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성인 천식 발생기전에서 포도상구균 장독소 역할 연구

Staphylococcal enterotoxin in the pathogenesis of adult-onset asthma

2017년 1월

서울대학교 대학원

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Staphylococcal enterotoxin in the pathogenesis of adult-onset asthma

By Woo-Jung Song

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국문초록

서론: 포도상구균은 코점막과 피부에 흔히 상재하는 균으로, 만성부비동염과 아토피피부염의 위험인자로 잘 알려져 있으며, 최근에는 천식의 발생 및 중증도의 위험인자일 가능성이 제시되고 있다. 성인기 발생 천식은 소아 청소년기 발생 천식과 다르며 아토피 외 다양한 병태생리가 관여할 가능성이 제시되고 있다. 포도상구균 장독소는 초항원으로 작용하여 IgE 면역반응을 유도하고 증폭할 수 있으므로 비아토피성 IgE 매개 알레르기 질환의 병태생리에 중요할 가능성이 있다. 따라서 본 연구에서는 포도상구균 장독소가 성인기 발생 천식의 위험인자일 가능성에 대해 검증하고자 한다.

방법: 네 가지 다른 방법의 순차적 연구를 통해 가설을 검증하고자 한다. 첫째, 체계적 문헌고찰 및 메타분석을 통해 포도상구균 장독소 IgE 감작과 천식의 연관성을 탐색하고자 한다. 둘째, 한국 지역사회 일반인구집단 역학조사 샘플을 활용하여, 포도상구균 장독소 IgE 감작과 성인기 발생 천식의 연관성과 그 독립성을 확인하고자 한다. 셋째, 성인기 발생 천식의 이질성을 고려하여, 포도상구균 장독소 IgE 감작과 특히 관련되는 천식의 표현형을 구체화하고자 한다. 넷째, 천식동물실험모델을 통해, 포도상구균 장독소 B 의 천식 발생에 관여하는 효과를 분석하고자 한다.

결과: 첫째, 체계적 문헌 고찰을 통해 관련성이 확인된 문헌 7 편을 메타분석 하였고, 그 결과 위험도(odds ratio) 2.95 (95% 신뢰구간 2.28-3.82)로 포도상구균 장독소 IgE 감작과 천식이 중요하게 연관될 가능성을 확인하였다. 둘째, 1080 명의 창원 산청 지역사회 코호트를 분석하여, 포도상구균 장독소 IgE 감작율이 일반

인구집단 내에서 27.0% 정도로 높고, 아토피와 달리 연령이 증가함에도 장독소 IgE 감작율은 여전히 높거나 증가하는 특징을 관찰하였다. 또한, 성인기 발생 천식과의 독립적 연관성도 유의한 것으로 확인되었다. 셋째, 249 명의 노인천식 환자와 98 명의 대조군 분석을 통해, 포도상구균 장독소 IgE 감작은 천식의 중증도, 호산구성 기도염증, 만성부비동염 동반 여부와 유의하게 관련됨을 확인하였다. 또한 다중 대응분석을 통해, 이러한 요소들의 상호 관련성이 서로 밀접함을 확인하였다. 넷째, 저농도 집먼지진드기 항원을 포도상구균 장독소 B 와 함께 투여한 동물실험을 통해, 장독소 B 는 알레르겐 감작기에 함께 투여되었을 때 천식의 표현형 발생을 증가시키며, 이는 집먼지진드기 항원의 감작을 증진시키는 보조제 역할에 기인한 것으로 확인되었다.

결론: 포도상구균 장독소는 성인기 천식 발생의 위험 인자이며, 특히 중증 천식과 관련 있다. 천식 발생에 관여하는 구체적인 기전에 대한 연구가 향후 추가적으로 필요하며, 또한 포도상구균 및 장독소 제거의 천식 예방 및 치료에 효과적일 가능성에 대한 추가 연구가 필요하다.

주요어: 천식, 포도상구균

학 번: 2010-30597

Abstract

Introduction: *Staphylococcus aureus* is a frequent colonizer in human nasal mucosa and skin, and the colonization has been suggested as a risk factor for chronic rhinosinusitis and atopic dermatitis. Recent evidence suggests that *S. aureus* is also related to asthma development and severity. Unlike childhood-onset asthma, adult-onset asthma appears to be a heterogeneous condition resulting from complex interactions between host and environment. The present study was conducted to test the hypothesis that *S. aureus* is a risk factor for adult-onset asthma.

Methods: A stepwise approach was made to test the hypothesis. First, a systematic review and meta-analysis was performed to explore the associations between staphylococcal enterotoxin IgE (SE-IgE) sensitization and asthma. Second, we investigated to determine whether SE-IgE sensitization is an independent risk factor for adult-onset asthma, using a general population survey sample. Third, considering the heterogeneity of adult-onset asthma, we examined which asthma phenotype is specifically related to SE-IgE sensitization, using an elderly asthma cohort study sample. Finally, we carried out an animal model experiment to examine if SE has causative effects for asthma development.

Results: First, 7 relevant studies (5 case-control and 2 population-based studies) were identified in systematic review, and pooled odds ratio (OR) of SE-IgE sensitization for asthma was 2.95 (95% confidence interval 2.28-3.82). Second, a cross-sectional database of 1,080 Korean adults in the community population survey was analyzed. The prevalence of SE-IgE sensitization was 27.0%, and unlike inhalant allergen sensitization, it increased with aging. Multivariate logistic regression analyses demonstrated that SE-IgE sensitization was significantly related to adult-onset asthma, independently of confounders. Third, we analyzed 249 elderly asthma patients and 98 healthy controls; we found that SE-IgE sensitization was

significantly associated with asthma severity, sputum eosinophilia, and chronic rhinosinusitis comorbidity. Multiple correspondence analyses supported their close inter-relationships. Finally, in an animal experimental model, intra-nasal administration of staphylococcal enterotoxin B promoted the development of allergic sensitization to house dust mite and subsequent allergic asthma, suggesting the adjuvant effects.

Conclusion: The present findings suggest that S aureus is a risk factor for adult-onset asthma, particularly for severe eosinophilic phenotype. These findings warrant further investigation for the mechanisms of associations and clinical relevance.

Keywords: asthma, Staphylococcus aureus

Student Number: 2010-30597

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1. Introduction

Emerging evidence suggests that asthma is a heterogeneous clinical entity [1]. In the past, asthma was mainly associated with inhalant allergen sensitization (atopic asthma); however, recently it has been found that various risk factors are involved in the complex pathophysiology of asthma [2]. Particularly, adult-onset asthma is regarded as having more heterogeneous mechanisms and phenotypes, and has frequently a non-atopic nature, previously called as intrinsic asthma [3].

Staphylococcus aureus (SA) is a human commensal microorganism that is often responsible for various invasive infectious diseases [4]. However, SA is also a frequent colonizer in upper airways and skin among healthy individuals [4], and superantigenic properties of Staphylococcal enterotoxins (SE) [5] have been also associated with allergic diseases in the skin [6] and upper airways [7]. In patients with atopic dermatitis, skin colonization with SA is positively related to severity [8]. In patients with chronic rhinosinusitis (CRS) and/or nasal polyp (NP), SE-IgE sensitization is associated with tissue eosinophilia, refractory inflammation, and recurrence after endoscopic sinus surgery [9]. Furthermore, recent observations [10, 11] suggested that SE-specific IgE (SE-IgE) is a potential risk factor for asthma. In the literature, the history of studies on the role of bacterial antigens for asthma go back to about 100 years ago [12]. Since then, numerous researchers have long been interested in the roles of bacteria, particularly SA, in the pathogenesis of asthma but with controversy [13-20]. It is just until recently that their significant associations have come into the spotlight.

Here we hypothesized that SE has a pathogenic role in the pathogenesis of adult-onset asthma. To test this hypothesis, we took a stepwise approach (Figure 1); first we conducted a systematic review to summarize previous reports on the associations between asthma and SE-

IgE sensitization. Second, we examined if the associations of SE-IgE sensitization with adult asthma hold true in a general population sample and are independent of confounders. Third, we investigated which of clinical phenotypes of adult-onset asthma is specifically related to SE-IgE sensitization, considering the heterogeneity of adult asthma, using an elderly asthma cohort sample. Finally, we conducted an animal model experiment to examine if SE has causative effects for asthma pathogenesis.

2. Material and Methods

2.1. Systematic review and meta-analysis

First, we conducted a systematic review and meta-analysis of previous literatures to summarize the associations between SE-IgE sensitization and asthma.

Literature search

A systematic literature review was performed on Pubmed and Embase databases to identify peer-reviewed articles reporting the prevalence of SE-IgE sensitization in asthmatics and healthy controls, published from January 1960 until February 2013, without language restriction. The search utilized the keywords 'asthma OR wheeze OR wheezing' AND 'Staphylococcal OR Staphylococcus'. Additional articles were manually sought through the reference lists of the retrieved articles. The review process followed the recommendations of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [21], as presented in Figure 2. Inclusion criteria were 1) the population-based or case-control studies which compared the prevalence of SE-IgE sensitization in asthmatics with non-asthmatic controls. Exclusion criteria were 1) the articles without peer-reviewed full-text (i.e., conference abstracts) and 2) the studies which did not determine the positivity of SE-IgE sensitization in a standardized manner.

The literature search and review process was performed by two researchers. In cases of disagreement during the selection of relevant studies, it was resolved by discussion within all the authors. The outcome data extracted were: study design, subjects, region/population, and the definition and prevalence of SE-IgE positivity. If prevalence of SE-IgE positivity was not described, corresponding authors were contacted to obtain the data.

Statistical analyses

A pooled estimate of risk for asthma by SE-IgE positivity was calculated by using the fixed-effect models with Mantel-Haenszel methods. The results were expressed as odds ratio (OR) with 95% confidence intervals (CI). Homogeneity testing was performed using the I^2 test. The analysis was performed using the "metan" command in STATA package (release 12.0; StataCorp., Texas, USA).

2.2. General population sample analysis

Next, we examined the associations of SE-IgE sensitization with asthma in Korean general adult population samples. Also we examined the epidemiologic features of and risk factors for SE-IgE sensitization, which has not been reported so far.

Study population

We analyzed a database collected from cross-sectional surveys on adults living in two regions in Korea in 2007 [22]. The two study regions are endemic for *Clonorchiasis* and in Gyeongnam Province: Shinan-meon in Sancheong and Buk-meon in Changwon. Both regions are mainly agricultural areas, but Changwon is more urbanized than Sancheong. The surveys were conducted by collaboration between the Clinical Research Center for Chronic Obstructive Airway Disease of Seoul National University Hospital and Seoul National University Bundang Hospital and the National Cancer Center of Korea. The study protocol was reviewed and approved by the institutional review board of the National Cancer Center, Korea (IRB reference NCCNCS-07-080).

Study participants were recruited in collaboration with Public Health Centers in the study regions. Briefly, a leaflet and information materials detailing the study purpose and protocols were distributed to residents by the head of each village. The residents were contacted directly or by telephone calls for enrollment. In total, 1,116 subjects who were at least 30 years of age recruited voluntarily from a target population of 17,494 residents (616 subjects from 5,526 Sancheong residents and 500 subjects from 11,968 Changwon residents). All participants provided written informed consent. Our final analysis included 1,080 subjects (96.8%) who had serum available for IgE measurements.

Questionnaires

Interviews were performed by trained researchers who were certified to conduct epidemiological surveys. The structured questionnaires included demographic parameters, medical history and allergic disease history. Medical histories included items on 40 common chronic illnesses. The presence of allergic diseases was assessed using questionnaires on asthma and rhinitis, as described previously [23]. Rhinitis was defined by the question "Have you had a problem with sneezing or a runny or blocked nose without a cold in the last 12 months?"

Definition of asthma

Current asthma was defined positive if the subjects had positive methacholine airway hyperresponsiveness (AHR) and current wheeze ("Have you had a wheezing or whistling in the chest during the last 12 months?"). Past asthma was defined by prior asthma diagnosis in history, but having no current wheeze or AHR. Asthma was further classified by a participant-recalled symptom onset of 18 years as 'childhood-onset' or 'adult-onset'.

Methacholine challenge tests

Methacholine challenge tests were performed using a modification of Chai's method to measure AHR. Lung functions were measured by a portable spirometer (Micro Spirometer, Micro Medical, Kent, UK). Subjects with respiratory infections within the previous two weeks were excluded to avoid false-positive AHR. Subjects with a baseline forced expiratory volume 1 s (FEV1) of lower than either 1,200 mL or 50% of the predicted value were also excluded because of the presence of chronic obstructive pulmonary disease. A Rosenthal-French dosimeter (Laboratory for Applied Immunology, Baltimore, MD, USA) was used to deliver aerosols generated by a nebulizer (DeVilbliss, Carlsbad, CA, USA). Subjects were instructed to inhale five inspiratory capacity breaths of increasing methacholine concentration (1.25, 2.5, 6.25, 12.5, and 25 mg/mL) until the FEV1 decreased to < 80% of the baseline value or until the highest concentration was reached. Triplicate FEV1 measurements were recorded at 90 or 180 s after each inhalation, and the highest value was selected for analysis. Methacholine AHR was scored as positive if the concentration of methacholine provoking a 20% decrease in FEV1 was < 16 mg/mL.

Allergen skin prick test

Skin testing was performed using a panel of 10 common inhalant allergens, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, dog epithelia, *Blatella germanica*, fungus mixture, tree pollen mixture 1 (alder, hazel, popular, elm, and willow), tree pollen mixture 2 (birch, beech, oak, and plane tree), grass pollen mixture (velvet grass, orchard grass, rye grass, timothy grass, Kentucky blue grass, and meadow grass), mugwort, and ragweed. Positive (1 mg/mL histamine) and negative (0.9% sodium chloride) controls were also tested. The skin response was defined positive if the allergen wheal was larger than or equal in size to the histamine wheal and the diameter of the allergen wheal was at least 3

mm. Inhalant allergen sensitization was determined when the subjects had a positive skin response to any of the tested allergens.

Serum total IgE and specific IgE measurements for staphylococcal enterotoxin

Serum samples were assayed for total IgE and SE-IgE levels using the ImmunoCAP 250 (Thermo Fischer, Uppsala, Sweden) according to the manufacturer's instructions. SE-IgE was measured using a Staphylococcal enterotoxin mix (SEA, SEC and TSST-1; Thermo Fischer, Uppsala, Sweden), as previously described [10].

Other assessments

Height and weight were measured to the nearest 0.1 cm or 0.1 kg in barefoot individuals wearing light clothing. Body mass index (BMI) was calculated by dividing weight by height squared. Stool samples were analyzed for *Clonorchis sinensis* infection using the Kato-Katz method, and the intensity of infection was described as eggs per gram of feces (EPG) as previously described [22].

Statistical analyses

Descriptive data were calculated as the mean ± standard deviation, median [interquartile range (IQR)], or percentages. Data were compared by a t-test, Mann-Whitney test, one-way ANOVA test, or chi-squared test. Bivariate correlations were examined by Spearman tests. Logistic regression tests were performed to identify independent risk factors for SE sensitization or asthma. In multivariate logistic regression tests for SE sensitization, confounders included variables with $P < 0.10$ in univariate tests (age, sex, smoking status, alcohol status, diabetes mellitus, inhalant allergen sensitization, and clonorchis infection) and also demographic factors (residence area and BMI). Multiple correspondence analyses were used to confirm the relationships between SE sensitization and the risk factors identified in

multivariate logistic regression tests. For the asthma logistic regression test, the control group was defined as never having asthma; multivariate logistic regression tests were performed with adjustment for factors with $P < 0.10$ in univariate tests (age, BMI, smoking status, alcohol status, inhalant allergen sensitization, and rhinitis) and a demographic factor (sex). Linear regression models were used to identify determinants for total IgE levels. As total IgE and SE-IgE levels were not normally distributed (Shapiro–Wilk W test: $W < 0.001$), they were transformed to logarithmic scales for linear regression tests. All the statistical analyses were performed using the Stata 12.0 software package (Stata Corporation, College Station, TX, USA).

2.3. Clinical sample analysis

Next, we examined the associations of SE-IgE sensitization with clinical characteristics among elderly patients, using the database of an elderly asthma cohort study.

Study design

We conducted a case-control study of elderly asthma patients and non-asthmatic controls (aged ≥65 years old) in 2013. Elderly asthma patients were recruited from a previously reported cohort study [24]. Briefly, the cohort was established as a prospective observational multicenter study with planned 5-year follow-up (2009–2013). The cohort consisted of 1,031 elderly asthma patients recruited from nine referral hospitals in Korea. The diagnosis of asthma was made by allergy physicians, based on clinical history and the documented presence of a reversible airway obstruction (a positive bronchodilator response or ≥12% and 200 mL improvement in forced expiratory volume in 1 second [FEV1] after anti-asthmatic

treatments). Of the cohort participants, the present study included the patients recruited at two institutions (Seoul National University Hospital [SNUH] and Seoul National University Bundang Hospital [SNUBH]) as they underwent specific assessments for CRS and NP; CRS and NP are known to be associated with asthma, particularly by interaction with SE-IgE [25]. Finally, 249 elderly asthma patients from 370 patients recruited at SNUH and SNUBH were included in the present study; the reasons for exclusion were no serum collection (n=59), several comorbidities which could affect respiratory symptoms or serum IgE levels (n=19), and no follow-up (n=43). The flow chart of patient inclusion is presented in Supplementary Figure 3.

As a control group, non-asthmatic elderly people were recruited using bulletin board advertisement. The absence of asthma was confirmed by both questionnaires and methacholine challenge test (MCT). Questionnaires included the items “Have you ever had asthma?” and “Have you ever had wheezing or whistling in your chest?” Controls were recruited if they gave negative responses to both questions and also had a negative MCT (PC20 >16 mg/ml by a five-breath dosimeter method [26]).

To focus on relationships between asthma and SE-IgE levels, several comorbid conditions that could affect lower respiratory tract symptoms or serum IgE levels were excluded. The exclusion criteria were applied to all of asthma patients and controls and were congestive heart failure, Churg-Strauss syndrome, allergic bronchopulmonary aspergillosis, chest X-ray abnormality, eczema, malignancy, recent anti-IgE monoclonal antibody treatment and recent systemic corticosteroid therapy for conditions other than asthma or rhinosinusitis. Comorbid medical conditions were checked by questions of the following type: “Have you ever been diagnosed with or treated for (specific disease) by a physician” or “Have you been treated with (specific medication) by a physician within the last year?” Chest X-ray abnormality

included bronchiectasis, emphysema, tuberculosis, or other grossly abnormal parenchymal lesions.

All interviews were performed by research nurses experienced in geriatric assessment. All assessments were performed at the participating institutions. All participants were fully informed about the protocols, and provided a written statement of informed consent. The study protocol was approved by the institutional review board of all participating institutions.

Baseline assessment

All participants underwent a baseline assessment at recruitment. Demographic factors and clinical history were assessed by self-administered questionnaires. Late-onset asthma was defined as development of asthma after the age of 40 years [27]. Body mass index (BMI) was calculated by measuring weight and height and using the formula BMI=weight/height squared. Diabetes mellitus (DM) and gastroesophageal reflux disease (GERD) were evaluated as comorbidities using the structured questionnaires mentioned above.

CRS was determined by the persistence of two or more of the following symptoms for ≥3 months during the last year: nasal discharge, nasal obstruction, facial pain and/or anosmia [28]. Subjects who had any of these symptoms underwent nasal endoscopy for NPs. NPs were also deemed positive if the subject had a history of NP surgery. Thus the subjects were classified as no CRS, CRS without NP (CRSsNP), or CRS with NP (CRSwNP).

Atopy was assessed by skin prick tests for 55 common inhalant allergens in Korea. Atopy was defined positive if the subject had a positive skin response to any of the tested allergens (defined by an allergen-induced wheal 3 mm or greater than the negative control) [29]. They were instructed to withhold anti-histamines and over-the-counter drugs for common colds for 5 days before skin prick tests. Predicted percentage values for FEV1 ($FEV1\%_{pred}$) and FVC

($FVC\%_{pred}$) were obtained using the methods of Morris [30]. Peripheral blood eosinophil counts were measured using an automated hematology analyzer (XE-2100, Sysmex, Kobe, Japan), and blood eosinophilia was defined by ≥ 450 cells/μL [31].

In asthma patients, induced sputum tests were performed as previously described [32], and were successful in 186 patients (success rate: 74.7%). Briefly, after the basal FEV1 measurement, patients were pretreated with 200 μg of salbutamol aerosol inhalation. Sputum was induced by 4.5% hypertonic saline inhalation using an ultrasonic nebulizer with output set at 4.5 mL/min (Omron Co., Tokyo, Japan) for 5 min, up to four times (for a total of 20 min or until their FEV1 decreased by ≥ 10%). After each hypertonic saline inhalation, they were asked to blow the nose, rinse the mouths, and encouraged to expectorate sputum into a sterile petri dish plate. Sputum was processed within 4 hours of induction. The equal volume of 0.01 M dithioerythritol (DTE) was added to sputum samples and gently mixed. They were filtered with 100 μm cell strainer or mesh on 15 mL tube and centrifuged with 1,000 or 2,000 rpm for 10 minutes at 4°C. Cell pellets were then resuspended in phosphate buffer saline to a volume equal to the original sputum plus DTE. Total cell counting was carried out using a hemocytometer and cell concentrations were then adjusted to 1.0×10^6 cells/mL. Cytospins were prepared by adding 60 μL of this cell suspension to Shandon II cytocentrifuge cups and spun for 5 min at 42 g. Slides were stained with Diff Quick solution (Sysmex Co., Kobe, Japan). Leukocytes, bronchial epithelial cells and squamous cells were counted. To determine cell differentiation, 300 nucleated cells per slide were counted and these counts were expressed as percentages of intact round nucleated cells, excluding squamous epithelial cells. Sputum samples containing more than 20% squamous epithelial cells were not analyzed. Finally, sputum eosinophilia was defined by ≥3% [33]. They withheld theophylline and montelukast for 2 days and inhaled corticosteroids for 3 days before induced sputum analyses.

Asthma outcomes

The asthma patients were followed up and their adherence was checked according to the original cohort protocols. They were treated by allergy physicians according to Global Initiative for Asthma (GINA) guidelines [34], and were instructed to visit at 3-month intervals regularly or visit urgently if exacerbated. Medication adherence and inhaler technique were checked by research nurses at every visit, to maintain good adherence and correct techniques; patients were asked to respond to by a questionnaire item "How often did you take your inhalers on days when you are not having asthma symptoms?" (all of the time, most of the time, some of the time, occasionally, or never) [35], and were repeatedly educated to ensure their medication adherence to be 'all of the time' or 'most of the time'. They were also asked to bring their inhalers to check dose counters and inhaler techniques, to ensure their proper usage. Formal education was followed whenever they were determined to have difficulties in using inhalers.

Asthma 'exacerbation' was defined according to the official American Thoracic Society/European Respiratory Society Statement: (1) use of systemic corticosteroids or an increase from the stable maintenance dose for at least 3 days due to an asthma attack (defined as 'systemic corticosteroid rescue therapy') or (2) hospitalization or an emergency room visit requiring systemic corticosteroids for asthma [36]. The present analysis included asthma outcomes during the 12 months after the baseline assessment. The difference between the best and worst $FEV1\%_{pred}$ values during the 12-month period was defined as '$FEV1\%_{pred}$ variability'. Medication details were also collected. On the basis of the asthma outcomes during the 12-month follow-up period, severe asthma was defined as the disease which requires the GINA steps 4-5 medications (high-dose inhaled corticosteroid and long-acting beta2-agonist or leukotriene modifier/theophylline) or systemic corticosteroid therapy for ≥50%

of the year to maintain control or to remain uncontrolled despite the high-intensity treatment [37].

Serum IgE assessment

Baseline sera were analyzed for total IgE and SE-IgE levels, using an ImmunoCAP 250 analyzer (Thermo Fischer, Uppsala, Sweden) according to the manufacturer's instructions. SE-IgE levels were measured using staphylococcal enterotoxin mix (SEA, SEC and TSST-1; Thermo Fischer), as described previously [38]. SE-IgE sensitization was classified into three groups: negative (<0.10 kU/L), moderate (0.10–0.35 kU/L), and high (≥0.35 kU/L).

Statistical analyses

Descriptive data are presented as the means ± standard deviation (SD), medians (interquartile range [IQR]), or percentages. Groups were compared by *t*-test, Mann–Whitney *U* test, chi-squared test, one-way ANOVA or Kruskal–Wallis test. Bivariate correlations for non-parametric variables were tested by Spearman's test. Correlations between sputum eosinophil percentage and other variables were examined using general linear models, with adjustment for demographic factors (age, gender, BMI, and smoking status) and clinical variables with $p<0.10$ in univariate tests. Multivariate logistic regression was used to investigate relationships between severe asthma and other variables; confounders included demographic factors (age, gender, BMI, and smoking status) and variables for which p was <0.10 in univariate tests. As sputum eosinophil percentage, blood eosinophil counts and serum SE-IgE levels were not normally distributed ($W<0.001$, Shapiro–Wilk W test), they were log-transformed in the statistical analyses. Finally, multiple correspondence analyses were performed to present the pattern of relationships between asthma severity, SE-IgE sensitization and related factors (CRS and sputum eosinophilia). All statistical analyses were

carried out using Stata 12.0 (Stata Corporation, College Station, TX, USA). All statistical tests were two-sided and $p<0.05$ was considered statistically significant.

2.4. Animal model experiments

Here we aimed to examine if staphylococcal enterotoxin B (SEB) has pathogenic effects in the development of asthma, using a low-dose house dust mite allergen-induced mouse asthma model experiment.

Animals

Six weeks old BALB/c mice (18-20 g) were purchased from SLC Inc. (Hamamatus, Kotoh-cho, Japan). All experiments were performed with the approval of the Institutional Animal Care and Use Committee of the Institute (IACUC) of Laboratory Animal Resources at Seoul National University.

Experimental design

A total of 36 mice were divided into 6 groups to examine the effects of SEB in low-dose *Der p* allergen-induced asthma model (Figure 4): group A (control group), group B (*Der p* sensitization and *Der p* challenge group), group C (SEB sensitization and *Der p* challenge group), group D (*Der p* sensitization and SEB challenge group), group E (*Der p* sensitization and *Der p*+SEB co-challenge group), and group F (*Der p*+SEB co-sensitization and *Der p* challenge group).

Der p extracts were purchased from Yonsei University Hospital (Seoul, Korea) [39]. *Der p* was re-suspended in phosphate-buffered saline (PBS) and were intra-nasally given to mice.

The dose of *Der p* was determined by our previous *in vitro* and *in vivo* exploration for the effects of *Der p*, where 30 ug of *Der p* did not induce profound inflammatory responses from bronchial epithelial cells or did not induce allergic sensitization when administered alone in naïve mice. SEB was purchased from Toxin Technology, Inc. (Sarasota, USA). The intranasal administration dose of SEB 10 ug/mL was based on previous reports [40] and our experiments.

Measurement of outcomes

One day after the last challenge with *Der p* and/or SEB, methacholine AHR was measured with a barometric plethysmographic chamber (OCP 3000, Allmedicus, Anyang, Korea). The three-min enhanced pause (Penh) was measured.

Twenty-four hours after the assessment of airway hyperresponsiveness, mice were sacrificed and bronchoalveolar lavage (BAL) fluid and lung tissue were obtained. BAL fluid inflammatory cells were obtained as previously described [41]. Briefly, BAL was carried out by inserting a cannula into the bronchi and infusing 2 mL of PBS. BAL fluid was recovered by syringe aspiration. Cells were separated from the BAL fluid, and microscope slides were prepared using Cytospin 3 (Thermo Scientific, Pittsburgh, PA, USA) and stained using the Diff-Quik staining kit (Sysmex Co., Kobe, Japan). A total of 300 cells/slide were examined by light microscopy using specific criteria for quantification.

Lung tissues were obtained from the middle zone of left lung to examine histopathological changes of lung parenchyma. Tissues were fixed in 4% neutral buffered formalin, processed, paraffin embedded, sectioned at 3-mm intervals, and stained with hematoxylin-eosin (H&E).

Lung cytokine responses were measured using lung lysates. Total RNA (2 ug) was reverse transcribed into cDNA using a single-strand DNA synthesis kit (Promega, Madison, WA, USA). Gene expression was measured using an ABI 7500 real-time polymerase chain

reaction (RT-PCR) system (Applied Biosystems, Foster, CA, USA) with cDNA, gene-specific primers, and SYBR Green master mix. The expression of each gene was normalized against β-actin and presented as fold changes compared to controls.

Blood samples were obtained by cardiac puncture, and total IgE and *Der p*-specific IgG1 and IgE antibody levels were measured. Immunoglobulin levels were measured by enzyme-linked immunosorbent assay (ELISA) using *Der p* for coating and anti-mouse isotype-specific antibodies (Southern Biotechnology Associates, Birmingham, AL, USA) for detection.

Statistical analysis

Data were analyzed using Stata 14.1 software (Stata Corporation, College Station, TX, USA). All experiments were repeated at least three times. Comparison between groups was made using the Kruskal-Wallis, or the Mann-Whitney test. P values <0.05 were considered statistically significant.

3. Results

3.1. Systematic review and meta-analysis

Of 683 potentially relevant publications identified through the literature search, 659 papers were excluded after reading the abstract and title. Further 17 papers were excluded after reading the full text. Finally, 5 case-control studies and 2 population-based studies from peer-reviewed journals were included (Figure 1). The summary of seven included studies is described in Table 1 and 2.

The prevalence of SE-IgE positivity among asthmatics widely varied with study populations, ranging from 14.9% to 79.1%; however, the rates showed trends to increase in older subjects and in more severe asthmatics. In non-asthmatic controls, the rate of SE-IgE sensitization also ranged widely, from 3.8% to 41.3%. Collectively, all the included articles consistently showed that SE sensitization is more frequent among asthmatics than controls, irrespective of study populations. In the meta-analysis, the pooled odds ratio of SE-IgE sensitization for asthma was 2.95 (95% CI 2.28–3.82, Figure 5).

3.2. General population sample analysis

In total, 1,080 subjects were included in the analysis (Table 3). The population had a mean age of 60.2 ± 11.5 yrs, was predominantly female (62.8%) and had an average BMI for Korean adults (mean 23.6 ± 3.2 kg/m^2), with no differences between the Sancheong and Changwon areas. The prevalence of current asthma and past asthma was 5.8% and 2.6%, respectively. The prevalence of SE-IgE sensitization was 27.0% when defined by the ≥ 0.35 kU/L cutoff (or 55.7% by the ≥ 0.10 kU/L cutoff) without significant difference between

areas. The prevalence of inhalant allergen sensitization was 12.0%. The prevalence of current asthma, inhalant allergen and SE-IgE sensitization was presented in Figure 6 by age groups.

First, we explored risk factors for SE-IgE sensitization. In univariate chi-squared tests, the relevant factors were identified as male sex, advanced age, current smoking, current alcohol use, diabetes mellitus, inhalant allergen sensitization and *Clonorchis* infection. Using multivariate logistic regression tests (Table 4), independent associations with SE-IgE sensitization were tested. Male sex, advanced age (≥ 61 years), current smoking, and inhalant allergen sensitization were identified as independent risk factors for SE-IgE sensitization. In Spearman tests, serum SE-IgE levels showed a positive correlation with pack-year smoking history ($r = 0.247$, $P < 0.001$). Diabetes mellitus was the only comorbid condition that was related to SE-IgE sensitization in univariate tests but only marginally related in multivariate tests ($P = 0.057$). Obesity (defined as BMI ≥ 27.5 kg/m^2 [42]) tended to increase the risk of SE-IgE sensitization ($P = 0.085$). Multiple correspondence analyses were performed to confirm the relationships between SE-IgE sensitization and six relevant factors that were identified in multivariate logistic regression tests ($P < 0.10$). SE-IgE sensitization was closely correlated with male sex and smoking status (Figure 7).

The relationships between inhalant allergen and SE-IgE sensitizations were further investigated. Statistical significance was found for most tested allergens including house dust mites, cockroaches, grass pollen, and tree pollens. The number of allergen sensitizations was also significantly correlated with SE-IgE sensitization (likelihood-ratio $P = 0.002$; Table 5). After inclusion of SE-IgE sensitization as an additional independent variable, risk factors for inhalant allergen sensitization were investigated in multivariate logistic regression tests. To study dose relationships, SE-IgE levels were categorized into tertile-based groups above 0.10 kU/L (T1: 0.10–0.22; T2: 0.22–0.61; and T3: 0.61–50.68 kU/L). High SE-IgE showed a

significant association with inhalant allergen sensitization [T3 vs. negative; odds ratio (OR) 3.13, 95% confidence interval (CI) 1.79–5.48, $P < 0.001$] (Table 6).

The correlations between total IgE and SE-IgE levels were evaluated in linear regression models. In univariate linear regressions, relevant factors for log-transformed total IgE levels were log-SE-IgE ($P < 0.001$), age ($P = 0.022$), sex ($P < 0.001$), smoking status ($P < 0.001$), BMI ($P = 0.069$), alcohol use ($P < 0.001$), inhalant allergen sensitization ($P < 0.001$) and *Clonorchis* infection ($P < 0.001$). Multivariate linear regression analyses demonstrated that log-SE-IgE was a stronger determining factor (Table 7) than other parameters including male sex, current smoking, alcohol use, *Clonorchis* infection (or infection intensity defined by EPG) and inhalant allergen sensitization. The correlation between SE-IgE and total IgE levels ($r = 0.758$, $P < 0.001$) was also presented in a scatter plot, as log-transformed (Figure 8).

Subjects with current asthma had a significantly higher rate of SE-IgE sensitization, or higher levels of SE-IgE compared to those with never asthma or past asthma (Table 8). To investigate independent associations between SE-IgE sensitization and current asthma, multivariate logistic regression tests were performed as described above. Current asthma was significantly associated with SE-IgE sensitization (OR 2.52, 95% CI 1.36–4.67, $P = 0.003$). Current smoking was related to current asthma in a univariate test (vs. never smoking; OR 2.34, 95% CI 1.08–5.04, $P = 0.020$); however, the relationship was not significant in multivariate models co-adjusted with SE-IgE sensitization (OR 1.47, 95% CI 0.66–3.26, $P = 0.347$).

Of interest, current asthma was mostly adult-onset (mean onset: 60.5 ± 10.3 years). Adult-onset current asthma (n=60) had higher levels of SE-IgE (median 0.31, IQR 0.17–0.91 kU/L) compared to childhood-onset current asthma (n=3; median 0.06, IQR 0.03–0.27 kU/L), past asthma, or never asthma (Table 4). In multivariate logistic regression, adult-onset current

asthma showed independent associations with SE-IgE sensitization (vs. never asthma; OR 2.46, 95% CI 1.34–4.54, P = 0.004). The positive associations between adult-onset current asthma and SE-IgE levels were further supported by the dose relationships, using the tertile-based SE-IgE categories (Table 9).

3.3. Clinical sample analysis

The baseline characteristics are presented in Table 10. Mean age at asthma onset was 64.3 years (range: 29–83 years) and none had a history of childhood-onset asthma. The proportion of late-onset asthma (onset after the age of 40 years [27]) was 98.8%, and mean duration of asthma was 7.5±6.8 years. There was no significant difference in the distribution of age, gender, smoking status, and serum total IgE between elderly patients and controls. Serum SE-IgE levels were significantly higher in elderly patients compared to controls (median 0.16 [IQR 0.04–0.53] vs. 0.10 [0.01–0.19], p<0.001).

The comparison of elderly asthma patients according to serum SE-IgE levels is shown in Table 11. Compared to the SE-IgE-negative group (<0.10 kU/L), the SE-IgE-high group (≥0.35 kU/L) had significantly more CRS (CRSsNP 48.8% vs. 35.1% and CRSwNP 23.2% vs. 4.3%, p<0.001), higher blood eosinophil counts (median 296 vs. 128 cells/μL, p<0.001), higher total IgE levels (median 290.2 vs. 24.5 kU/L, p<0.001) and a higher rate of sputum eosinophilia (87.7% vs. 34.4%, p<0.001).

In Spearman's tests, serum SE-IgE level showed positive correlations with total IgE level (r=0.825, p<0.001), blood eosinophil count (r=0.373, p<0.001) and sputum eosinophil percentage (r=0.584, p<0.001) but not with sputum neutrophil percentage (r=-0.100,

p=0.310). The correlation between sputum eosinophil percentage and blood eosinophil count was also significant (r=0.502, p<0.001). The significant correlation of serum SE-IgE level with sputum eosinophil percentage remained independent of confounders in the multivariate analysis (coefficient estimate 0.29, 95% confidence interval [CI] 0.07–0.52, p=0.010; Table 12).

Severe asthma was more frequent in the SE-IgE-high group (53.7% vs. 13.8%, p<0.001). SE-IgE-high group showed more $FEV1\%_{pred}$ variability (21.9±10.5% vs. 14.5±9.0%, p<0.001), more frequent asthma exacerbation (2.0±1.8 vs. 0.3±0.8, p<0.001), and more frequent systemic corticosteroid rescue therapy (2.2±2.0 vs. 0.5±1.0, p<0.001), compared to the SE-IgE-negative group.

Multivariate logistic regression analyses were performed to test if the associations between SE-IgE sensitization and asthma severity were independent of confounders. SE-IgE sensitization showed significant associations with severe asthma in a dose-dependent manner (SE-IgE-high vs. SE-IgE-negative group: odds ratio [OR] 7.47, 95% CI 1.86–30.03, p=0.005; Table 13). Atopy was not related to severe asthma in the univariate test (p=0.800), and did not affect the relationship between SE-sensitization and severe asthma when included as a confounder in the multivariate analyses (specific data not shown).

As patients with severe asthma had significantly more sputum eosinophils compared to non-severe asthma patients (Table 14), we repeated the multivariate logistic regression analyses in a subgroup of 186 asthma patients for which induced sputum data was available. The association with severe asthma remained significant when sputum eosinophilia was added as a confounder (SE-IgE-high vs. SE-IgE-negative group: OR 5.01, 95% CI 1.32–19.04, p=0.018; Table 15).

Multiple correspondence analyses were performed to demonstrate the relationships between severe asthma, CRS, sputum eosinophilia and serum SE-IgE level (Figure 9); severe asthma was located near high SE-IgE levels, CRSwNP, and sputum eosinophilia. However, non-severe asthma was situated close to moderate or negative SE-IgE levels, no CRS, and sputum non-eosinophilia. These results confirmed the significant relationships between asthma severity, SE-IgE sensitization, CRS, and sputum eosinophilia, which were shown in multiple logistic regression analyses (Table 13 and 15).

These analyses described above were replicated within the subgroup of late-onset asthma patients (n=246; 98.8% of total asthmatics), and pointed to the same conclusions (specific data not shown).

3.4. Animal model experiments

Methacholine AHR was significantly increased in group F only, where SEB was co-administered with *Der p* during the sensitization period (Figure 10). No significant differences were observed between other groups.

In BAL fluid analyses, groups B-F showed increased inflammatory cells counts in total, compared to group A (control). In particular, the increase of eosinophil counts was significantly found in group F (Figure 11). In histopathological analyses of lung tissue section, peri-bronchial and perivascular infiltration of inflammatory cells, particularly eosinophils, were more profoundly observed in group F, which is in line with the BAL fluid findings (Figure 12).

In RT-PCR analyses, levels of several cytokines analyzed here, including IL-4, IL-5, IL-13, IL-17, and IFN-γ were observed to be significantly increased in group F particularly, compared to other groups (Figure 13 A-E). Similar findings were observed in the measurement of serum total IgE and *Der p*-specific IgG1 and IgE levels. Only group F showed significant increase of these immunoglobulin levels, suggesting the adjuvant effects of SEB during allergic sensitization period (Figure 14 A-C).

4. Discussion

4.1. Systematic review and meta-analysis

The systematic review and meta-analysis demonstrated that SE-IgE sensitization has significant associations with asthma. Also, it was suggested to have relationships with the clinical reactivity and severity of asthma by individual studies.

In a sense, it may not be surprising that SA may contribute to the pathogenesis of asthma, as it has already been consistently associated with other allergic disorders like atopic dermatitis [43] or CRSwNP [44]. The studies by Tee and Pepys (1981) [15], although not included in the present analyses, were the first to compare sIgE to bacterial antigens (SA, *Streptococcus pneumoniae* and *Haemophilus influenzae*) in subjects with various allergic diseases. The reasons for exclusion were that they did not define the positivity of SE-IgE sensitization in a standardized manner and did not report the prevalence of SE-IgE sensitization and thus were not directly comparable to other included studies. Methodologically, they utilized the radio-allergosorbent tests (RAST) and the RAST score ratio (=patient's specific RAST score/cord blood specific RAST score) for comparisons. In their reports, IgE RAST scores for SA were slightly higher in asthmatics (n=20, mean 1.2) than controls (n=20, mean 1.0), but without statistical significance.

Meanwhile, it should be noted that the definition of SE-IgE positivity was considerably heterogeneous among the studies identified in the systematic review. To specify, 2 case-control studies (Bachert 2012 [10] and Kowalski 2011 [11]) and 2 population studies [45, 46] utilized the SE mix (SEA, SEC, TSST-1) antigen kits; but three other case-control studies [47-49] used each enterotoxin specific IgE tests (Table 1). Moreover, the cut-off value for positivity also varied. Two recent case-control studies (Bachert 2012 [10] and Kowalski 2011

[11]) adapted ≥ 0.1 kU/L, whereas other 5 previous case-control or population-based studies used ≥ 0.35 kU/L as the cutoff levels.

Considering the methodological heterogeneity, the pooled odds ratio was calculated respectively for similarly designed study collection. For two case-control studies (Bachert 2012 [10] and Kowalski 2011 [11]) utilizing the cutoff of 0.1 kU/L for SE mix, the odds ratio was 5.61 (95% CI 3.27–9.63); in three case-control studies (Lee 2006 [47], Lee 2005 [48] and Rossi 2004 [49]) with different definition (as any of tested SE IgE ≥ 0.35 kU/L), the odds ratio was 4.67 (95% CI 2.25–9.68). In two population-based studies (Hollams 2010 [45] and Semi-Jusufagic 2007 [46]), the pooled odds ratio was relatively lower (OR 1.81, 95% CI 1.28–2.56) than case-control studies but was also significant. The odds ratio for each study was presented in Figure 5.

The studies by Rossi and Monasterolo (2004) [49] were conducted in allergic rhinitis and/or asthma patients with house dust mite sensitization. Particularly, they reported the correlation between SE-IgE sensitization and serum ECP levels, indicating that SE-IgE is a potential marker for clinical severity of allergic diseases.

The studies by Jyh-Hong Lee et al. in Taiwanese children/adolescents (2005) [48] reported that the association with SE-IgE is more related to asthma or airway hyper-responsiveness (AHR) than allergic rhinitis alone. Jae-Young Lee et al. (2005) [47] also found significantly high prevalence of SE-IgE sensitization among Korean asthmatic adults. Additionally, they revealed that SE-IgE sensitization is related to the degree of AHR (methacholine PC20).

Two later case-control studies extend the previous findings to severe asthma. In the studies by Kowalski (2011) [11], they found significantly higher serum levels of SE-IgE among severe asthmatics than non-severe counterparts (1.39 ± 0.30 vs. 0.38 ± 0.07 kU/L; P = 0.01), despite similar rates of SE-IgE positivity (76.1% vs. 71.1%). They also found that the presence of

SE-IgE sensitization significantly correlated with various lung function parameters, when adjusted for age. In recent studies by Bachert (2012) [10], the association between SE sensitization and severe asthma was clearly demonstrated by utilizing various sophisticated statistical models. Particularly, they found that SE-IgE was more closely related to asthma severity than house dust mite or grass pollen sIgE was.

Two population-based studies were available for children/adolescents; however, their associations with asthma were significant but less strong than the case-control studies. In the studies by Semi-Jusufagic (2007) [46] on UK children aged 5 years, SE sensitization significantly correlated with current wheeze, wheeze frequency and persistence, and dry air bronchial reactivity. Later studies by Hollams (2010) [45] on Australian children aged 14 years found dose-dependent relationships of SE-IgE for asthma (in univariate analyses), and particularly for AHR (also in multivariate analyses).

A limitation of this systematic review should be considered in the interpretation of findings. All the included studies reported the positive associations between asthma and SE sensitization, which might be indicative of publication bias. Small numbers of included studies (n=7) and substantial heterogeneity (I^2=65.8%) warrants careful interpretation of the results. Particularly in adults, large-scale community population-based studies are necessary to confirm the findings.

To summarize, the systematic review and meta-analyses of current literatures demonstrated that Staphylococcal enterotoxin sensitization was significantly associated with asthma. These findings warrant further studies for elucidating mechanisms, and for confirming their relationships in large-scale populations.

4.2. General population sample analysis

The present analysis demonstrated the epidemiology and the significance of SE-IgE sensitization in the community-based general adult populations. Smoking history, male sex, older age (≥ 61 years), and inhalant allergen sensitization were risk factors for SE-IgE sensitization. SE-IgE level was a strong determinant factor for total IgE level. The sensitization showed independent associations with adult-onset current asthma.

Despite recent evidence suggesting that SE-IgE is an emerging marker for asthma [10, 11], there was a paucity of evidence in general populations as summarized in the systematic review. In children/adolescents, two studies have been published on the association between SE-IgE sensitization and asthma (510 five-year-old children in the UK [50] and 1,380 fourteen-year-old children in Australia [45]). In adults, the GA^{2}LEN survey has recently found the significant links with asthma in European general populations (n=2,908, mean age 48.9 years) [51]. In this regard, the present study is addition to the knowledge, conducted for the first time in Asian general adult population. From the homogeneous results of various European centers in GA^{2}LEN, this present study supports the notion that the asthma-SE association is universally significant across continents.

Moreover, we found that SE-IgE sensitization was significantly related to adult-onset current asthma in the community-based adult population. As the number of current asthma was very small among childhood-onset asthma, we could not draw conclusions on childhood-onset longstanding asthma. The low prevalence of childhood-onset asthma could have been attributed to recall bias, as the information was collected by questionnaires. The finding could also have been related to less symptomatic features of childhood-onset asthma compared to adult-onset asthma in older adults [52], or related to the characteristics of the study population (mean age: 60.2 years). Despite the shortcomings, we suggest a potential role of

SE-IgE sensitization in the pathogenesis of adult-onset asthma, as the associations were independently significant.

Despite the significance of SE-IgE sensitization, the epidemiology is still under investigated. In the current literature, there are only a few studies to report the prevalence in community-based populations [45, 50, 51]. Nevertheless, it seems more clear that SE-IgE sensitization is prevalent in the community; it ranged from 9.6% to 19.3% in pediatric populations (by ≥ the 0.35 kU/L cutoff) [45, 50], and was 29.3% in European adult populations (by ≥ 0.10 kU/L) [51]. Together with the present findings (27.0% by ≥ 0.35 kU/L, or 55.7% by ≥ 0.10 kU/L), we suggest the substantial burden of the sensitization in allergic disorders.

In this regard, risk factors for the sensitization need to be investigated. Interestingly, smoking was a major risk factor. The mechanisms of action are unclear, but cigarette smoke may promote allergen penetration into human bronchial epithelia and thus elicit allergen-specific responses from resident basophils [53]. As SA/SE is inhaled in indoor dust or frequently colonizes the upper airways [4, 54], we postulate that epithelial damage caused by smoke exposure increases the risk of staphylococcal enterotoxin penetration into subepithelial layers, thereby leading to SE-mediated stimulation of immune cells and sensitization. Another interesting finding was that positive effects of smoking on asthma risk disappeared when co-adjusted with SE-IgE levels in multivariate logistic regressions. These findings warrant further investigations; however, we postulate that the pathogenic role of smoking on adult asthma pathogenesis [55] is at least partly mediated by SE-IgE sensitization.

Male sex as a risk factor may seem unusual, considering that female sex has been more frequently associated with allergic diseases [56]; however, the findings were in line with the European adult population study [51]. As males had longer or more intense smoking histories than females, the male preponderance may have resulted from certain lifestyle factors such as

smoking. However, in the present study, male sex and current smoking were associated with SE-IgE sensitization, independently from each other.

The relationship between older age (≥ 61 years) and SE sensitization is another interesting finding. As skin from older individuals has a reduced barrier function or an increased epidermal permeability to exogenous antigens [57], we presume that the risk of sensitization increases with aging. Compared with previous population-based studies, the SE sensitization rate was significantly higher among our control subjects (mean age 59.7 years, 26.1%) than among younger controls in the previous two studies (mean age 14 years, 17.9% in Australian controls [45] and mean age 5 years, 7.5% in UK children [50]). Although these data are not directly comparable, age could have an effect on SE-IgE sensitization, such that 'older age' may be a risk factor for the sensitization and for later developing asthma when combined by co-factors. In the literature, SE-IgE sensitization rate ranges from 38 to 76% of adult asthmatics [58], indicating the presence of specific asthma subtype mediated by SE-IgE sensitization. We have previously shown that SE-IgE is specifically related to severe non-atopic asthma in adults [10]. Collectively, we suggest a potential role of SE-IgE in the pathogenesis of adult-onset non-atopic asthma among older adults.

Our findings on the association with inhalant allergen sensitization confirm previous results from children/adolescent population studies [45, 50] and extend this knowledge into older adults. Atopic subjects may be more prone to SA colonization and sensitization, as M2 macrophages, which are more frequent in the Th2 milieu, have reduced phagocytotic activity, which leads to increased risk of bacterial survival [59]. Conversely, there is also experimental evidence that nasal SEB administration enhances allergen sensitization in mice [40], and in *in vitro* studies using human dendritic cells (DC), SEB caused DCs to drive the Th2 polarization of naïve T cells [60]. Clinically, our cross-sectional findings could suggest that inhalant

allergen sensitization during early life predisposes to the SE sensitization at later life, as they showed discordant patterns with regard to aging; however, further longitudinal studies are necessary to clarify the relationships.

Correlations between SE-IgE and total IgE levels also need to be discussed. Total IgE, which was a traditional indicator of allergy or atopy, has been associated with demographic or environmental factors [61, 62], genetic factors [63], inhalant allergen sensitization [62] or parasitic infection [64]. We performed a comprehensive analysis of IgE in areas with high rates of endemic *Clonorchis* infection to identify determinants of total IgE levels. Interestingly, SE-IgE levels were the most significant determinant, most likely because SE is a superantigen that promotes polyclonal IgE production [5]. These findings suggest that potential effects of SE-IgE need to be taken into consideration when interpreting total IgE levels, particularly in older adult populations. Considering the heterogeneity of adult-onset asthma and the significance of endotype approaches [65, 66], we feel that SE-IgE is a useful biomarker to be included in clinical or epidemiological studies for adult asthma.

There still remains possibility that SE-IgE sensitization is a ‘surrogate marker’ just to reflect the effects of still ‘undiscovered risk factors’. In fact, SA and various bacteria may co-exist in indoor dust [67], and serum SE-IgE could be a surrogate marker that reflects high exposure to various microbial antigens, although this possibility has been partially excluded [45]. High indoor levels of bacteria and mold spores have already been associated with asthma severity [68]. Moreover, serum antibody levels do not directly represent the local inflammation in the airways. However, serum IgE levels correlate with local airway SE-IgE levels or are functional [69]. Further studies would be necessary to delineate whether it is a surrogate marker or the causative factor.

The present study has several limitations. By using a cross-sectional survey, we could not determine causal relationships between parameters. The results could not be generalized because the survey was not conducted nationwide. However, the prevalence of asthma was comparable to that in other community-based random population surveys in Korean older adults [23, 70]. Because the study participants were not randomly recruited but were volunteers from communities, selection biases may have existed. Recall biases also need to be taken into account, as our phenotype definition was partly based on self-administered questionnaires. However, the present study has strengths in that the demographic features were different from previous community population-based studies [45, 50, 51], and we included additional analyses by age of asthma onset. Thus, the present findings are new addition to previous knowledge.

To summarize, we report the epidemiology and the significance of Staphylococcal sensitization in the community-based adult populations. Our identification of risk factors for SE-IgE sensitization may be useful in understanding the pathophysiology of SE-mediated allergic diseases. The strong correlation between SE-IgE and total IgE levels also warrants further investigation. The independent relationships with adult-onset asthma warrant further studies to clarify the precise pathogenic roles of Staphylococcal sensitization.

4.3. Clinical sample analysis

The present clinical epidemiological study demonstrated that SE-IgE sensitization was significantly related to asthma in the elderly. In particular, it was closely related to late-onset severe eosinophilic asthma, comorbid with CRSwNP. Our findings indicate a potential implication of SE in the high morbidity burden of asthma in the elderly, and also suggest important clues to the pathogenesis of late-onset severe eosinophilic asthma in the aged group.

The pathogenesis of late-onset asthma in the elderly remains elusive, despite the characteristics have been reported in the literature since the 1970s. Lee *et al.* described 15 cases of elderly patients who developed asthma after the age of 60 years [71]. Interestingly, these cases were frequently eosinophilic (12/15) but non-atopic (11/15), which is similar to our findings. However, the reason for airway eosinophilic inflammation remains to be clarified in non-atopic elderly subjects. In the present study, sputum eosinophilia was frequent (58.6%) in elderly asthma patients, but did not significantly correlate with atopic status (p=0.227). Rather, serum SE-IgE level had significant correlations with sputum eosinophilia. Thus, our findings lead to the speculation that SE-IgE sensitization may be involved in the pathogenesis of eosinophilic airway inflammation in late-onset non-atopic elderly asthma patients. The roles of SE sensitization in eosinophilic airway inflammation among non-atopic or late-onset asthma patients have been also suggested by a recent Japanese cohort study [72]; SE IgE sensitization was significantly related to high serum periostin levels and rapid decrease in FEV1 [72].

We found significant relationships between SE-IgE sensitization and severe asthma in the elderly, confirming two recent reports from younger adult asthma patients. Kowalski *et al.* found 3-fold higher levels of SE-IgE in patients with severe asthma (n=109, mean age: 50.9 years) compared to patients with non-severe asthma (n=101, mean age 38.4 years) [73]. We

also previously demonstrated that SE-IgE sensitization was significantly associated with severe asthma in younger adults (n=166, mean age: 46.5 years), independently of atopy and total IgE [74]. However, it has never been examined which severe asthma patients are related to SE-IgE sensitization; those two studies did not evaluate asthma onset age, sputum profiles or comorbidities. Thus, the novelty of the present study is that it does not only extend the relationships of SE-IgE sensitization with asthma severity into the elderly population, but also specifically defines its associations with severe late-onset eosinophilic asthma comorbid with sinusitis.

We found close relationships between SE-IgE sensitization and severe late-onset eosinophilic asthma comorbid with CRS in the elderly. Interestingly, this phenotype has repeatedly been described in recent studies of younger adult patients. A late-onset eosinophilic asthma phenotype (mean age at onset 32.6 years) was observed in cluster analyses of UK refractory asthma patients in secondary care [75]. In the Netherlands, Amelink *et al.* reported associations between severe adult-onset asthma (mean age at onset 40.2 years) and nasal polyposis, sputum eosinophil count, exhaled nitric oxide, blood neutrophil count and absence of atopy [76]. Van Veen *et al.* also reported difficult-to-treat asthma characterized by persistent sputum eosinophilia, non-atopy and extensive sinus disease [77]. These findings are supported by more recent unsupervised phenotyping studies using the Severe Asthma Research Program cohort, where a specific cluster characterized by older age, later onset, less atopy, more sinus disease, more eosinophilia, and more severe asthma was described [78]. Thus, our findings demonstrated here indicate that this phenotype is clinically significant also in the elderly patients, and further suggest for the first time that SE-IgE sensitization is involved in the pathogenesis of this asthma phenotype. To prove this, our findings warrant replication in different population samples.

The inter-relationships of SE-IgE with late-onset asthma and CRS are supported by observational studies. CRS had a significant association with adult-onset asthma in European general populations, irrespective of nasal allergy [79]. SE-IgE sensitization was also significantly related to adult-onset asthma independently of rhinitis symptom in Korean community populations, although CRS was not studied then [38]. As SA is a major colonizer in the nasal cavity of patients with CRS and/or NP [80], the relationships of SE-IgE and late-onset asthma could be confounded by comorbid CRS. However, in the literature reported so far, the relationships of SE-IgE with asthma were independent of rhinitis, CRS or NP comorbidity [38, 73, 81]. Thus we hypothesize that SE is a mechanistic link between asthma and CRS, either as a direct cause or disease modifier for late-onset asthma.

Recently, it has been reported that nasal colonization with SA is also positively associated with risks of wheeze, asthma, or severe asthma [82, 83]. In the United States National Health and Nutrition Examination Survey (NHANES) 2001-2002 (age 6-85 years), SA nasal colonization rate was 28.4% [95% CI 27.3-29.6%] and more prevalent in males. Of note, it was positively associated with emergency room visit for wheezing, asthma diagnosis ever, or asthma attack in past year [82]. In addition, in a school-based cohort study of Norwegian adolescents (age 18-19 years), nasal SA carriage was found in 51.3% of 868 study participants, and significantly associated with severe asthma (OR 3.34 [95% CI 1.33-8.37]) or severe allergic rhinitis (OR 1.65 [95% CI 1.06-2.55]) but not with any asthma (OR 1.19 [0.77-1.83]) or any allergic rhinitis (OR 1.26 [95% CI 0.92-1.73]) in univariate analyses [83]. The positive associations between nasal SA carriage and severe asthma or allergic rhinitis remains significant in multivariate logistic regression analyses [83]. These findings support our conclusions that SE-IgE is more relevant to asthma comorbid with nasal pathology. However, in the literature so far, no studies have examined both of nasal SA carriage and serum SE-IgE levels in relation to asthma outcomes. In a study of CRS patients and controls,

nasal SA colonization rates paralleled the presence of SE-IgE in nasal tissue homogenates; however, nasal carriage rate was higher than SE-IgE prevalence in each subjects [84], suggesting the possibility that nasal SA carriage is an antecedent event to SE-IgE sensitization. Further investigation of both markers would help to understand the relationships between SA/SE-IgE and the pathogenesis of asthma.

Several major limitations need to be considered in interpreting the present findings. First, this analysis had a correlative nature, and thus could not address causal relationships of SE-IgE sensitization. Second limitation is a potential selection bias, as the participants were recruited from specialist allergy clinics at tertiary institutions with exclusion of several conditions such as heart failure or lung parenchymal diseases. As the present study only included the patients from two referral clinics (SNUH and SNUBH), another selection bias issue may be raised; thus we further examined serum SE-IgE levels available from 365 patients recruited at other participating institutions (data not described). This cohort included significantly more female patients (66.3% vs. 53.2%, p=0.001), with earlier age of asthma onset (64.3±8.7 vs. 65.9±7.1 years, p=0.001), slightly more atopy and lower total IgE levels. However, we observed no significant difference in serum SE-IgE levels (patients from SNUH and SNUBH; median 0.16 [IQR 0.04-0.53] vs. patients from other institutions; 0.16 [0.04-0.57], p=0.603) and SE-IgE sensitization status (percentage of SE-IgE-high group: 32.9% vs. 34.0%, p=0.963) between two patients' groups. We also found the relationships between SE-IgE sensitization and late-onset asthma to be consistent in the analyses of whole available samples (n=711). Thus, the risk of forementioned selection bias may not be significant. Considering the interactions of SE-IgE with asthma and CRSwNP [25], we believe our analyses of 249 patients characterized for CRSwNP were more appropriate for the present study purpose than whole available sample analyses without relevant information. Third, our study cohort had the characteristics of being predominantly late-onset asthma (98.8%), and thus we could not

compare with childhood-onset longstanding asthma in the elderly. Our findings on SE-IgE associations need to be interpreted in the context of late-onset disease. However, the predominance of late-onset asthma was consistent over nine different referral clinics which originally participated in our entire cohort study (about 95%; specific data not shown here). As our study participants were recruited from tertiary referral hospitals, they may have been likely to have recent-onset asthma with more severity, compared to asthma patients in the community. Fourth, considering the heterogeneity within CRS and NP subgroups which relates to the risk of comorbid asthma [85], further studies need to characterize CRS phenotypes. In addition, the prevalence of NP may have been underestimated in the present study, as smaller polyps (score <2 in the Davos classification [86]) may have been missed by flexible nasal endoscopy.

Nevertheless, the present study has strength, and provides important implications for further studies. This is the first report on the specific relationships of SE-IgE with late-onset severe asthma in the elderly. We analyzed a well-characterized group of elderly patients with asthma, including asthma severity, comorbidity and sputum profiles, which is a new and detailed extension to previous reports on the general associations between SE-IgE and asthma severity among younger adult patients [73, 74]. Clinical relationships of SE-IgE sensitization demonstrated here may indicate SE to be a new potential therapeutic target to reduce morbidity burden of asthma in the elderly, and also suggest clues to understand the pathophysiology of late-onset severe eosinophilic asthma in the elderly.

4.4. Animal model experiments

The present experimental study demonstrated that SEB facilitates the development of asthma, via adjuvant effects during allergen sensitization period. The mechanisms that SEB exerts the roles as adjuvant in allergic sensitization warrant further investigation, but previously have been suggested to include the promotion of dendritic cell migration and maturation [40].

There are several experimental studies to suggest the pro-allergic roles of SEB or SA in airway diseases. *In vitro* experiments have revealed that SEB induces the corticosteroid insensitivity in human peripheral blood mononuclear cells (PBMC) [87], modulates dendritic cells to drive Th2 polarization [60], and influences nasal epithelium to secrete granulocyte migration and survival factors [88]. Moreover, SA may directly induce epithelial cell-derived cytokines such as TSLP and IL-33 from human nasal polyp tissues and BEAS-2B cells, which could implicate the mechanistic roles of SA in steroid-resistant type 2 inflammation [89]. *In vivo* experiments have demonstrated that nasal SEB administration can promote allergen sensitization and airway inflammation in ovalbumin-induced murine asthma [40], or induce non-allergic eosinophilic asthma by itself [90]. In another animal model using epicutaneous SEB exposure, it enhanced ovalbumin-induced experimental 'atopic march' from dermatitis to asthma, supporting its pathophysiological plausibility [91]. Our experiment is the addition to previous reports, by demonstrating that SEB promoted the development of allergic sensitization and asthma in response to nasal exposure of *Der p*, a conventional allergen in humans.

Recent experimental studies have discovered pathogenic effects of staphylococcal serine protease-like proteins (Spl) in the development of allergic airway diseases [92]. Spl successfully induced IgE sensitization to Spl itself and allergic airway disease phenotypes in mouse experiments. Also, Spl-specific IgE levels were significantly higher in human asthma

patients than in non-asthmatic controls. Thus, Spl is now supposed to play potentially key roles in non-atopic but allergic diseases. Further studies are warranted to validate and further elucidate the roles of Spl in the pathogenesis of asthma, particularly in terms of asthma phenotypes and endotypes.

Collectively, along with our findings that SEB promote the allergic sensitization of *Der p* and subsequent development of allergic asthma in mice, further works need to be done to clarify the mechanisms that SE is involved in the pathogenesis of asthma.

5. Conclusion

Emerging evidence suggests that SE has a pathogenic role in adult-onset asthma. First, our systematic review showed that SE-IgE sensitization is consistently associated with asthma. Secondly, our general population sample analyses demonstrated that SE-IgE sensitization is an independent risk factor for adult-onset asthma. Third, our clinical sample analyses suggested that SE-IgE sensitization was a risk factor for specific phenotype of late-onset asthma, which was severe eosinophilic asthma comorbid with CRSwNP. Finally, SEB showed adjuvant effects in low dose *Der p*-induced allergic asthma mouse models. In addition, recent experimental evidence suggested that unconventional staphylococcal proteins, such as Spl, may also have potentially important roles in SA-induced allergic airway diseases. These findings collectively suggest strong potential for SE or staphylococcal proteins to be involved in the pathogenesis of asthma in adults. These findings also generate new questions, whether decolonization of SA or blockade of SE-mediated immune pathways could be a new therapeutic option in preventing, or managing asthma in adults.

Tables

Table 1. Summary of hospital-based case-control studies on the association between Staphylococcal enterotoxin sensitization and asthma

Author (year) [reference]	Subjects	Region	Measures of SE-IgE (positive cutoff)	SE positivity (%)
Bachert (2012) [10]	Severe asthma=166; Nonsevere asthma=152; Control=69 (mean age 41.3 yrs)	UK and Germany	SE mix (SEA, SEC, TSST-1) sIgE (≥ 0.1 kU/L)	59.6% (99/166) in severe asthma 40.8% (62/152) in nonsevere asthma 13.0% (9/69) in controls
Kowalski (2011) [11]	Severe asthma=109; Nonsevere asthma=101; Control=45 (mean age 42.4 yrs)	Poland	SE mix (SEA, SEC, TSST-1) sIgE (≥ 0.1 kU/L)	76.1% (79/104) in severe asthma 71.1% (64/90) nonsevere asthma 41.3% (12/29) in controls
Lee (2006) [47]	Aspirin-intolerant asthma=80; Aspirin-tolerant asthma=62; Control=52 (mean age 41.1 yrs)	Korea	SEA, SEB, TSST-1 sIgE (≥ 0.35 kU/L)	38.0% (54/142) in asthma 17.1% (7/41) in controls

Lee (2005) [48]	Allergic rhinitis/asthma=188; Control=53 (mainly < 20 yrs)	Taiwan	SEA, SEB sIgE (≥ 0.35 kU/L)	29.4% (5/17) in asthma 3.8% (2/53) in controls
Rossi (2004) [49]	Allergic rhinitis and/or asthma=198; Control=25 (mean age 22.9 yrs)	Italy	SEA, SEB, SEC, SED, TSST-1 sIgE (≥ 0.35 kU/L)	35.5% (22/62) in asthma 4% (1/25) in controls

Abbreviations: SE, Staphylococcus aureus enterotoxin; SEA-D, Staphylococcal enterotoxin A-D; TSST-1, toxic shock syndrome toxin-1; sIgE, specific IgE

Table 2. Summary of population-based studies on the association between Staphylococcal enterotoxin sensitization and asthma

Author (year) [reference]	Subjects	Region	Asthma definition (prevalence)	Measures of SE-IgE (cutoff level)	SE positivity (%)
Hollams (2010) [45]	1,380 children (the West Australian Pregnancy Cohort study; aged 14 yrs)	Australia	Current asthma; recent symptoms + asthma medication + ever doctor diagnosis (prevalence 10.5%)	SE mix (SEA, SEC, TSST-1) sIgE (≥ 0.35 kU/L)	27.1% (38/140) in asthma; 17.9% (214/1195) in controls
Semi-Jusufagic (2007) [46]	510 children (the Manchester Asthma and Allergy Study; aged 5 yrs)	UK	Current wheeze (ISAAC, prevalence 22.1%) Physician-diagnosed asthma (prevalence 20.1%)	SE mix (SEA, SEC, TSST-1) sIgE (≥ 0.35 kU/L)	14.4% (16/111) in current wheeze; 14.9% (15/101) in physician-diagnosed asthma;

					7.5% (23/307) in controls (never wheezer)

Abbreviations: SE, Staphylococcus aureus enterotoxin; SEA-C, Staphylococcal enterotoxin A-D; TSST-1, toxic shock syndrome toxin-1; sIgE, specific IgE; ISAAC, the International Studyof Asthma and Allergies in Childhood questionnaire

Table 3. Baseline characteristics of study population in the community surveys

Parameters	Sancheong (n=605)	Changwon (n=475)	*P* value
Age (yrs)	59.9 ± 12.1	60.6 ± 10.8	0.306
Female sex (%)	64.0	61.3	0.362
BMI (kg/m^2)	23.5 ± 3.2	23.8 ± 3.2	0.130
Smoking status (%)			
Never smoker	64.4	65.0	0.435
Ex-smoker	17.3	19.3	
Current smoker	18.3	15.7	
Alcohol status (%)			
Never drinker	50.5	54.0	0.080
Ex-drinker	8.9	5.3	
Current drinker	40.6	40.7	
Diabetes mellitus (%)	8.4	9.3	0.631
Rhinitis* (%)	16.9	16.1	0.706
Current asthma† (%)	5.6	6.1	0.836
Inhalant allergen sensitization‡ (%)	12.3	11.4	0.649
Clonorchis infection§ (%)	33.8	14.6	< 0.001
FVC%pred	92.4 ± 15.9	94.6 ± 17.2	0.037
FEV1%pred	108.3 ± 20.7	108.6 ± 20.6	0.891
FEV1/FVC%	84.7 ± 8.4	82.9 ± 9.4	0.002
SE-IgE			
Levels (kU/L)	Median 0.14 (IQR	Median 0.11 (IQR	0.372

	0.05–0.41)	0.04–0.37)	
≥ 0.35 kU/L (%)	27.6	26.3	0.636
Total IgE (kU/L)	Median 129.1 (IQR 43.4–358.2)	Median 98.6 (IQR 35.9–307.3)	0.781

Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; SE-IgE, Staphylococcal enterotoxin specific IgE

*Rhinitis was defined by the question "Have you had a problem with sneezing, or a runny, or blocked nose without a cold in the last 12 months?".

†Current asthma was defined as positive if the subjects had current wheeze (Have you had a wheezing or whistling in the chest during the last 12 months?) and methacholine airway hyperresponsiveness.

‡Inhalant allergen sensitization was defined as positive if the subjects had a positive skin response to any of tested 10 inhalant allergens.

§Clonorchis infection was defined by stool sample analyses for the presence of eggs.

P values were determined by t-tests, Mann-Whitney tests, or chi-squared tests.

Table 4. Multivariate logistic regression for Staphylococcal enterotoxin sensitization*

SE positive sensitization (n=292) vs. negative sensitization (n=788)	Adjusted OR (95% CI)†	*P* value
Residence area		
Sancheong	Reference	
Changwon	1.02 (0.74–1.39)	0.922
Age group		
≤ 40 yrs	Reference	
41–50 yrs	1.77 (0.78–4.01)	0.173
51–60 yrs	2.17 (0.99–4.75)	0.053
61–70 yrs	2.74 (1.27–5.90)	0.010
> 70 yrs	2.95 (1.33–6.57)	0.008
Sex		
Female	Reference	
Male	1.93 (1.21–3.07)	0.006
BMI (kg/m^2)		
< 23	Reference	
23.0–24.9	0.85 (0.58–1.25)	0.414
25.0–27.4	1.19 (0.79–1.79)	0.418
≥ 27.5	1.58 (0.94–2.65)	0.085
Smoking status		
Never smoker	Reference	
Ex-smoker	1.36 (0.82–2.25)	0.232
Current smoker	3.26 (2.03–5.24)	< 0.001

Alcohol status		
Never drinker	Reference	
Ex-drinker	0.92 (0.50–1.71)	0.800
Current drinker	1.13 (0.78–1.64)	0.516
Diabetes mellitus		
No	Reference	
Yes	1.61 (0.99–2.64)	0.057
Inhalant allergen sensitization		
No	Reference	
Yes	2.28 (1.45–3.59)	< 0.001
Clonorchis infection		
No	Reference	
Yes	1.16 (0.83–1.68)	0.366

Abbreviations: SE, Staphylococcal enterotoxin; OR, odds ratio; CI, confidence interval; BMI, body mass index

P values were determined by multivariate logistic regression tests.

[*]Staphylococcal enterotoxin sensitization was defined by serum Staphylococcal enterotoxin mix (SEA, SEC and TSST-1) specific IgE level ≥ 0.35 kU/L.

[†]Adjusted for residence area, age group, sex, BMI category, smoking status, alcohol status, diabetes mellitus, inhalant allergen sensitization, and *Clonorchis* infection.

Table 5. Relationships between inhalant allergen and Staphylococcal enterotoxin sensitization

	SE sensitization% (≥ 0.35 kU/L)	*P* value
Inhalant allergen sensitization		
No, n=937	24.9	< 0.001
Yes, n=127	43.3	
Number of sensitized inhalants		
0, n=937	24.9	0.002
1, n=72	40.3	
2, n=31	45.2	
3, n=12	50.0	
≥ 4, n=12	50.0	

Abbreviation: SE, Staphylococcal enterotoxin

P values were determined by likelihood-ratio chi-squared tests.

Table 6. Multivariate logistic regression tests for inhalant allergen sensitization

	Adjusted OR (95% CI)*	*P* value
Residence area		
Sancheong	Reference	
Changwon	1.27 (0.83–1.94)	0.263
Age	0.95 (0.93–0.97)	< 0.001
Sex		
Female	Reference	
Male	1.94 (1.07–3.54)	0.029
BMI (kg/m^2)		
< 23	Reference	
23.0 – 24.9	0.94 (0.56–1.55)	0.801
25.0 – 27.4	1.11 (0.64–1.91)	0.711
≥ 27.5	1.28 (0.65–2.52)	0.482
Smoking		
Never smoker	Reference	
Ex-smoker	0.45 (0.22–0.95)	0.036
Current smoker	0.63 (0.32–1.23)	0.173
Clonorchis infection		
No	Reference	
Yes	1.81 (1.16–2.84)	0.010
SE-IgE levels		
Negative (< 0.10 kU/L)	Reference	
Lowest tertile (0.10–0.22 kU/L)	1.40 (0.77–2.55)	0.275

Mid-tertile (0.22–0.61 kU/L)	1.60 (0.90–2.84)	0.107
Highest tertile (0.61–50.68 kU/L)	3.13 (1.79–5.48)	< 0.001

Abbreviations: OR, odds ratio; CI, confidence interval; BMI, body mass index; SE-IgE, Staphylococcal enterotoxin specific IgE

P values were determined by multivariate logistic regression tests.

*Adjusted for residence area, age, sex, BMI category, smoking status, clonorchis infection, and tertile-based SE-IgE category

Table 7. Multivariate linear regression for total IgE levels (kU/L)

For log-total IgE	*P* values*	β	Std. Err.	t
Log-SE-IgE (kU/L)	< 0.001	0.58	0.02	29.5
Male sex	< 0.001	0.49	0.10	5.05
Age	< 0.001	0.01	0.003	3.94
BMI	0.013	0.02	0.01	2.49
Smoking status				
Ex-smoker	0.457	-0.08	0.11	-0.74
Current smoker	0.904	-0.13	0.11	-0.12
Alcohol status				
Ex-drinker	0.374	0.11	0.13	0.89
Current drinker	< 0.001	0.32	0.07	4.37
Inhalant allergen sensitization	0.001	0.33	0.09	3.43
Clonorchis infection	0.008	0.19	0.07	2.67

Abbreviations: SE-IgE, Staphylococcal enterotoxin specific IgE; BMI, body mass index

*P values were determined by multivariate linear regression tests with adjustment for log-transformed SE-IgE, sex, age, BMI, smoking status, alcohol status, inhalant allergen sensitization, and *Clonorchis* infection.

Table 8. Comparison by asthma onset and current activity

	Never asthma[*] (n=989)	Past asthma[†] (n=28)	Current asthma (n=63)	*P* values
Age (yrs)	59.7 ± 11.6	63.6 ± 10.3	66.4 ± 7.7[ll]	< 0.001[§]
Adult-onset asthma[‡] (%)	-	50%	95.2%	0.003[**]
Female sex (%)	62.7	75.0	58.7	0.327[**]
BMI (kg/m^2)	23.6 ± 3.1	22.8 ± 3.0	24.1 ± 4.1	0.161[§]
Smoking status (%)				
Never smoker	65.0	67.9	58.1	0.708[**]
Ex-smoker	17.9	21.4	21.0	
Current smoker	17.1	10.7	21.0	
Rhinitis (%)	15.7	28.0	24.6	0.058[**]
Inhalant allergen sensitization (%)	12.5	11.1	3.3	0.104[**]
SE-IgE (kU/L)	Median 0.12 (IQR 0.04–0.37)	Median 0.09 (IQR 0.03–0.15)	Median 0.31 (IQR 0.13–0.91)[ll, ¶]	0.004[§]
SE-IgE ≥ 0.35 kU/L (%)	26.1	14.3	44.4	< 0.001[**]

Abbreviations: BMI, body mass index; SE-IgE, Staphylococcal enterotoxin specific IgE; IQR, inter-quartile ranges

[*]Never asthma was defined by no asthma diagnosis in history and no current asthma.

[†]Past asthma was defined by prior asthma diagnosis history but no current asthma.

[‡]Adult-onset asthma was defined by a participant-recalled asthma starting after 18 years old.

[§]*P* values were determined by one-way ANOVA tests; [ll]$P < 0.05$ between never asthma and current asthma; and [¶]$P < 0.05$ between past asthma and current asthma by *post hoc* analyses.

[**]*P* values were determined by chi-squared tests.

Table 9. Multivariate logistic regression tests for adult-onset current asthma*

Adult-onset current asthma (n=60) vs. never asthma (n=989)	Adjusted OR (95% CI)†	*P* value
Age	1.07 (1.04–1.11)	< 0.001
Sex		
Female	Reference	
Male	1.05 (0.44–2.52)	0.912
BMI (kg/m^2)		
< 23	Reference	
23.0 – 24.9	0.98 (0.45–2.13)	0.964
25.0 – 27.4	1.02 (0.45–2.32)	0.966
≥ 27.5	2.55 (1.11–5.84)	0.027
Smoking status		
Never smoker	Reference	
Ex-smoker	0.84 (0.33–2.15)	0.715
Current smoker	1.01 (0.39–2.60)	0.987
Alcohol status		
Never drinker	Reference	
Ex-drinker	2.65 (1.05–6.64)	0.039
Current drinker	0.84 (0.40–1.73)	0.629
Inhalant allergen sensitization		
No	Reference	
Yes	0.26 (0.06–1.13)	0.072
Rhinitis		

No	Reference	
Yes	2.02 (1.01–4.08)	0.049
SE-IgE levels		
Negative (< 0.10 kU/L)	Reference	
Lowest tertile (0.10–0.22 kU/L)	2.38 (0.91–6.25)	0.078
Mid-tertile (0.22–0.61 kU/L)	5.07 (2.17–11.8)	< 0.001
Highest tertile (0.61–50.68 kU/L)	6.02 (2.49–14.5)	< 0.001

Abbreviations: OR, odds ratio; CI, confidence interval; BMI, body mass index; SE-IgE, Staphylococcal enterotoxin specific IgE

P values were determined by multivariate logistic regression tests.

*Adult-onset current asthma was defined by current asthma (defined by current wheeze and methacholine airway hyperresponsiveness) with a participant-recalled onset after 18 years old.

†Adjusted for age, sex, BMI category, smoking status, alcohol status, inhalant allergen sensitization, and rhinitis

Table 10. Baseline characteristics of elderly control and asthma patients

	Elderly control (n=98)	Elderly asthma (n=249)	*p* values
Female (%)	68.4	66.3	0.708
Age (years)	70.6±4.8	71.5±5.1	0.168
Asthma onset age (years)		64.3±8.7	
BMI (kg/m^2)	23.6±3.6	24.4±3.3	0.057
Smoking status (%)			
Never	72.5	74.7	0.480
Former	20.4	15.7	
Current	7.1	9.6	
DM (%)	7.1	12.9	0.130
GERD (%)	12.2	20.4	0.078
CRS (%)			<0.001
No CRS	82.7	41.4	
CRSsNP	17.3	45.8	
CRSwNP	0.0	12.9	
Atopy (%)	13.4	42.5	<0.001
Pre-BD $FEV1\%_{pred}$	117.0±23.6	85.1±19.5	<0.001
Pre-BD $FVC\%_{pred}$	95.0±16.1	86.7±18.0	<0.001
Pre-BD FEV1/FVC%	84.8±8.9	75.3±12.5	<0.001
Blood eosinophils (/μL)	129 (IQR 78–213)	200 (IQR 108–350)	<0.001
Blood eosinophilia (≥ 450 cells/μL, %)	4.9%	16.2%	<0.001

Sputum eosinophilia (≥ 3%, %)		58.6%	
Serum total IgE (kU/L)	91.5 (IQR 28.9–218.2)	83.3 (IQR 31.4–220.0)	0.934
Serum SE-IgE (kU/L)	0.10 (IQR 0.01–0.19)	0.16 (IQR 0.04–0.53)	<0.001
SE-IgE sensitization (%)			
Negative (<0.10 kU/L)	48.0	37.8	0.008
Moderate (0.10–0.35 kU/L)	35.7	29.3	
High (> 0.35 kU/L)	16.3	32.9	

Abbreviations: BMI, body mass index; DM, diabetes mellitus; GERD, gastroesophageal reflux disease; CRS, chronic rhinosinusitis; NP, nasal polyp; pre-BD, pre-bronchodilator; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; SE, staphylococcal enterotoxin; IQR, interquartile range

Results were presented as percentage, mean ± standard deviation, median (interquartile range), or otherwise specified.

p values were determined by chi squared tests, t tests, or Mann-Whitney tests.

Table 11. Comparison of 249 elderly asthma patients according to serum staphylococcal enterotoxin specific IgE levels

	Negative SE-IgE group (<0.10 kU/L; n=94)	Moderate SE-IgE group (0.10–0.35 kU/L; n=73)	High SE-IgE group (≥0.35 kU/L; n=82)	*p* value
Baseline characteristics				
SE-IgE (kU/L)	0.03 (IQR 0.01–0.06)	0.17 (IQR 0.13–0.24)	0.92 (IQR 0.54–1.47)	<0.001
Female (%)	75.5	67.1	54.9	0.015
Age (years)	71.3±5.0	71.8±5.3	71.4±5.2	0.842
Asthma onset age (years)	64.1±9.7	64.5±8.0	63.9±8.1	0.895
BMI (kg/m^2)	24.9±3.4	24.5±3.2	23.8±3.2	0.774
Smoking (%)				
Never	80.9	74.0	68.3	0.448
Former	11.7	16.4	19.5	
Current	7.4	9.6	12.2	
DM (%)	8.5	15.1	15.9	0.278
GERD (%)	19.5	23.4	18.9	0.778
CRS (%)				
No CRS	60.6	48.0	28.0	<0.001
CRSsNP	35.1	39.7	48.8	
CRSwNP	4.3	12.3	23.2	
Atopy (%)	26.4	52.9	47.6	0.014

Blood eosinophils (/μL)	128 (IQR 89–230)	175 (IQR 86–325)	296 (IQR 184–436)	<0.001
Blood eosinophilia (≥ 450 cells/μ, %)	8.2%	16.0%	25.0%	0.026
Serum total IgE (kU/L)	24.5 (IQR 9.6–61.5)	92.9 (IQR 54.4–176.1)	290.2 (IQR 133.1–620.1)	<0.001
Sputum neutrophils (%), n=186	26.0 (IQR 12.7–30.0)	26 (IQR 9.7–31.3)	15.3 (IQR 8.5–36.8)	0.599
Sputum eosinophils (%), n=186	1.3 (IQR 0.3–4.3)	2.5 (IQR 1.0–5.0)	9.7 (IQR 5.5–18.3)	<0.001
Sputum eosinophilia (≥ 3%, %), n=186	34.4	46.2	87.7	<0.001
Baseline $FEV1\%_{pred}$	98.1±24.7	98.4±26.6	83.9±21.4	<0.001
Clinical observations during the study period				
$FEV1\%_{pred}$ variability during study period	14.5±9.0	16.5±8.9	21.9±10.8	<0.001
Exacerbation frequency (median [mean±SD])	0 (0.4±0.8)	0 (0.8±1.3)	2 (2.0±1.8)	<0.001
OCS rescue frequency (median [mean±SD])	0 (0.5±1.0)	0 (0.9±1.4)	2 (2.2±2.0)	<0.001
Mean ICS dose (mcg, fluticasone equivalent/day)	522.2±281.6	586.7±235.2	733.5±256.0	<0.001
Regular OCS use (%)	7.3	12.0	19.7	0.089

Severe asthma (%)	13.8	28.8	53.7	<0.001

Abbreviations: SE, staphylococcal enterotoxin; BMI, body mass index; DM, diabetes mellitus; GERD, gastroesophageal reflux disease; CRS, chronic rhinosinusitis; NP, nasal polyp; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; SD, standard deviation; OCS, oral corticosteroid; IQR, interquartile range

Results were presented as percentage, mean ± standard deviation, median (interquartile range), or otherwise specified.

p values were determined by chi squared tests, one-way ANOVA tests, or Kruskal-Wallis tests.

Table 12. General linear models for sputum eosinophil percentage among 186 elderly asthma patients

For log-sputum eosinophil percentage	*p* values	Coef.	Std. Err.	z	95% CI
Age (years)	0.698	-0.01	0.10	-0.39	-0.02–0.02
Male gender	0.681	0.06	0.14	0.41	-0.21–0.32
BMI (kg/m^2)	0.979	0.01	0.02	0.03	-0.04–0.04
Former smoker	0.847	-0.03	0.16	-0.19	-0.35–0.29
Current smoker	0.908	0.02	0.19	0.12	-0.35–0.40
CRSsNP	0.047	0.24	0.12	1.99	0.00–0.47
CRSwNP	0.075	0.29	0.16	1.78	-0.03–0.62
Atopy	0.854	-0.02	0.10	-0.18	-0.22–0.19
Log-blood eosinophils (/μL)	0.001	0.47	0.15	3.19	0.18–0.76
Log-total IgE (kU/L)	0.460	-0.09	0.13	-0.74	-0.35–0.16
Log-SE-IgE (kU/L)	0.010	0.29	0.11	2.59	0.07–0.52

Abbreviations: CI, confidence interval; BMI, body mass index; CRS, chronic rhinosinusitis; NP, nasal polyp; SE, staphylococcal enterotoxin

p values were determined by general linear models with adjustment for age, gender, BMI, smoking status, CRS, atopy, log-blood eosinophil, log-total IgE, and log-SE-IgE.

Table 13. Multivariate logistic regression analyses for asthma severity among 249 elderly asthma patients

Severe asthma (n=78) vs. non-severe asthma (n=171)	Adjusted OR	95% CI	*p* values
Age (years)	1.04	0.95–1.14	0.370
Male gender	0.17	0.04–0.66	0.011
BMI (kg/m^2)	0.98	0.86–1.12	0.754
Smoking status			
Never	Reference		
Former	1.09	0.22–5.35	0.912
Current	6.02	1.03–35.30	0.047
GERD	8.24	2.74–24.76	<0.001
CRS			
No CRS	Reference		
CRSsNP	2.49	0.93–6.65	0.069
CRSwNP	7.41	1.82–30.19	0.005
Blood eosinophilia (≥ 450/μL)	2.52	1.38–12.81	0.012
Total IgE ≥ 100 kU/L	1.14	0.36–3.60	0.828
SE-IgE sensitization			
Negative (<0.10 kU/L)	Reference		
Moderate (0.10–0.35 kU/L)	1.82	0.52–6.39	0.348
High (≥ 0.35 kU/L)	7.47	1.86–30.03	0.005

Abbreviations: OR, odds ratio; CI, confidence interval; BMI, body mass index; GERD, gastroesophageal reflux disease; CRS, chronic rhinosinusitis; NP, nasal polyp

p values were determined by multivariate logistic regression tests with adjustment for age, gender, BMI, smoking, GERD, CRS, blood eosinophilia (≥ 450 vs. < 450 cells/μL), total IgE (≥ 100 vs. < 100 kU/L), and SE-IgE sensitization status.

Table 14. Comparison by asthma severity in 249 elderly asthma patients

	Non-severe (n=171)	Severe (n=78)	*p* value
Female (%)	67.3	64.1	0.626
Age (years)	71.3±5.0	71.8±5.3	0.506
Asthma onset age (years)	64.3±8.5	63.8±9.2	0.633
BMI (kg/m^2)	24.5±3.4	24.1±3.1	0.360
Smoking (%)			
Never	76.0	71.8	0.759
Former	14.6	18.9	
Current	9.4	10.3	
DM (%)	10.5	17.9	0.105
GERD (%)	12.9	37.1	<0.001
CRS (%)			
No CRS	58.5	19.2	<0.001
CRSsNP	33.3	57.7	
CRSwNP	8.2	23.1	
Atopy (%)	41.9	44.0	0.800
Blood eosinophils (/μL)	200 (IQR 110–263)	312 (IQR 169–510)	<0.001
Sputum neutrophil percentage, n=186	26.0 (IQR 12.6–35.7)	12.0 (IQR 5.3–36.3)	0.088
Sputum eosinophil percentage, n=186	2.6 (IQR 0.6–6.7)	7.0 (IQR 4.0–15.0)	<0.001
Sputum eosinophilia (≥ 3%), n=186 (%)	47.0	78.3	<0.001

Serum total IgE (kU/L)	68.3 (IQR 22.7–168.8)	140.0 (IQR 57.9–368.2)	<0.001
High total IgE (≥ 100 kU/L) (%)	36.3	62.8	<0.001
Serum SE-IgE (kU/L)	0.11 (IQR 0.03–0.32)	0.45 (IQR 0.13–0.93)	<0.001
SE-IgE sensitization (%)			
Negative (< 0.10 kU/L)	47.4	16.7	<0.001
Moderate (0.10-0.35 kU/L)	30.4	26.9	
High (≥ 0.35 kU/L)	22.2	56.4	

Abbreviations: BMI, body mass index; DM, diabetes mellitus; GERD, gastroesophageal reflux disease; CRS, chronic rhinosinusitis; NP, nasal polyp; SE, staphylococcal enterotoxin; IQR, interquartile range

Results were presented as percentage, mean ± standard deviation, median (IQR), or otherwise specified.

p values were determined by chi squared tests, t tests, or Mann-Whitney tests.

Table 15. Multivariate logistic regression analyses for severe asthma among 186 elderly asthma patients who had induced sputum data available

Severe asthma (n=69) vs. non-severe asthma (n=117)	Adjusted OR	95% CI	*p* values
Age (years)	1.07	0.97–1.18	0.184
Male gender	0.21	0.06–0.77	0.018
BMI (kg/m^2)	0.98	0.85–1.13	0.785
Smoking status			
Never	Reference		
Former	1.28	0.31–5.41	0.733
Current	6.44	1.08–38.26	0.041
GERD	16.65	4.64–59.80	<0.001
CRS			
No CRS	Reference		
CRSsNP	6.85	2.33–20.11	<0.001
CRSwNP	6.88	1.67–28.30	0.008
Sputum eosinophilia (≥ 3%)	2.26	0.81–6.25	0.118
Total IgE ≥ 100 kU/L	1.44	0.52–3.99	0.479
SE-IgE sensitization			
Negative (<0.10 kU/L)	Reference		
Moderate (0.10–0.35 kU/L)	1.91	0.58–5.34	0.218
High (≥ 0.35 kU/L)	5.01	1.32–19.04	0.018

Abbreviations: OR, odds ratio; CI, confidence interval; BMI, body mass index; GERD, gastroesophageal reflux disease; CRS, chronic rhinosinusitis; NP, nasal polyp

p values were determined by multivariate logistic regression tests with adjustment for age, gender, BMI, smoking status, GERD, CRS, sputum eosinophilia, total IgE (≥ 100 vs. < 100 kU/L), and SE-IgE sensitization.

Figures

Figure 1. Stepwise approach to test the hypothesis

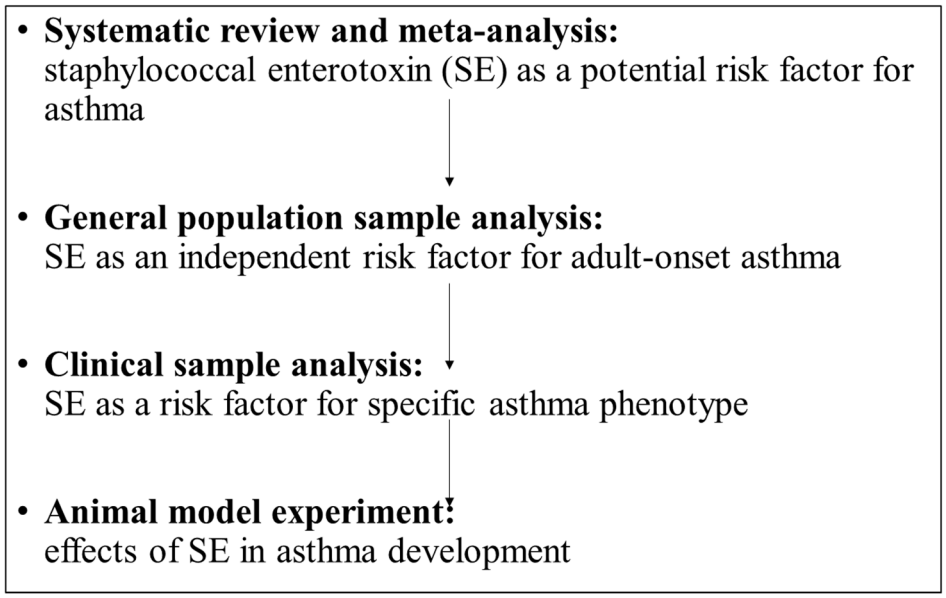


Figure 2. PRISMA flow chart for systematic review and meta-analysis

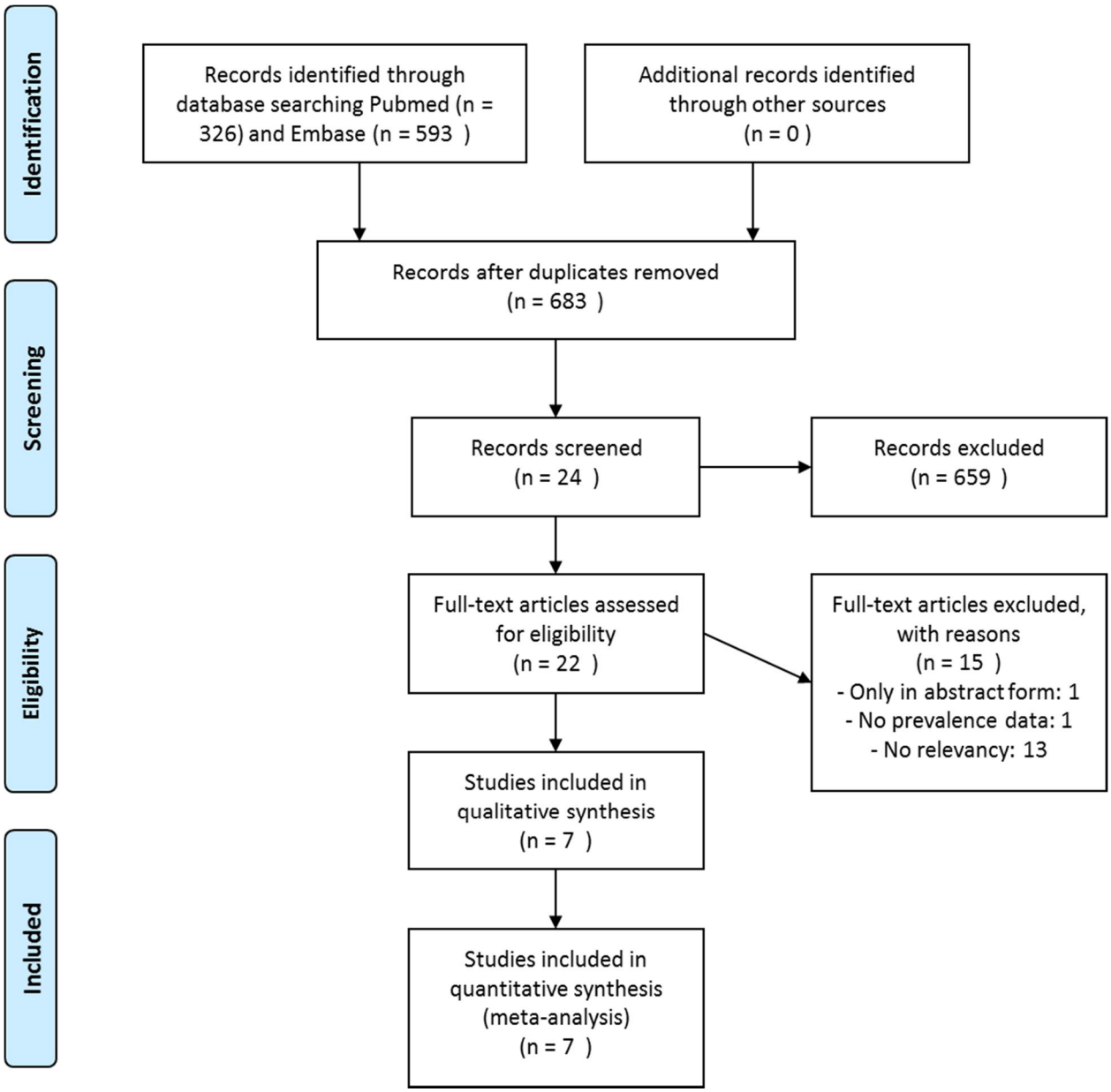


Figure 3. Flow chart for patient inclusion in the elderly asthma case-control study

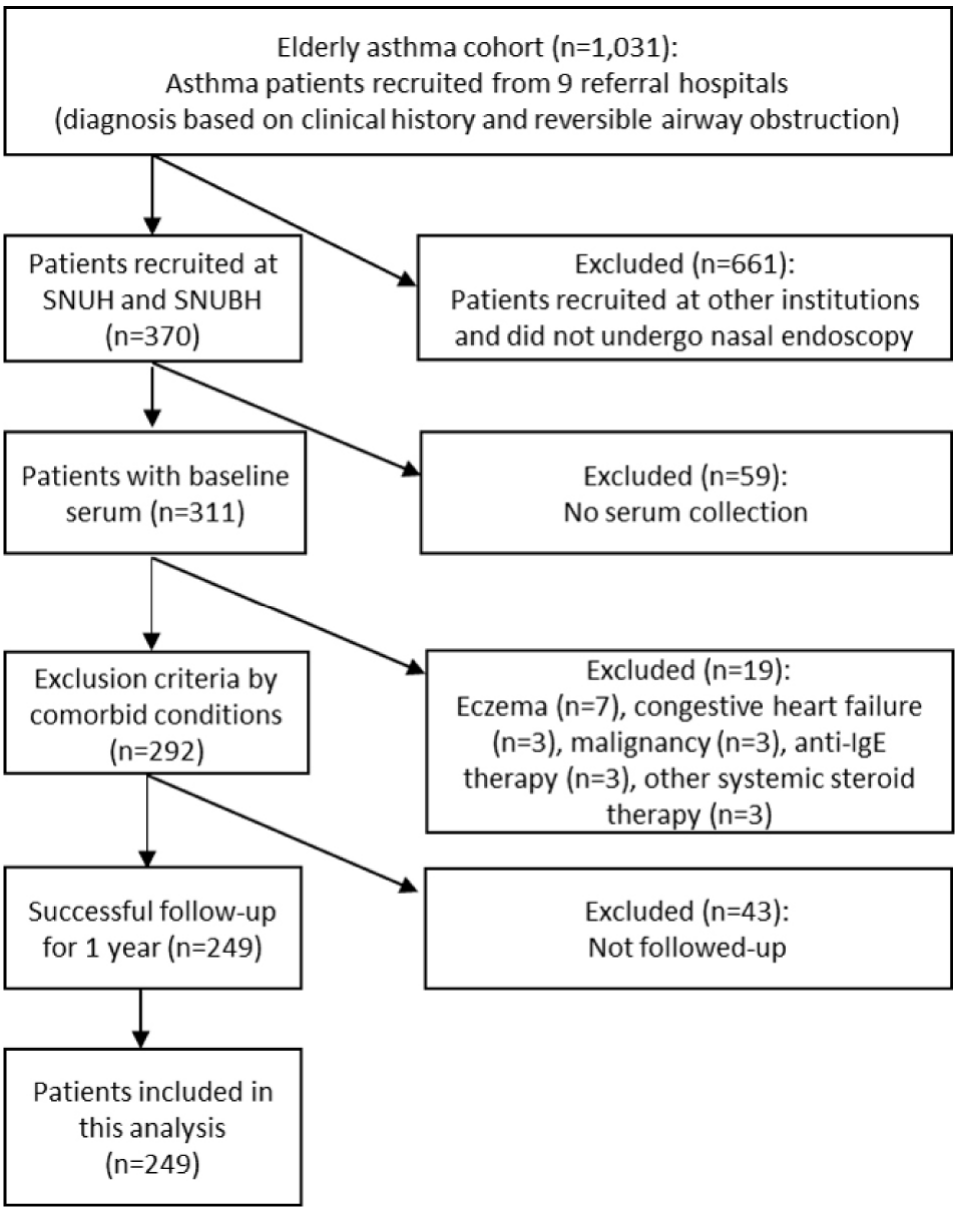
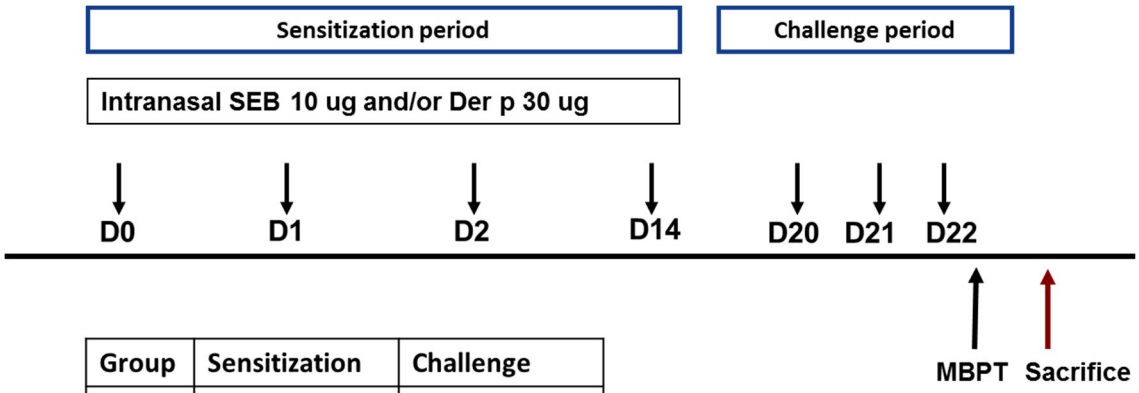


Figure 4. Study design of animal experimental model of allergic asthma



Group	Sensitization	Challenge
A	PBS	PBS
B	Der p	Der p
C	SEB	Der p
D	Der p	SEB
E	Der p	Der p+SEB
F	Der p+SEB	Der p

Figure 5. Forest plots of studies comparing the frequency of Staphylococcal enterotoxin sensitization in asthmatics and controls. Gray squares represent individual studies, and the size of squares is proportional to the number of subjects in the study. Horizontal lines indicate 95% confidence interval ranges. Vertical dotted lines and diamond shapes represent pooled summary estimates for the analysis (the width of the diamond represents the 95% confidence intervals). Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; M-H, Mantel-Haenzel test; D+L, DerSimonian and Laird method

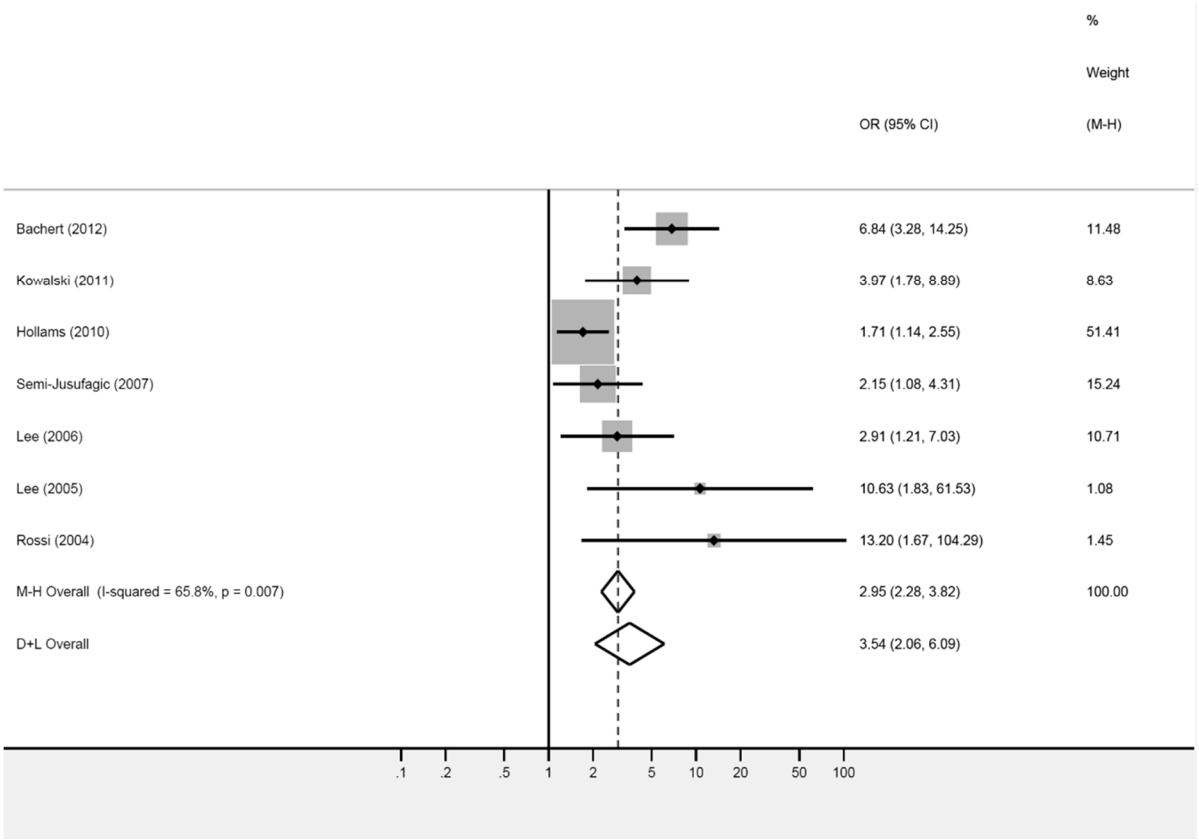


Figure 6. Prevalence of current asthma, inhalant allergen sensitization and Staphylococcal enterotoxin sensitization by age groups.

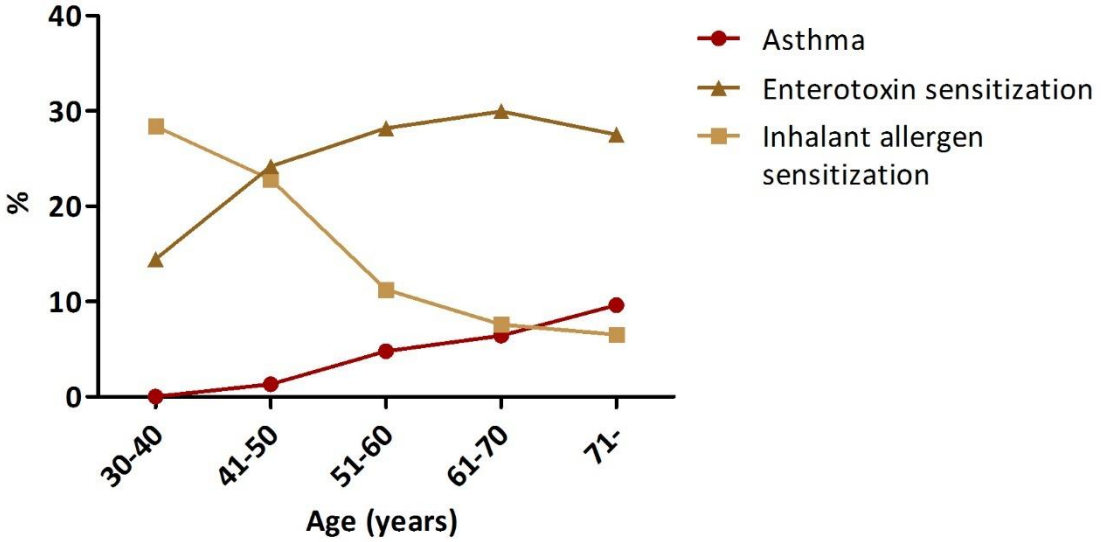


Figure 7. Multiple correspondence analysis map representing the relationships between Staphylococcal enterotoxin sensitization and six relevant factors that were identified in multivariate logistic regression tests.

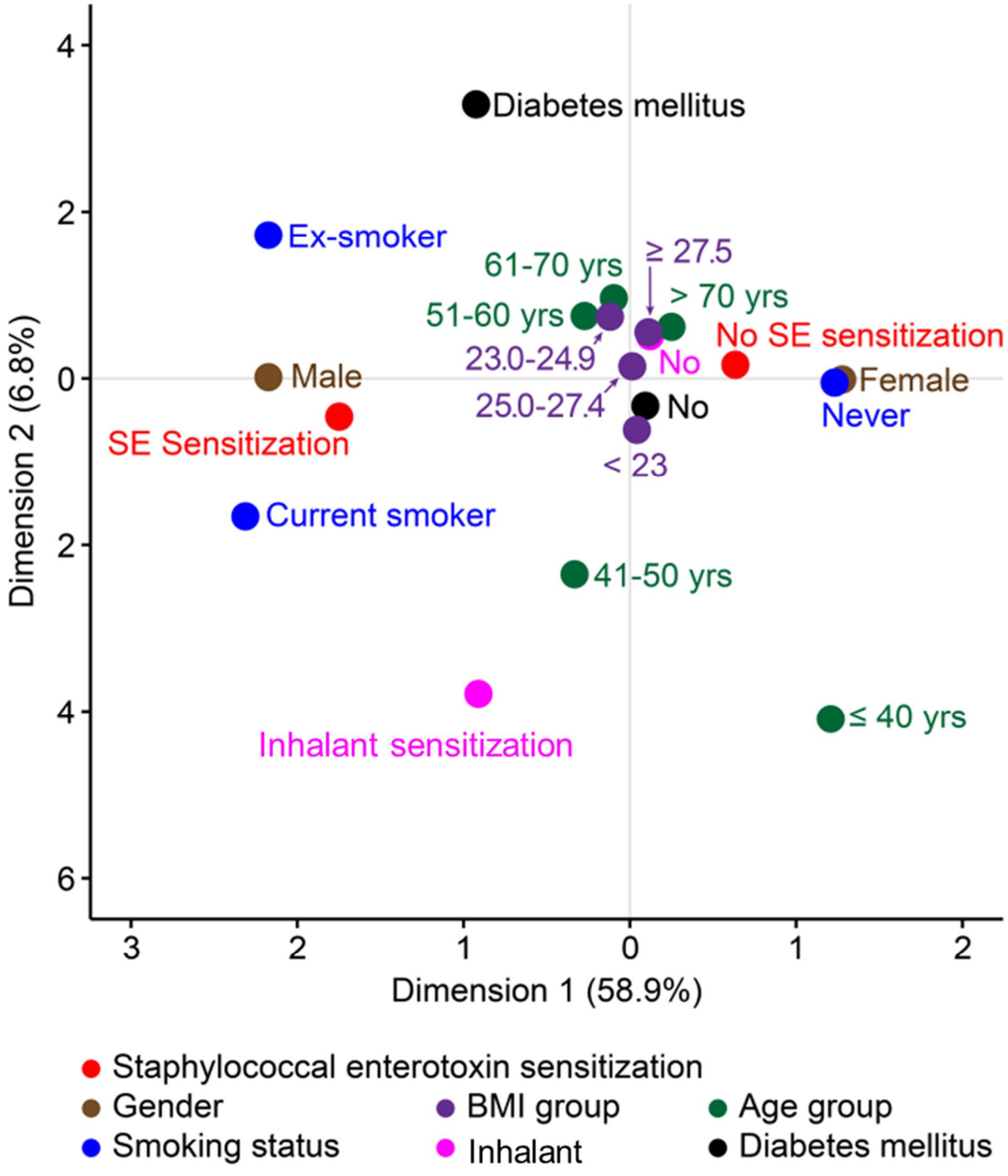


Figure 8. Correlation between log-transformed total IgE and Staphylococcal enterotoxin specific IgE (SE-IgE) levels. The scatter plot is shown with the 95% confidence interval (CI) and the regression line.

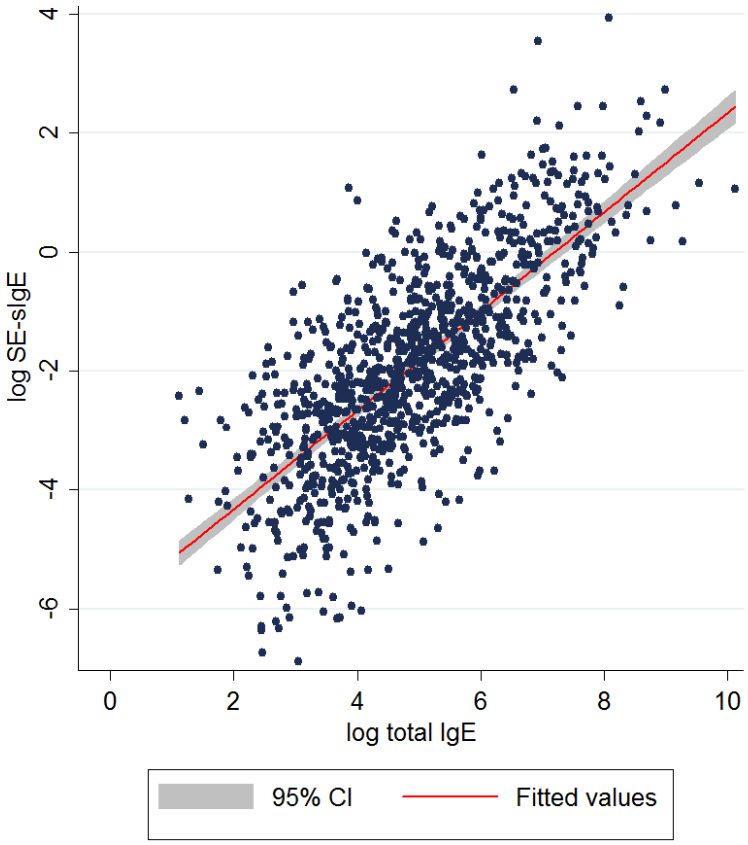


Figure 9. Multiple correspondence analyses plot for the inter-relationships between severe asthma, chronic rhinosinusitis, sputum eosinophilia and serum staphylococcal enterotoxin IgE levels among 186 elderly asthma patients. Severe asthma is situated close to high SE-IgE (≥ 0.35 kU/L), CRSwNP, and sputum eosinophilia (≥ 3%).

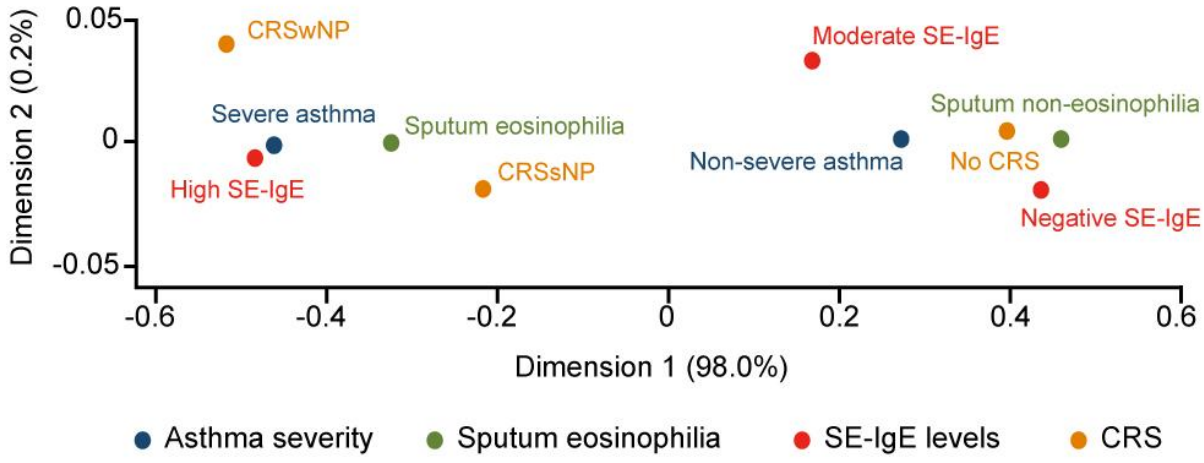


Figure 10. Comparison of airway hyperresponsiveness to methacholine between experimental groups.

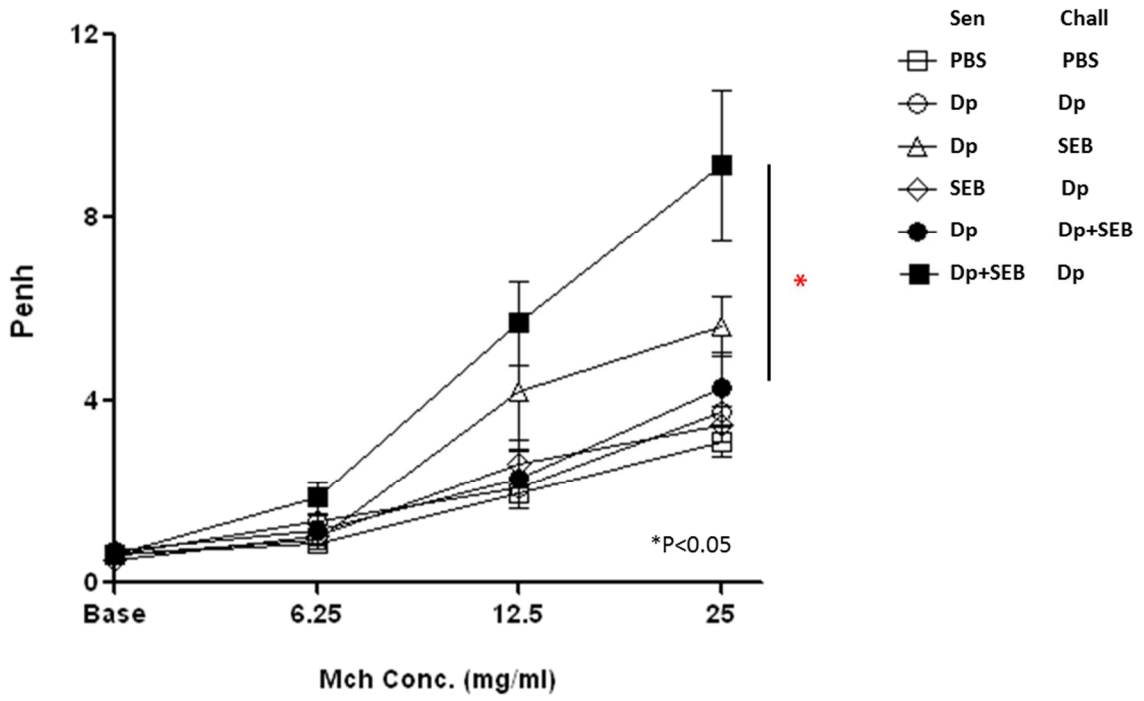


Figure 11. Comparison of inflammatory cell counts in bronchoalveolar lavage fluids between experimental groups.

Abbreviations: BAL, bronchoalveolar lavage; Mac, macrophage; Neu, neutrophil; Eos, eosinophil; Lym, lymphocyte; Sen, sensitization; Chall, challenge; PBS, phosphate-buffered saline; Dp, Dermatophagoides pteronyssinus; SEB, staphylococcal enterotoxin B

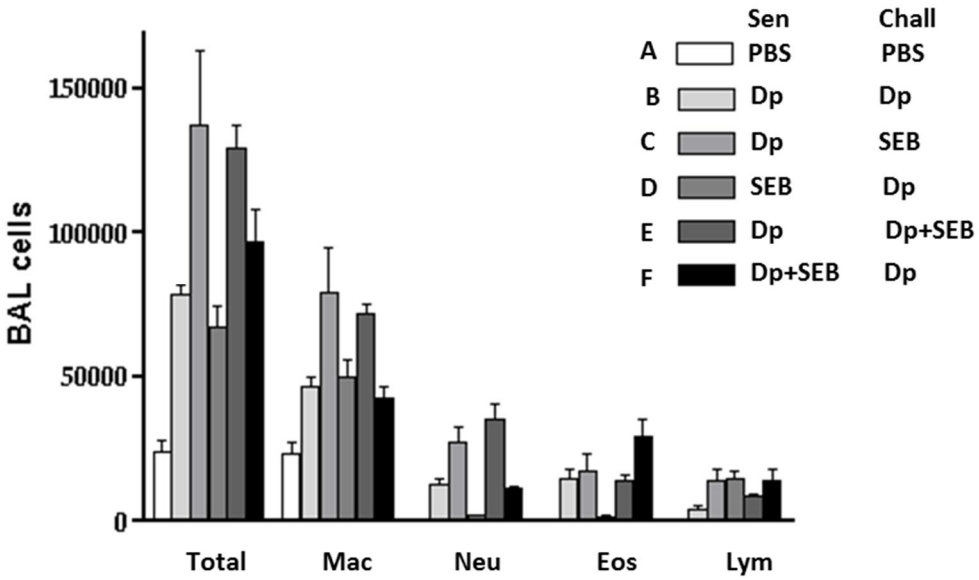


Figure 12. Comparison of histopathological findings between experimental groups.

Abbreviations: Sen, sensitization; Chall, challenge; PBS, phosphate-buffered saline; Dp, Dermatophagoides pteronyssinus; SEB, staphylococcal enterotoxin B

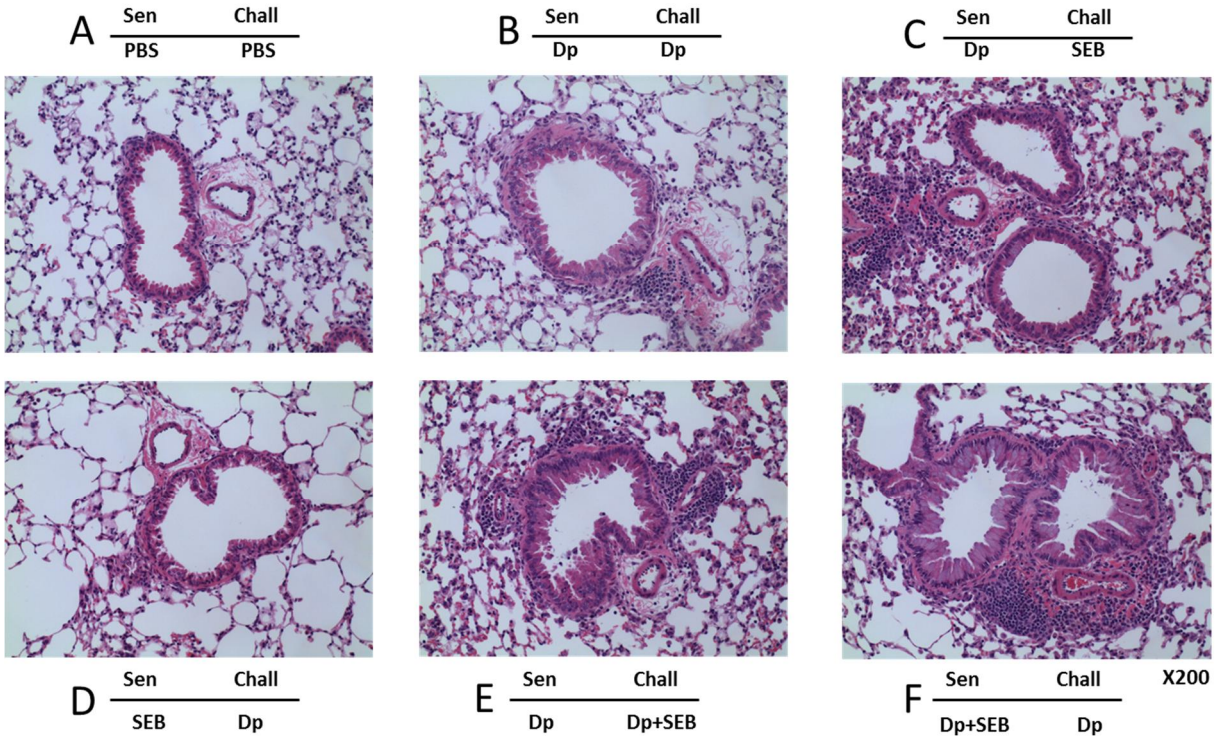
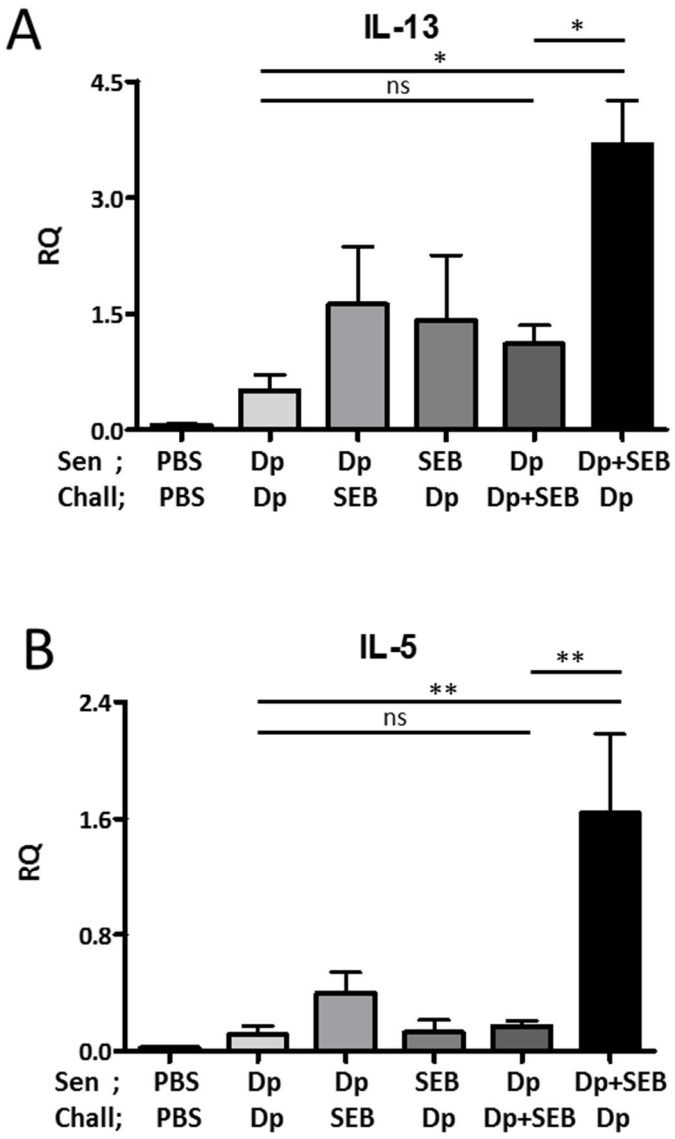
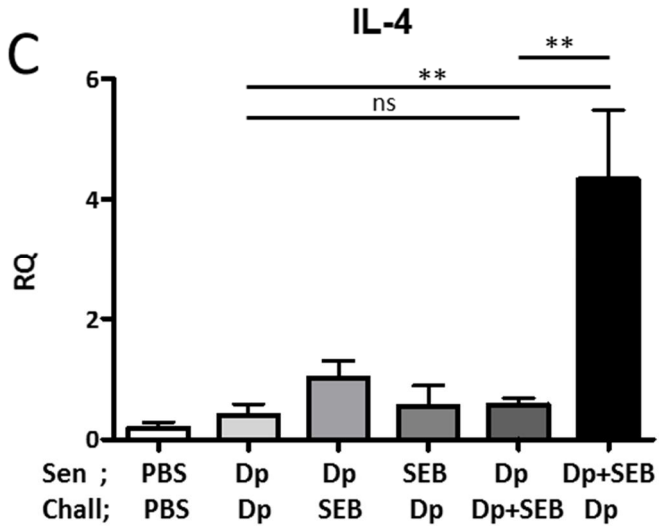


Figure 13. Comparison of inflammatory cytokine gene expression in lung tissue lysates measured by RT-PCR; (A) IL-13, (B) IL-5, (C) IL-4, (D) IFN-γ, (E) IL-17. *P<0.05, **P<0.01. ns, not significant

Abbreviations: RQ, relative quantity; Sen, sensitization; Chall, challenge; PBS, phosphate-buffered saline; Dp, Dermatophagoides pteronyssinus; SEB, staphylococcal enterotoxin B

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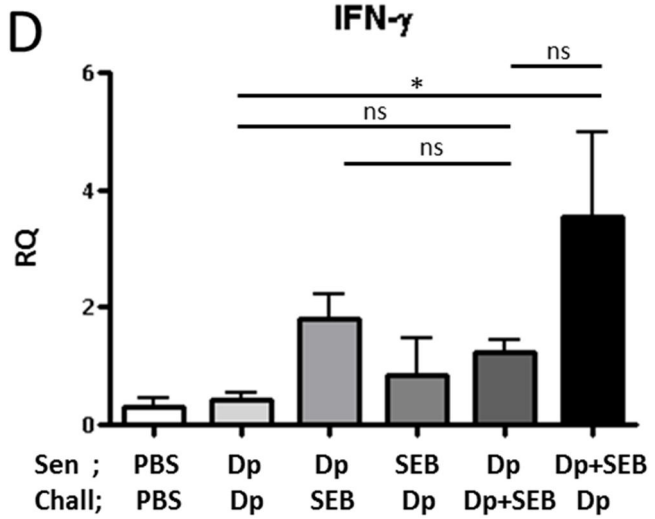
Chall; PBS Dp SEB Dp Dp+SEB Dp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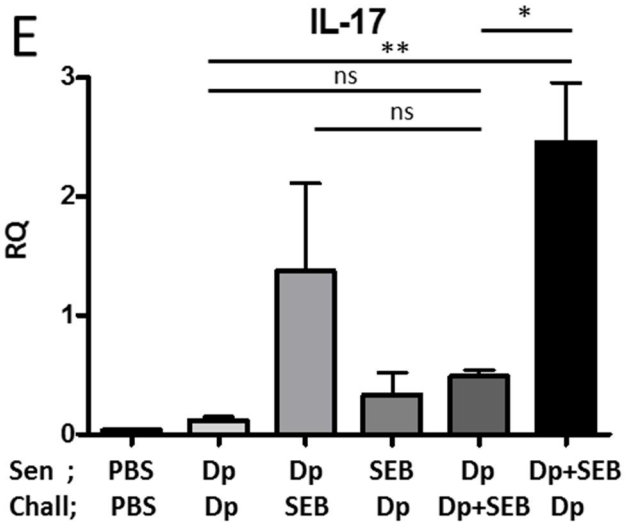
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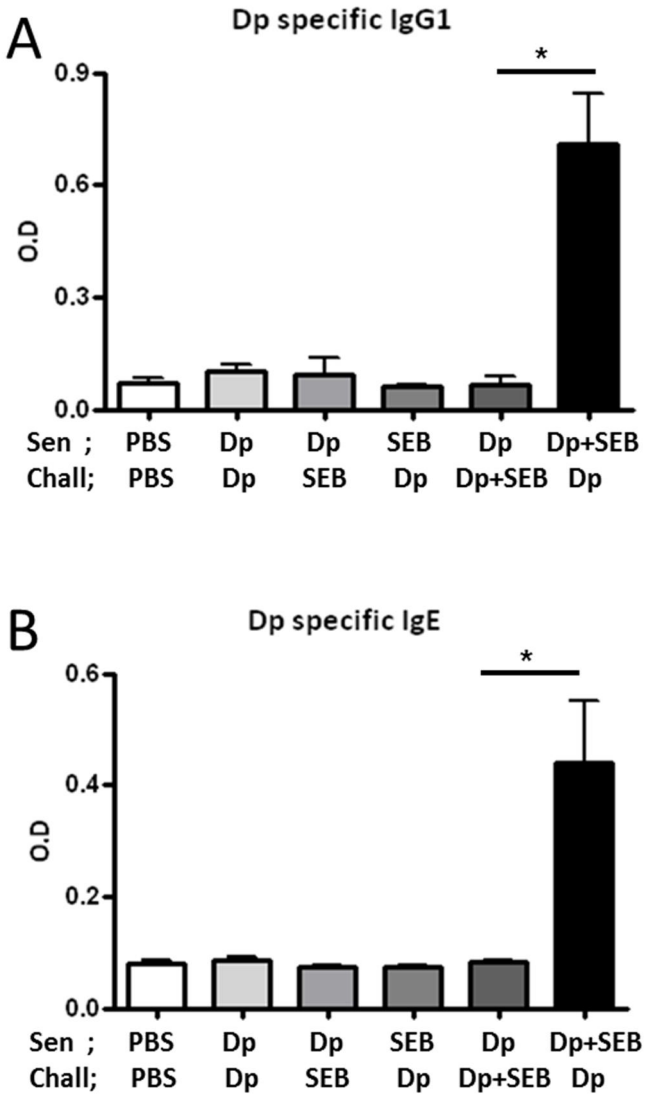
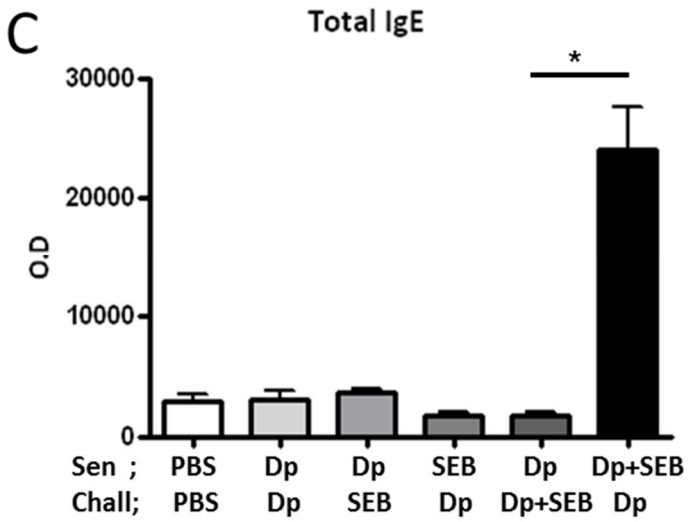
Chall; PBS Dp SEB Dp Dp+SEB Dp

Figure 14. Comparison of serum immunoglobulin levels measured by ELISA between experimental groups; (A) Der p-specific IgG1, (B) Der p-specific IgE, (C) total IgE. *P<0.05

Abbreviations: OD, optical density; Sen, sensitization; Chall, challenge; PBS, phosphate-buffered saline; Dp, Dermatophagoides pteronyssinus; SEB, staphylococcal enterotoxin B

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0
O.D
Sen ; PBS Dp Dp SEB Dp Dp+SEB
Chall; PBS Dp SEB Dp Dp+SEB Dp

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