y	59 (12) [31–84]	58 (13) [34–77]	.83
Disease duration, y	17 (23)	11 (14)	.62
Current or ex-smokers, %	8/26	15/14	.02
Pack-years	20 [5.3–114]	25 [6.5–82]	.46
Duration after smoking cessation, y	12.6 (12.4)	6.9 (12.3)	.01
Allergic rhinitis, %	55	52	.80
Atopic dermatitis, %	6	11	.53
Blood eosinophils, /L	325 (287)	438 (492)	.24
Blood neutrophil, /L	3,777 (1,352)	5,096 (4,127)	.047
Total serum IgE, IU/mL	57 [5–600]	380 [19–11,000]	<.001
Sensitization, %			
Mixed molds	3	29	.008
House dust mite	32	48	.20
Cat dander	12	21	.33
Dog dander	9	38	.007
Japanese cedar pollen	35	59	.06
Mixed grass pollens	15	29	.18
Weed	0	21	.007
FEV_1, % predicted	98.6 (21.5)	81.5 (25.2)	.005
FEV_1/FVC, %	75.4 (9.4)	68.4 (11.6)	.01
$FEF_{25\%-75\%}$, % predicted	68.0 (30.5)	48.9 (29.7)	.01
Airway reversibility	5.0 (9.0)	7.3 (12.1)	.34
ICS daily maintenance dose, μg[b]	409 (305)	591 (375)	.02
GINA treatment step 5, %	0	17	.02

Abbreviations: $FEF_{25\%-75\%}$, forced expiratory flow between 25% and 75%; FeNO, fractional concentration of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; SEA, *Staphylococcus aureus* type A; SEB, *Staphylococcus aureus* type B (plus sign, positive; minus sign, negative).
[a]Data are presented as mean (SD) or mean (SD) [range] except for pack-years and IgE.
[b]Equivalent to fluticasone propionate.

eTable 3
Patient Characteristics Stratified According to Sensitization to SEB and SEA in Never Smokers With Asthma[a]

Characteristic	SEB(−) (n = 88)	SEB(+) (n = 30)	*P* value	SEA(−) (n = 97)	SEA(+) (n = 21)	*P* value
Sex, F/M	69/19	24/6	.85	77/20	16/5	.75
Age at asthma onset, y	40 (24) [0–86]	43 (22) [0–75]	.54	41 (24) [0–86]	41 (25) [3–75]	.83
Age at enrollment, y	52 (18) [16–87]	55 (17) [14–79]	.37	52 (17) [17–87]	54 (19) [14–76]	.49
Disease duration, y	11 (15)	11 (15)	.86	11 (15)	13 (15)	.20
Allergic rhinitis, %	47	46	.96	43	63	.13
Atopic dermatitis, %	20	7	.23	19	5	.14
Blood eosinophils, /L	225 (195)	390 (451)	.06	244 (297)	373 (230)	.003
Blood neutrophil, /L	3,958 (1,779)	4,243 (1,874)	.62	4,029 (1,791)	4,037 (1,885)	.62
Total serum IgE, IU/mL	81 [5–870]	430 [33–3,900]	<.001	83 [5–790]	730 [39–3,900]	<.001
Sensitization, %						
Mixed molds	10	23	.07	11	24	.13
House dust mite	42	73	.003	45	71	.03
Cat dander	13	23	.15	12	29	.06
Dog dander	16	30	.09	14	43	.006
Japanese cedar pollen	49	83	.001	54	76	.06
Mixed grass pollens	20	40	.03	22	38	.12
Weed	9	20	.11	10	19	.009
FEV_1, % predicted	96.7 (22.7)	86.1 (23.3)	.03	96.7 (23.0)	81.7 (20.2)	.007
FEV_1/FVC, %	78.1 (9.5)	73.2 (14.6)	.04	78.2 (10.2)	70.7 (13.5)	.005
$FEF_{25\%-75\%}$, % predicted	73.4 (30.6)	59.8 (30.4)	.06	74.4 (30.6)	49.1 (24.3)	<.001
Airway reversibility	3.9 (7.9)	6.0 (7.6)	.13	3.5 (7.8)	8.7 (6.9)	<.001
ICS daily maintenance dose, μg[b]	471 (330)	503 (336)	.62	464 (304)	549 (434)	.58
GINA treatment step 5, %	6	10	.42	5	19	.03

Abbreviations: $FEF_{25\%-75\%}$, forced expiratory flow between 25% and 75%; FeNO, fractional concentration of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; SEA, *Staphylococcus aureus* type A; SEB, *Staphylococcus aureus* type B (plus sign, positive; minus sign, negative).

[a]Data are presented as mean (SD) or mean (SD) [range] except for pack-years and IgE.

[b]Equivalent to fluticasone propionate.