

# Specific IgE against *Staphylococcus aureus* enterotoxins: An independent risk factor for asthma

Claus Bachert, MD, PhD,<sup>a,\*</sup> Kristel van Steen, PhD,<sup>b,c,e</sup> Nan Zhang, MD,<sup>a</sup> Gabriele Holtappels,<sup>a</sup> Tom Cattaert, PhD,<sup>b,c</sup> Bärbel Maus, PhD,<sup>b,c,e</sup> Roland Buhl, MD, PhD,<sup>d</sup> Christian Taube, MD, PhD,<sup>d,i</sup> Stephanie Korn, MD,<sup>d</sup> Marek Kowalski, MD,<sup>f</sup> Jean Bousquet, MD, PhD,<sup>g</sup> and Peter Howarth, MD<sup>h</sup> Ghent and Liège, Belgium, Lodz, Poland, Mainz, Germany, Montpellier, France, Southampton, United Kingdom, and Leiden, The Netherlands

**Background:** The role of IgE in patients with severe asthma is not fully understood.

**Objective:** We sought to investigate whether IgE to *Staphylococcus aureus* enterotoxins might be relevant to disease severity in adult asthmatic patients.

**Methods:** Specific IgE antibody concentrations in serum against enterotoxins, grass pollen (GP), and house dust mite allergens and total IgE levels were measured in adult cohorts of 69 control subjects, 152 patients with nonsevere asthma, and 166 patients with severe asthma. Severe asthma was defined as inadequately controlled disease despite high-dose inhaled corticosteroids plus at least 2 other controller therapies, including oral steroids.

**Results:** Enterotoxin IgE positivity was significantly greater in patients with severe asthma (59.6%) than in healthy control subjects (13%,  $P < .001$ ). Twenty-one percent of patients with severe asthma with enterotoxin IgE were considered nonatopic. Logistic regression analyses demonstrated significantly increased risks for enterotoxin IgE-positive subjects to have any asthma (OR, 7.25; 95% CI, 2.7-19.1) or severe asthma (OR, 11.09; 95% CI, 4.1-29.6) versus enterotoxin IgE-negative

subjects. The presence of GP or house dust mite IgE antibodies was not associated with either significantly increased risk for asthma or severity. Oral steroid use and hospitalizations were significantly increased in patients with enterotoxin IgE and nonatopic asthma. GP IgE was associated with a higher FEV<sub>1</sub> percent predicted value, and enterotoxin IgE was associated with a lower FEV<sub>1</sub> percent predicted value.

**Conclusions:** Staphylococcal enterotoxin IgE antibodies, but not IgE against inhalant allergens, are risk factors for asthma severity. We hypothesize that the presence of enterotoxin IgE in serum indicates the involvement of staphylococcal superantigens in the pathophysiology of patients with severe asthma. (J Allergy Clin Immunol 2012;130:376-81.)

**Key words:** Asthma, asthma severity, hospitalizations, FEV<sub>1</sub>, IgE, *Staphylococcus aureus*, enterotoxins, superantigens

Asthma is a global health problem associated with high morbidity and socioeconomic burden.<sup>1</sup> Within the United States, asthma affects an estimated 1 in 15 persons. These patients make a combined 10 million outpatient doctor's office visits per year and account for one quarter of all emergency department visits. Although disease can be controlled with optimal therapy for most of these patients,<sup>2</sup> many patients have uncontrolled asthma.<sup>3</sup> Patients with severe uncontrolled asthma are at risk for severe exacerbations and account for a significantly disproportionate amount of asthma-related health care costs. Such treatment-resistant asthmatic patients with persistent disease represent a major unmet need, and novel treatments based on a better understanding of disease are required.<sup>1,4</sup>

Phenotypic characterization of populations with severe asthma has identified a greater preponderance of aeroallergen-specific IgE-negative subjects (nonatopic asthma) and a greater prevalence of comorbid rhinosinusitis than in patients with nonsevere asthma.<sup>5-7</sup> Despite this, however, and based on evidence gained from patients with atopic dermatitis<sup>8-10</sup> and those with nasal polyposis,<sup>11-13</sup> as well as from our own preliminary findings in asthmatic patients and a systematic review of the literature and meta-analysis,<sup>14,15</sup> we have hypothesized that IgE responses orchestrated by enterotoxins from *Staphylococcus aureus* might provide an explanation for disease persistence and severity in asthmatic patients, even in those with classically considered nonatopic asthma. Enterotoxins generated by *S aureus* can act both as nominal antigens, stimulating specific IgE responses (*Staphylococcus aureus* enterotoxins [SE] IgE), and as superantigens, promoting a polyclonal IgE response reflected by an increase in total IgE (tIgE) levels. We have previously identified increased serum concentrations of SE IgE in asthmatic patients,<sup>14</sup> a finding supported by a recent study in Poland,<sup>16</sup> and have linked its presence

From <sup>a</sup>the Upper Airway Research Laboratory (URL), Ghent University Hospital; <sup>b</sup>the Systems and Modelling Unit, Montefiore Institute, University of Liège; <sup>c</sup>Bioinformatics and Modelling, GIGA-Research, University of Liège; <sup>d</sup>the Pulmonary Department, Mainz University Hospital; <sup>e</sup>the Center for Medical Genetics, Ghent University Hospital; <sup>f</sup>the Department of Allergy, Immunology and Rheumatology, Medical University of Lodz; <sup>g</sup>University Hospital and INSERM 1080, Montpellier; <sup>h</sup>Clinical and Experimental Sciences, Faculty of Medicine, Southampton University and National Institute of Health Respiratory Biomedical Research Unit, Southampton General Hospital; and <sup>i</sup>the Department of Pulmonology, Leiden University Medical Center.

\*C. Bachert is currently affiliated with the Division of ENT Diseases, Clintec, Karolinska Institutet, Stockholm, Sweden.

Supported by grants to C.B. from the Flemish Scientific Research Board, FWO, nos. A12/5-HB-KH3 and G.0436.04, and the Interuniversity Attraction Poles Programme (IUAP), Belgian state, Belgian Science Policy P6/35, and to P.H. from the UK Medical Research Council, G0800649 (Wessex Severe Asthma Cohort), and by the Global Allergy and Asthma European Network (GA<sup>2</sup>LEN). T.C. is a Postdoctoral Researcher of the Fonds de la Recherche Scientifique (FNRS). T.C. and K.S. acknowledge research support by the Belgian Network BioMAGNet (Bioinformatics and Modelling: from Genomes to Networks), funded by the Interuniversity Attraction Poles Programme (Phase VI/4), and by the IST Programme of the European Community under the PAS-CAL2 Network of Excellence (Pattern Analysis, Statistical Modelling and Computational Learning), IST-2007-216886.

Disclosure of potential conflict of interest: J. Bousquet has received honoraria from Stallergenes, Actelion, Almirall, AstraZeneca, Chiesi, GlaxoSmithKline, Merck, Novartis, OM Pharma, Sanofi, Teva, and Uriach. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication January 6, 2012; revised May 6, 2012; accepted for publication May 11, 2012.

Available online June 26, 2012.

Corresponding author: Claus Bachert, MD, PhD, Upper Airway Research Laboratory (URL), Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium. E-mail: claus.bachert@ugent.be.

0091-6749/\$36.00

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doi:10.1016/j.jaci.2012.05.012

#### Abbreviations used

BMI: Body mass index  
ECP: Eosinophil cationic protein  
GP: Grass pollen (*Phleum pratense*)  
HDM: House dust mite (Der p 1)  
OR: Odds ratio  
SE: *Staphylococcus aureus* enterotoxins  
tIgE: Total IgE

to higher tIgE levels. However, the relevance of these findings to the risk of expressing asthma, particularly severe asthma, and their relevance in relationship to other specific IgE responses to aeroallergens has not been determined.

The primary hypothesis for this project is that IgE to SEs is relevant to disease severity in adult asthma. To address this, serum concentrations of SE IgE and levels of specific IgE against house dust mite (HDM [Der p 1]) and grass pollen (GP [*Phleum pratense*]) allergens were measured in adult cohorts of asthmatic patients (both nonsevere and severe asthma), as well as in healthy control subjects, to examine their risk potential as a biomarker to distinguish both asthma and severe asthma phenotypes, as well as to explore their relationship to measures of tIgE. Furthermore, the inclusion in the asthmatic population of both atopic and nonatopic asthmatic patients based on the presence or absence of specific IgE against the aeroallergens evaluated allowed the exploration of the relevance of SE IgE to nonatopic asthma.

## METHODS

Sixty-nine nonasthmatic control subjects, 152 patients with nonsevere asthma, and 166 patients with severe treatment-resistant asthma were recruited at the Departments of Pneumology in Southampton and Mainz. Recruitment was from departmental research databases and the Wessex Severe Asthma Cohort. All asthmatic patients had established disease, and none of the nonasthmatic control subjects had a current or past history of asthma-related symptoms. Severe treatment-resistant asthma was defined as inadequately controlled disease despite high-dose inhaled steroid therapy plus at least 2 other controller therapies, including oral steroids.<sup>4</sup> Nonsevere asthma was either mild (not steroid treated) or moderate (low-dose inhaled steroid) based on requirements for asthma treatment to achieve disease control. The study was approved by the local ethics committees, and all subjects provided written informed consent. Basic clinical characteristics of both populations were comparable with respect to smoking habits, age, and sex distribution.

## Procedures

Subjects attended for 1 visit during which clinical and questionnaire characterization was undertaken and spirometry was performed by using a calibrated Vitalograph Compact II (Vitalograph, Buckingham, United Kingdom). The highest values of 3 consecutive recordings were used for analysis. All standard asthma therapy was taken as usual, although short-acting bronchodilators were avoided for 8 hours and long-acting bronchodilators for 12 hours before attendance. Body mass index (BMI) was calculated according to the following formula:

$$\text{BMI} = \text{weight [kg]} / \text{height}^2 [\text{m}^2].$$

Skin prick testing was performed for the following allergens: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, tree pollens, mixed GPs, weed pollens, and *Alternaria tenuis*. A wheal diameter of 3 mm or greater in excess of that elicited by the negative control (0.9% saline) was considered a positive result.

Venous blood was drawn and serum was stored for assessment in a central laboratory (Ghent, Belgium). Levels of total serum IgE, eosinophil cationic protein (ECP), and specific IgE to SEs, HDM, and GP allergens were measured with the ImmunoCAP system (Phadia, Uppsala, Sweden). The lower limit of SE IgE detection was set at 0.1 kU/L and that of HDM and GP was set at 0.35 kU/L, respectively, as recommended by the manufacturer.

## Statistical methods

All data analyses were undertaken with the statistical software R version 2.13.0 (<http://www.r-project.org/>). Descriptive analyses compared control subjects and patients with nonsevere and severe asthma by using available demographic data (age, sex, BMI, and current smoking status), tIgE levels, specific IgE levels (for SE, HDM and GP), log-transformed ECP levels, and additional clinical data (oral steroid use, FEV<sub>1</sub>, and hospitalization). The tIgE level was entered as both a dichotomized variable (at 100 kU/L) and a continuous log-transformed variable. Specific IgE measurements were either dichotomized at their detection limit (0.1 kU/L for SE and 0.35 kU/L for HDM and GP) or log-transformed (restricted to strictly positive samples) on a continuous scale. Group comparisons with continuous (binary) variables were performed by using Kruskal-Wallis (Fisher exact) tests. The significance threshold was corrected for the number of group comparisons by using the Bonferroni procedure. In addition, the association between different specific IgE positivities was investigated, and significance was assessed by using Fisher exact tests. Within specific IgE-positive patients, the percentages of specific IgE to tIgE were compared across control subjects and patients with nonsevere and severe asthma by using the Kruskal-Wallis test.

Linear stepwise regression models were built to predict the logarithm of tIgE concentration, and logistic stepwise regression models were built to predict disease severity categories. Automated selection was based on the Akaike information criterion by using the stepAIC function from R-package MASS. Model building started from the null model and allowed for both forward and backward variable selection steps. For reasons of model comparability, only subjects with complete information on all proposed predictor variables were included in the model-building process. Proposed effects were SE IgE, HDM IgE, GP IgE, and all their higher-order interactions. To facilitate the interpretation of interaction terms in final models, we recoded any 2 interacting binary predictors into a single 4-level categorical variable. Oral steroid use, hospitalization within the last 12 months (logistic regression), and FEV<sub>1</sub> (linear regression) were incorporated into the modeling as indices of disease severity. These analyses were complemented with a multiple correspondence analysis<sup>17</sup> on disease severity and the specific IgEs by using the multiple correspondence analysis function from R-package FactoMineR. A 2-dimensional representation was used, and confidence ellipses around the points representing the categories were added by using the plot ellipses function from the same R-package.<sup>18</sup>

Finally, direct or indirect effects of dichotomized IgE-related measurements on disease severity were explored by using categorical Bayesian networks (R-package catnet).<sup>19</sup> Maximum likelihood estimation was used to fit these networks (catnet R function cnSearchOrder), and the best model was selected by using a Bayesian information criterion (catnet R function cnFindBIC).

## RESULTS

Demographic data, serum total and specific IgE and ECP distributions, and information on asthma severity (FEV<sub>1</sub> percent predicted, oral steroid use, and hospitalizations over the last 12 months) are summarized in Table I. Mean age and BMI increased with asthma severity, whereas current and ever smoking demonstrated no significant differences between control subjects and asthmatic patients. Oral steroid use and hospitalizations within the last 12 months were nearly exclusively observed in the severe asthma group, whose percent predicted FEV<sub>1</sub> values were significantly lower than those of the other groups. The number of patients with serum tIgE levels of 100 kU/L or greater increased with disease severity, as did tIgE values, although significant

**TABLE I.** Descriptive analysis of healthy control subjects and patients with nonsevere and severe asthma

Quantity	Control subjects (n = 69)	Patients with nonsevere asthma (n = 152)	Patients with severe asthma (n = 166)	Global P value	Patients with nonsevere asthma vs control subjects, P value	Patients with severe asthma vs control subjects, P value	Patients with severe asthma vs patients with nonsevere asthma, P value
Age (y), mean (SD)	34.23 (11.94)	38.85 (14.41)	46.51 (11.78)	<b>&lt;.001*</b>	.05†	<b>&lt;.001†</b>	<b>&lt;.001†</b>
Male sex, no. (%)	28 (40.6)	76 (50.0)	88 (53.0)	.22‡			
BMI (kg/m <sup>2</sup> ), mean (SD)	24.98 (4.85)	27.24 (5.85)	29.03 (6.14)	<b>&lt;.001*</b>	.01†	<b>&lt;.001†</b>	.01†
Ever smoker, no. (%)	20 (29.4)	38 (26.2)	53 (33.8)	.35‡			
Current smoker, no. (%)	8 (11.8)	12 (8.3)	11 (7.0)	.51‡			
Positive skin prick test response, no. (%)	28 (40.6)	123 (81.5)	109 (66.5)	<b>&lt;.001‡</b>	<b>&lt;.001‡</b>	<b>&lt;.001‡</b>	.003‡
Positive tIgE level (100 kU/L), no. (%)	19 (26.5)	85 (55.9)	107 (64.5)	<b>&lt;.001‡</b>	<b>&lt;.001‡</b>	<b>&lt;.001‡</b>	.14‡
tIgE (log), geometric mean (SD)	38.30 (4.06)	153.13 (5.27)	163.68 (5.23)	<b>&lt;.001*</b>	<b>&lt;.00†</b>	<b>&lt;.00†</b>	.40†
SE IgE positive (0.1 kU/L), no. (%)	9 (13.0)	62 (40.8)	99 (59.6)	<b>&lt;.001‡</b>	<b>&lt;.001‡</b>	<b>&lt;.001‡</b>	<b>.001‡</b>
SE IgE (log), geometric mean (SD)	0.32 (2.18)	0.66 (2.86)	0.73 (3.26)	.11			
HDM IgE positive (0.35 kU/L), no. (%)	19 (27.9)	86 (57.7)	75 (48.4)	<b>&lt;.001‡</b>	<b>&lt;.001‡</b>	.005‡	.11
HDM IgE (log), geometric mean (SD)	4.50 (5.67)	8.82 (5.85)	7.10 (5.89)	.37			
GP IgE positive (0.35 kU/L), no. (%)	17 (38.6)	69 (58.5)	72 (45.6)	.03‡	.03‡	.50‡	.04‡
GP IgE (log), geometric mean (SD)	5.26 (6.39)	6.87 (8.17)	4.30 (7.10)	.23			
ECP (log), geometric mean (SD)	6.84 (2.27)	12.79 (2.31)	15.05 (2.49)	<b>&lt;.001*</b>	<b>&lt;.001†</b>	<b>&lt;.001†</b>	.11†
Oral steroid use, no. (%)	0 (0.0)	7 (5.2)	99 (67.8)	<b>&lt;.001‡</b>	.19‡	<b>&lt;.001‡</b>	<b>&lt;.001‡</b>
FEV <sub>1</sub> , mean (SD)	98.48 (10.94)	91.78 (13.97)	62.84 (19.80)	<b>&lt;.001*</b>	<b>&lt;.001†</b>	<b>&lt;.001†</b>	<b>&lt;.001†</b>
Hospitalized, no. (%)	0 (0.0)	13 (9.7)	83 (69.2)	<b>&lt;.001‡</b>	.02‡	<b>&lt;.001‡</b>	<b>&lt;.001‡</b>

P values surviving Bonferroni correction are shown in boldface (global  $P < .05/18$ ; *post hoc* pairwise  $P < .05/54$ ).

\*Kruskal-Wallis test.

†Mann-Whitney U test.

‡Fisher exact test.

§Positive samples only.

||Geometric means and SDs at original scale correspond to usual (arithmetic) means at the log-transformed scale at which the analysis was carried out.

differences were only evident for asthmatic patients relative to nonasthmatic control subjects.

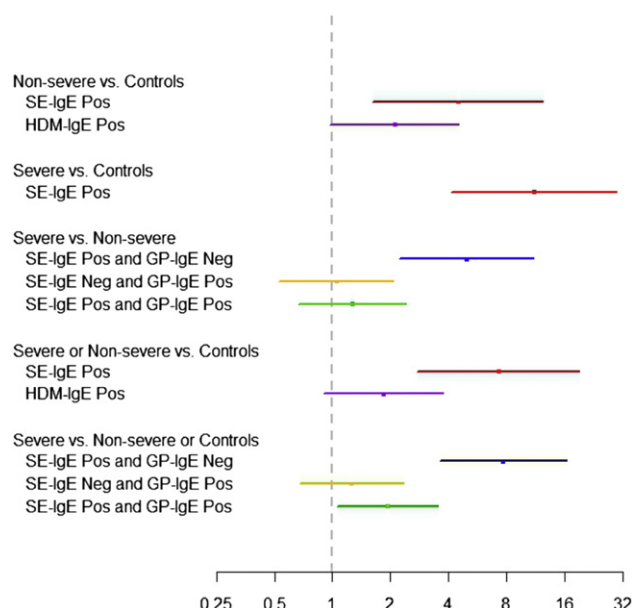
Mean serum SE IgE concentrations increased with disease severity, whereas concentrations for GP- or HDM-specific IgE did not. As an expression of polyclonality, mean SE IgE concentrations were less than 1 kU/L, and the mean fraction of SE IgE of tIgE was less than 0.5% independently of disease severity (see Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)); in contrast, mean HDM IgE levels ranged between 4.5 and 7.1 kU/L and accounted for 6% to 7% of tIgE, without differences between groups. Mean GP IgE levels ranged between 4.3 and 6.9 kU/L and represented, as a fraction of tIgE, 17% in the control subjects, 11.1% in the patients with nonsevere asthma, and only 4.6% in the patients with severe asthma ( $P \leq .01$  for severe vs nonsevere asthma). The odds ratios (ORs) for being SE IgE positive were significantly increased when sensitized to HDM; however, the odds of being positive to GP or HDM allergen was highest when 1 inhalant allergen-specific IgE was also present in serum. For details, see Table E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).

Linear regression models to estimate the effect of a specific IgE on total serum IgE levels are summarized in Table E3 in this

article's Online Repository at [www.jacionline.org](http://www.jacionline.org). The SE IgE level was associated with a significantly higher tIgE level than HDM or GP IgE; the combination of SE IgE with HDM IgE had an even greater effect.

Within the severe asthma group, patients were either negative for all 3 specific IgEs (17%), only positive for inhalant allergens (24%), only positive for SE IgE (21%), or positive for SE IgE and inhalant allergens (37%, see Table E4 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Whereas FEV<sub>1</sub> and BMI were not different between the severe asthma subgroups, the age of onset differed significantly between the only SE IgE-positive group (mean/SD  $36.68 \pm 14.09$  years), the SE IgE and inhalant allergen-negative subgroup ( $32.78 \pm 11.32$  years), and both inhalant allergen IgE-positive groups ( $20.03 \pm 14.53$  and  $24.90 \pm 20.73$  years, respectively;  $P < .001$ ).

By using univariate regression analysis, tIgE levels were associated with a significantly increased risk of having asthma (OR, 3.57; 95% CI, 2.35-5.45;  $P < .001$ ), but there was no effect on asthma severity (severe vs nonsevere asthma: OR, 1.06; 95% CI, 0.78-1.44;  $P = .72$ ). Logistic regression models were used to predict the various disease severity categories (Fig 1 and see Table E5 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).



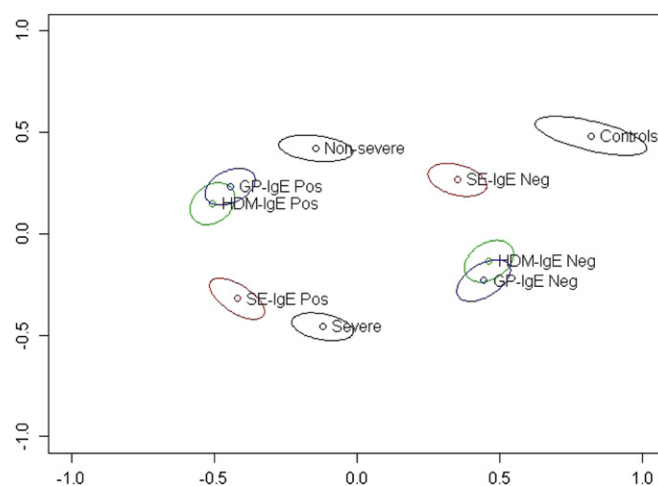
**FIG 1.** Logistic regression models for disease severity. ORs and 95% CIs are represented for the different comparisons. SE IgE levels in serum were associated with a significantly increased risk of asthma (OR, 7.26; 95% CI, 2.76-19.13;  $P < .001$ ) and especially severe asthma (OR, 11.09; 95% CI, 4.14-29.68;  $P < .001$ ).

jacionline.org). In contrast to tIgE, SE IgE levels in serum were associated with a significantly increased risk of asthma (OR, 7.26; 95% CI, 2.76-19.13;  $P < .001$ ) and especially severe asthma (OR, 11.09; 95% CI, 4.14-29.68;  $P < .001$ ). SE IgE levels also significantly increased the odds of severe versus non-severe asthma in GP IgE-negative subjects. Neither GP IgE nor HDM IgE levels were associated with a significantly increased risk for asthma or asthma severity; in contrast, the SE IgE level alone more than in combination with inhalant allergen IgE antibodies significantly increased the odds for nonsevere and severe asthma. The age of the patients did not have an influence on these risks.

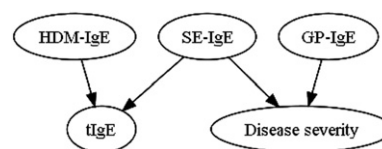
Multiple correspondence analyses with 95% confidence ellipses were constructed (Fig 2) to situate relationships between parameters and disease severity. The analyses confirmed that HDM-specific IgE and GP-specific IgE levels are closely related, whereas SE IgE status is independent from those antibodies. Moreover, the analysis demonstrated that severe asthma is situated together with SE-specific IgE levels.

Categorical Bayesian networks were used to clarify direct or indirect effects (Fig 3). The various specific IgEs were allowed to affect both tIgE levels and disease severity. The results suggest that specific HDM and SE IgE levels affect tIgE levels, whereas SE IgE and GP IgE levels affect disease severity directly and are not mediated through an effect on tIgE. SE IgE levels were associated with more severe disease, and GP IgE levels were associated with less severe disease.

Furthermore, we studied the relationship between IgE parameters and markers of disease severity, such as oral steroid use or hospitalization, within the last 12 months, and lung function was represented by FEV<sub>1</sub> (see Tables E6-E8 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Oral steroid use and hospitalizations were significantly increased in SE IgE-positive but GP IgE-negative subjects. Finally, GP IgE levels were associated



**FIG 2.** Multiple correspondence analyses factor map with 95% confidence ellipses situating relationships between parameters and disease severity. SE IgE is situated near severe asthma, whereas GP and HDM IgEs are situated near nonsevere asthma.



**FIG 3.** Categorical Bayesian network results suggest that SE IgE and GP IgE affect disease severity directly and are not mediated through an effect on tIgE. SE IgE was associated with more severe disease, and GP IgE was associated with less severe disease.

with a higher FEV<sub>1</sub> percent predicted value and SE IgE levels were associated with a lower FEV<sub>1</sub> percent predicted value versus those seen in specific IgE-negative subjects.

## DISCUSSION

This study identifies that subjects with serum specific IgE antibodies to SEs have a significantly increased risk of asthma and especially severe asthma. This is not purely a reflection of atopy because the presence of IgE against GP or HDM allergens was not an independent risk factor for either asthma or disease severity in this adult population. Furthermore, this study identified the presence of SE IgE in a significant proportion of patients with severe asthma who, according to standard aeroallergen skin prick test responses and serum specific IgE measurements, were nonatopic. Additionally, SE IgE positivity was identified as being significantly associated with high total serum IgE concentrations, need for oral steroid use, hospitalizations within the last 12 months, and impaired spirometric lung function. These findings suggest that the immune response to SEs might substantially contribute to asthma, particularly severe asthma, independently of classical atopy.

The findings build on 2 smaller studies in asthmatic patients that have identified the increased prevalence of SE IgE positivity in asthmatic patients in comparison with that seen in non-asthmatic control subjects<sup>14,16</sup> and a systematic review of the literature and meta-analysis evaluating the relevance of staphylococci and their enterotoxins to airways disease.<sup>15</sup>



Patients were recruited from 2 geographically distinct European sites to gather a sufficient number of subjects to perform a multivariate analysis. There were no major differences in demographic characteristics between these groups. The analysis demonstrated that SE IgE is different from GP and HDM IgE in terms of serum concentrations and fraction of tIgE levels, which is indicative of its polyclonal character. As such, SE IgE is associated with a higher total serum IgE level in comparison with GP and HDM IgE. The presence of serum SE IgE significantly increases the odds of having nonsevere and severe asthma versus control subjects and having severe versus nonsevere asthma. This is in contrast to the tIgE level, which moderately increases the risk of having asthma but has no effect on having severe versus nonsevere asthma. SE IgE was evident in asthmatic patients in both those with aeroallergen-specific IgE positivity and those who would classically be considered nonatopic. It is well recognized that a substantial group of patients with severe asthma, although nonatopic, have increased tIgE levels.<sup>20</sup> This study provides an explanation for this apparent discrepancy and raises the potential that immunologic responses against *S aureus* within the airways underlie the clinical disease expression in intrinsic or nonallergic asthma. The data also support the clinical finding of a frequent late-onset disease pattern in the intrinsic severe asthma subgroup, differentiating these patients from atopic patients with early-onset severe asthma. Our findings might also provide an explanation for the apparent similarity of the pathology in patients with nonatopic and atopic asthma, with both being associated with airway eosinophil recruitment.<sup>21</sup>

The present study does not localize the SE IgE production to the airways because the study is based on serum markers. However, there was no effect of former or present atopic dermatitis or the presence of reported nasal polyps on SE IgE or tIgE concentrations in a former study.<sup>16</sup> Furthermore, the findings have biological credibility in relationship to asthma pathology and the development of more severe disease. Staphylococcal enterotoxins have been shown to promote airway inflammation and bronchial hyperresponsiveness in animal models,<sup>22</sup> to activate cells in human tissue,<sup>23,24</sup> to promote overexpression of IgG<sub>4</sub> and IgE through B-cell activation and class-switching,<sup>25,26</sup> to induce T-cell activation that is poorly responsive to glucocorticoid regulation,<sup>27</sup> to inhibit regulatory T-cell function,<sup>28</sup> and to strongly amplify airway inflammation in combination with allergen exposure after sensitization.<sup>29,30</sup> Furthermore, in nasal polyp tissue specific IgE antibodies have been demonstrated to be functional in degranulating mast cells,<sup>31</sup> even in the absence of the same IgE specificities in the serum of the same patient. It has thus been speculated that the polyclonal IgE pattern allows for a continuous degranulation of tissue mast cells on contact with multiple and diverse environmental or local tissue allergens, including staphylococcal enterotoxins,<sup>31</sup> thereby supporting persistent mucosal inflammation in association with polyclonal activation of T cells.

There is some overlap between severe and nonsevere asthma in terms of SE IgE concentrations; however, we need to take into account that the serum SE IgE level is only a partial reflection of the mucosal tissue SE IgE level and might even be negative in serum but present in tissue.<sup>31</sup> SE IgE might be a reflection of current or former contact with *S aureus* enterotoxins, and the activity of IgE production can vary over time. We also have identified that a proportion of patients with mild or moderate asthma have SE IgE. Because this is a cross-sectional study, we cannot determine

whether the presence will determine a different course of disease in such patients compared with those who are SE IgE negative. In children followed from birth to the age of 5 years, it has been shown that the presence of SE IgE positivity at 5 years is associated with greater bronchial hyperresponsiveness than in those who have normal SE IgE measures.<sup>32</sup> To determine the relevance of SE IgE to disease progression in adults with asthma, prospective longitudinal studies are needed with evaluation of a range of outcome measures, including exacerbation tendency. Such studies would need to take into account both upper and lower airway disease expression.

In summary, our findings suggest that immune responses against SEs have a direct effect on disease severity, especially severe late-onset nonatopic (intrinsic) asthma. The link with tIgE levels suggests that this is mediated, at least in part, through the superantigenic effects that result in a polyclonal activation of T and B cells. This newly described mechanism might result in innovative therapeutic approaches in nonatopic asthmatic patients by using anti-IgE strategies.

**Clinical implications: Measurement of serum IgE antibodies against SEs in adult patients with severe asthma might be useful to differentiate superantigen-related polyclonal IgE from inhalant oligoclonal IgE.**

## REFERENCES

- Holgate S, Bisgaard H, Bjermer L, Haahtela T, Haughney J, Horne R, et al. The Brussels Declaration: the need for change in asthma management. *Eur Respir J* 2008;32:1433-42.
- Bateman ED, Boushey HA, Bousquet J, Busse WW, Clark TJ, Pauwels RA, et al. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma Control study. *Am J Respir Crit Care Med* 2004;170:836-44.
- Bousquet J, Boulet LP, Peters MJ, Magnussen H, Quiralte J, Martinez-Aguilar NE, et al. Budesonide/formoterol for maintenance and relief in uncontrolled asthma vs. high-dose salmeterol/fluticasone. *Respir Med* 2007;101:2437-46.
- Bousquet J, Mantzouranis E, Cruz A, Ait-Khaled N, Baena-Cagnani CE, Bleecker ER, et al. Uniform definition of asthma severity, control and exacerbations. Paper presented to WHO for a consultation on severe asthma. *J Allergy Clin Immunol* 2010;126:926-38.
- The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. European Network for Understanding Mechanisms of Severe Asthma. *Eur Respir J* 2003;22:470-7.
- Moore WC, Bleecker ER, Curran-Everett D, Erzurm SC, Ameredes BT, Bacharier L, et al. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol* 2007;119:405-13.
- Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010;181:315-23.
- Skov L, Olsen J, Giorno R, Schlievert P, Baadsgaard O, Leung D. Application of staphylococcal enterotoxin B on normal and atopic skin induces up-regulation of T cells by a superantigen-mediated mechanism. *J Allergy Clin Immunol* 2000;105:820-6.
- Bunikowski R, Mielke M, Skarabis H, Worm M, Anagnostopoulos I, Kolde G, et al. Evidence for a disease-promoting effect of *Staphylococcus aureus*-derived exotoxins in atopic dermatitis. *J Allergy Clin Immunol* 2000;105:814-9.
- Breuer K, Wittmann M, Bosche B, Kapp A, Werfel T. Severe atopic dermatitis is associated with sensitization to staphylococcal enterotoxin B (SEB). *Allergy* 2000;55:551-5.
- Bachert C, Gevaert P, Holtappels G, Johansson SG, van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol* 2001;107:607-14.
- Pérez-Novo C, Lowalski M, Kuna P, Ptánska A, Holtapels G, van Cauwenberge P, et al. Aspirin sensitivity and IgE antibodies to *Staphylococcus aureus* enterotoxins in nasal polyposis: studies on the relationship. *Int Arch Allergy Immunol* 2004;133:255-60.
- Van Zele T, Gevaert P, Watelet JB, Claeys G, Holtappels G, Claeys C, et al. *Staphylococcus aureus* colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. *J Allergy Clin Immunol* 2004;114:981-3.

14. Bachert C, Gevaert P, Howarth P, Holtappels G, van Cauwenberge P, Johansson SGO. IgE to *Staphylococcus aureus* enterotoxins in serum is related to severity of asthma. *J Allergy Clin Immunol* 2003;111:1131-2.
15. Pastacaldi C, Lewis P, Howarth P. Staphylococci and staphylococcal superantigens in asthma and rhinitis: a systematic review and meta-analysis. *Allergy* 2010;66:549-55.
16. Kowalski ML, Cieślak M, Pérez-Novo CA, Makowska J, Bachert C. Clinical and immunological determinants of severe/refractory asthma (SRA): association with Staphylococcal superantigen-specific IgE antibodies. *Allergy* 2010;66:32-8.
17. Greenacre M, Blasius J. Multiple correspondence analysis and related methods. London: Chapman & Hall/CRC; 2006.
18. Husson F, Lê S, Pagès J. Exploratory multivariate analysis by example using R. Computer science & data analysis. London: Chapman & Hall/CRC; 2010.
19. Ben-Gal I. Bayesian networks. In: Ruggeri F, Faltin F, Kenett R, editors. Encyclopedia of statistics in quality & reliability. Oxford: Wiley & Sons; 2007.
20. Beeh KM, Ksoll M, Buhl R. Elevation of total serum immunoglobulin E is associated with asthma in non allergic individuals. *Eur Respir J* 2000;16:609-14.
21. Gaga M, Lambrou P, Papageorgiou N, Koulouris NG, Kosmas E, Fragakis S, et al. Eosinophils are a feature of upper and lower airway pathology in non-atopic asthma, irrespective of the presence of rhinitis. *Clin Exp Allergy* 2000;30:663-9.
22. Herz U, Rückert R, Wollenhaupt K, Tschernig T, Neuhaus-Steinmetz U, Pabst R, et al. Airway exposure to bacterial superantigen (SEB) induces lymphocyte-dependent airway inflammation associated with increased airway responsiveness—a model for non-allergic asthma. *Eur J Immunol* 1999;29:1021-31.
23. Huvenne W, Callebaut I, Reekmans K, Hens G, Bobic S, Jorissen M, et al. *Staphylococcus aureus* enterotoxin B augments granulocyte migration and survival via airway epithelial cell activation. *Allergy* 2010;65:1013-20.
24. Patou J, Van Zele T, Gevaert P, Holtappels G, Van Cauwenberge P, Bachert C. *Staphylococcus aureus* enterotoxin B, protein A and lipoteichoic acid stimulations in nasal polyps. *J Allergy Clin Immunol* 2008;121:110-5.
25. Van Zele T, Gevaert P, Holtappels G, van Cauwenberge P, Bachert C. Local immunoglobulin production in nasal polyposis is modulated by superantigens. *Clin Exp Allergy* 2007;37:1840-7.
26. Mechtcheriakova D, Sobanov J, Knittelfelder R, Bachert C, Jensen-Jarolim E. Gene expression pattern associated with activation-induced cytidine deaminase, AID, in chronic paranasal disorders. *PLoS One* 2011;6:e25611.
27. Hauk PJ, Hamid QA, Chrousos GP, Leung DY. Induction of corticosteroid insensitivity in human PBMCs by microbial superantigens. *J Allergy Clin Immunol* 2000;105:782-7.
28. Cardona ID, Goleva E, Ou LS, Leung DY. Staphylococcal enterotoxin B inhibits regulatory T cells by inducing glucocorticoid-induced TNF receptor-related protein ligand on monocytes. *J Allergy Clin Immunol* 2006;117:688-95.
29. Hellings PW, Hens G, Meyts I, Bullens D, Vanoirbeek J, Gevaert P, et al. Aggravation of bronchial eosinophilia in mice by nasal and bronchial exposure to *Staphylococcus aureus* enterotoxin B. *Clin Exp Allergy* 2006;36:1063-71.
30. Huvenne W, Callebaut I, Plantinga M, Vanoirbeek J, Krysko O, Bullens D, et al. *Staphylococcus aureus* enterotoxin B facilitates allergic sensitization in experimental asthma. *Clin Exp Allergy* 2010;40:1079-90.
31. Zhang N, Holtappels G, Gevaert P, Patou J, Dhaliwal B, Gould H, et al. Mucosal tissue polyclonal IgE is functional in response to allergen and SEB. *Allergy* 2001;66:141-8.
32. Semic-Jusufagic A, Bachert C, Gevaert P, Holtappels G, Lowe L, Woodcock A, et al. *Staphylococcus aureus* sensitization and allergic disease in early childhood: population-based birth cohort study. *J Allergy Clin Immunol* 2007;119:930-6.

TABLE E1. Specific IgE fractions of tIgE for specific IgE–positive subjects

Quantity	Control subjects	Patients with nonsevere asthma	Patients with severe asthma	Global <i>P</i> value	Patients with nonsevere asthma vs control subjects, <i>P</i> value	Patients with severe asthma vs control subjects, <i>P</i> value	Patients with severe asthma vs patients with nonsevere asthma, <i>P</i> value
SE IgE/tIgE (%), mean (SD)*	0.34 (0.38)	0.22 (0.23)	0.48 (1.11)	.03†			
HDM IgE/tIgE (%), mean (SD)*	6.77 (6.58)	6.71 (7.46)	6.14 (8.12)	.35†			
GP IgE/tIgE (%), mean (SD)*	17.04 (19.89)	11.12 (12.44)	4.61 (7.07)	<.001†	.47‡	.01‡	<.001‡

\*Positive samples: only *P* values surviving Bonferroni correction are shown in boldface (global *P* < .05/3, *post hoc* pairwise *P* < .05/9).

†Kruskal-Wallis test.

‡Mann-Whitney *U* test.

**TABLE E2.** Association between specific IgEs

Association between specific IgEs	OR (95% CI)	<i>P</i> value
SE IgE positive (0.1 kU/L) and HDM IgE positive (0.35 kU/L)	2.56 (1.65-4.00)	<b>&lt;.001*</b>
SE IgE positive (0.1 kU/L) and GP IgE positive (0.35 kU/L)	1.74 (1.09-2.78)	.02*
HDM IgE positive (0.35 kU/L) and GP IgE positive (0.35 kU/L)	6.16 (3.70-10.40)	<b>&lt;.001*</b>

\*Fisher exact test: *P* values surviving Bonferroni correction are shown in boldface (*P* < .05/3).



**TABLE E3.** Linear regression model for tIgE (log)

Predictor	Multiple effect (95% CI)*	P value
SE IgE positive and HDM-IgE negative	6.75 (4.71-9.68)	<.001
SE IgE negative and HDM IgE positive	3.39 (2.35-4.88)	<.001
SE IgE positive and HDM IgE positive	14.56 (10.29-20.61)	<.001
GP IgE positive	2.14 (1.62-2.81)	<.001

\*Multiple effects at the original scale correspond to the usual additive effects at the log-transformed scale at which the analysis was carried out.

**TABLE E4.** Numbers (percentages) of specific IgE combinations within control subjects, patients with nonsevere asthma, and patients with severe asthma

Specific IgE combinations	Control subjects (n = 44)	Patients with nonsevere asthma (n = 118)	Patients with severe asthma (n = 155)
SE IgE negative, HDM IgE negative, and GP IgE negative	23 (52.3%)	28 (23.7%)	27 (17.4%)
SE IgE negative, HDM IgE and/or GP IgE positive	16 (36.4%)	42 (35.6%)	37 (23.9%)
SE IgE positive, HDM IgE negative, and GP IgE negative	1 (2.3%)	5 (4.2%)	33 (21.3%)
SE IgE positive, HDM IgE and/or GP IgE positive	4 (9.1%)	43 (36.4%)	58 (37.4%)

**TABLE E5.** Logistic regression models for disease severity

Comparison	Predictor	OR (95% CI)	P value
Patients with nonsevere asthma vs control subjects	SE IgE positive	4.47 (1.61-12.40)	<b>.004</b>
	HDM IgE positive	2.10 (0.97-4.54)	.06
Patients with severe asthma vs control subjects	SE IgE positive	11.09 (4.14-29.68)	<b>&lt;.001</b>
Patients with severe asthma vs patients with nonsevere asthma	SE IgE positive and GP IgE negative	4.98 (2.24-11.09)	<b>&lt;.001</b>
	SE IgE negative and GP IgE positive	1.05 (0.53-2.07)	.89
	SE IgE positive and GP IgE positive	1.27 (0.67-2.41)	.47
Patients with severe or nonsevere asthma vs control subjects	SE IgE positive	7.26 (2.76-19.13)	<b>&lt;.001</b>
	HDM IgE positive	1.84 (0.90-3.76)	.10
Patients with severe asthma vs patients with nonsevere asthma or control subjects	SE IgE positive and GP IgE negative	7.69 (3.61-16.37)	<b>&lt;.001</b>
	SE IgE negative and GP IgE positive	1.26 (0.67-2.34)	.47
	SE IgE positive and GP IgE positive	1.93 (1.06-3.51)	<b>.03</b>

**TABLE E6.** Logistic regression models for oral steroid use

Predictor	OR (95% CI)	P value
SE IgE positive and GP IgE negative	3.84 (1.83-8.02)	<.001
SE IgE negative and GP IgE positive	0.74 (0.35-1.55)	.42
SE IgE positive and GP IgE positive	0.95 (0.47-1.94)	.89

**TABLE E7.** Linear regression models for FEV<sub>1</sub>

Predictor	Effect (95% CI)	<i>P</i> value
SE IgE positive	−12.91 (−18.02 to −7.80)	<.001
GP IgE positive	5.81 (0.71 to 10.91)	.03

**TABLE E8.** Logistic regression models for hospitalization

Predictor	OR (95% CI)	P value
SE IgE positive and GP IgE negative	5.50 (2.49-12.17)	<.001
SE IgE negative and GP IgE positive	1.32 (0.62-2.80)	.47
SE IgE positive and GP IgE positive	1.60 (0.76-3.38)	.22