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

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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_1 percent predicted value, and enterotoxin IgE was associated with a lower FEV_1 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_1, IgE,* Staphylococcus aureus*, enterotoxins, superantigens*

Asthma is a global health problem associated with high morbidity and socioeconomic burden.[1] 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,[2] many patients have uncontrolled asthma.[3] 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.[1,4]

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.[5-7] Despite this, however, and based on evidence gained from patients with atopic dermatitis[8-10] and those with nasal polyposis,[11-13] as well as from our own preliminary findings in asthmatic patients and a systematic review of the literature and meta-analysis,[14,15] we have hypothesized that IgE responses orchestrated by enterotoxins from S*taphylococcus 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,[14] a finding supported by a recent study in Poland,[16] and have linked its presence

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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.[4] 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:

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

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_1, 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_1 (linear regression) were incorporated into the modeling as indices of disease severity. These analyses were complemented with a multiple correspondence analysis[17] 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.[18]

Finally, direct or indirect effects of dichotomized IgE-related measurements on disease severity were explored by using categorical Bayesian networks (R-package catnet).[19] 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_1 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_1 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