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Sensitization to *Staphylococcus aureus* enterotoxins in smokers with asthma

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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

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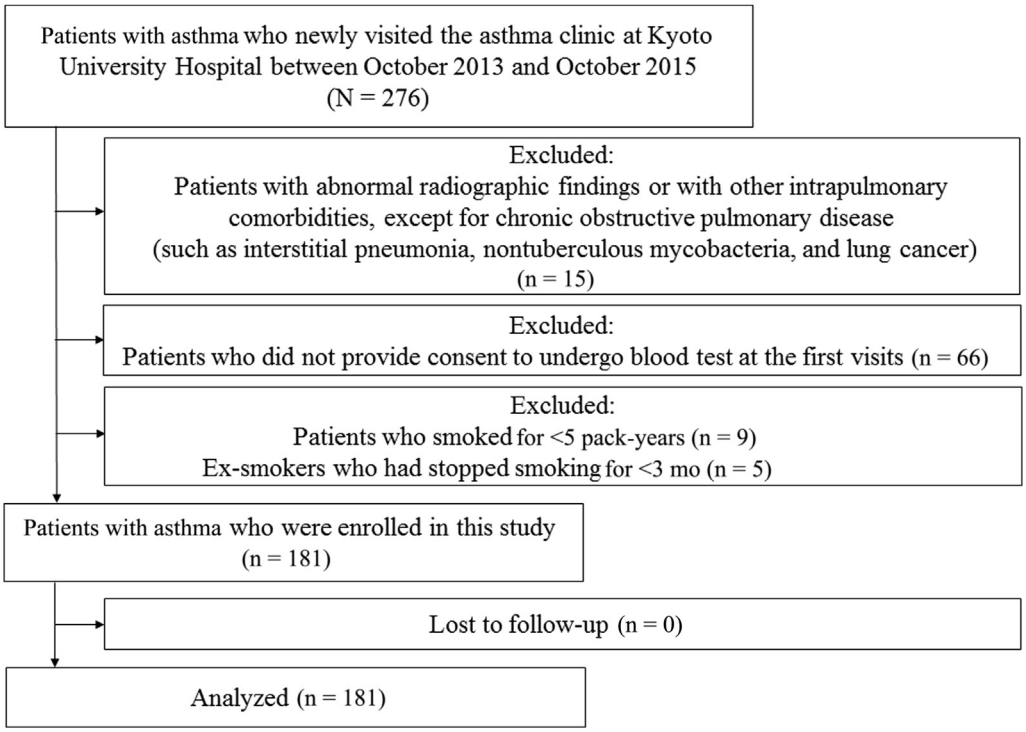

Figure 1. CONSORT flow diagram.

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