Specific IgE against staphylococcal enterotoxin (superantigens) in allergic rhinitis: An independent risk factor for severe bronchial asthma

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ABSTRACT
Objective: The study aimed to investigate whether IgE to Staphylococcus aureus enterotoxins might be relevant to disease severity in adult asthmatic patients.

Materials and Methods: Specific IgE antibody concentrations in serum against enterotoxins, grass pollen (GP), and house dust mite (HDM) allergens and total IgE levels were measured in 69 adult control subjects, 152 patients with non-severe 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: Statistical analysis demonstrated Enterotoxin IgE positivity which was significantly greater in patients with severe asthma (59.67%) than in healthy control subjects (13% P<.001). Twenty-one percent of patients with severe asthma showing positive enterotoxin IgE were considered non atopic. Also statistical analyses demonstrated significantly increased risks for enterotoxin IgE-positive subjects to have severe asthma (95%) versus enterotoxin IgE-negative subjects. The presence of GP or house dust 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 positive enterotoxin IgE and non-atopic asthma. GP IgE was associated with a higher FEV1 percent predicted value and enterotoxin IgE was associated with a lower FEV1 percent predicted value.

Conclusion: 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 bronchial asthma.

Key words: asthma, asthma severity, hospitalizations FEV1, IgE, staphylococcal aureus, enterotoxins, superantigens.

INTRODUCTION
Asthma is a global health problem associated with high morbidity and socioeconomic burden1. Within the United States, asthma affects an estimated one 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 patients2, still many patients have uncontrolled asthma3. 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 group, and novel treatments based on a better understanding of disease are required4.

Phenotypic characterization of populations with severe asthma has identified a greater preponderance of aeroallergen-specific IgE negative subjects (non-atopic asthma) and a greater prevalence of comorbid rhinosinusitis than in patients with non-severe asthma5. Despite this, however, and based on evidence gained from patients...
with atopic dermatitis\textsuperscript{4}, and those with nasal polyposis\textsuperscript{7}, as well as from our own preliminary findings in allergic rhinitis\textsuperscript{8} patients and from a systematic review of the literature and meta-analysis\textsuperscript{9}, we have hypothesized that IgE responses orchestrated by enterotoxins from \textit{Staphylococcus aureus} might provide an explanation for disease persistence and severity in asthmatic patients even in those with classically considered non-atopic asthma. Enterotoxins generated by \textit{S} \textit{aureus} can act both as nominal antigens, stimulating specific IgE responses (\textit{Staphylococcus aureus} enterotoxins [SE] IgE), and as superantigens, promoting a polyclonal IgE response reflected by an increase in total IgE (tIgE) levels. We and other authors have previously identified increased serum concentrations of SE IgE in asthmatic patients\textsuperscript{10,11}, a finding supported by a recent study in Poland\textsuperscript{12}, and have linked its presence 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 study was 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) and Grass Pollen (GP) allergens were measured in adult asthmatic patients (both non severe 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 non-atopic 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 non-atopic asthma.

MATERIALS AND METHODS

Sixty-nine non asthmatic control subjects, 152 patients with non-severe asthma, and 166 patients with severe treatment-resistant asthma were recruited at the Department of Internal medicine in Ain Shams University hospital in Cairo, Egypt. All asthmatic patients had established disease, and none of the non-asthmatic 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. Non severe 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 committee, and all subjects provided written informed consent. Basic clinical characteristics of both population were comparable with respect to smoking habits, age, and gender distribution.

During the first visit the clinical and questionnaire administration was undertaken and spirometry was performed by using a calibrated Vitalograph. The highest values of three 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 BMI=weight in kg/(height in m\textsuperscript{2}). Skin prick testing was performed for the following allergens: \textit{Dermatophagoides pteronyssinus}, \textit{Dermatophagoides farinae}, cat, dog, tree pollens, mixed GPs, weed pollens, and \textit{Alternaria}. 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 Immunology laboratory of
correspondence analysis on disease severity and the specific IgE levels by using the multiple correspondence analysis function from R-package FactoMineR. Finally, direct or indirect effects of dichotomized IgE-related measurements on disease severity were explored by using categorical Bayesian networks (R-package catnet). 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 cn Find B1C).

RESULTS
Demographic data, serum total and specific IgE distributions, and information on asthma severity (FEV1 percent predicted, oral steroid use, and hospitalizations over the last 12 months) are summarized in Table 1. 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 FEV1 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 differences were only evident for asthmatic patients relative to non-asthmatic control subjects.

Mean serum SE IgE concentrations increased with disease severity, whereas concentrations for GP or HDM-specific IgE did not any increase in levels.

To estimate the effect of a specific IgE on total serum IgE levels showed that the SE IgE level was associated with a significantly higher tIgE level than HDM or GP IgE. The results suggest that specific HDM and SE IgE levels affect...
Table 1 Descriptive analysis of healthy control subjects and patients with Non severe and severe asthma

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Control subjects (n = 69)</th>
<th>Patients with non-severe asthma (n = 152)</th>
<th>Patients with severe asthma (n = 166)</th>
<th>P value Non-severe asthma vs control</th>
<th>P value Severe asthma vs control</th>
<th>P value Severe asthma vs non-severe asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y), mean (SD)</td>
<td>34.23 (11.94)</td>
<td>38.85 (14.41)</td>
<td>46.51 (11.78)</td>
<td>&lt;.001*</td>
<td>&lt;.05 †</td>
<td>&lt;.001 †</td>
</tr>
<tr>
<td>Male sex, no. (%)</td>
<td>28 (40.6)</td>
<td>76 (50.0)</td>
<td>88 (53.0)</td>
<td>NS †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²), mean (SD)</td>
<td>24.98 (4.85)</td>
<td>27.24 (5.85)</td>
<td>29.03 (6.14)</td>
<td>&lt;.001*</td>
<td>&lt;.01 †</td>
<td>&lt;.001 †</td>
</tr>
<tr>
<td>Ever smoker, no. (%)</td>
<td>20 (29.4)</td>
<td>38 (26.2)</td>
<td>53 (33.8)</td>
<td>NS †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker, no. (%)</td>
<td>8 (11.8)</td>
<td>12 (8.3)</td>
<td>11 (7.0)</td>
<td>NS †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive skin prick test response, no. (%)</td>
<td>28 (40.6)</td>
<td>123 (81.5)</td>
<td>109 (66.5)</td>
<td>&lt;.001 †</td>
<td>&lt;.001 †</td>
<td>&lt;.001 †</td>
</tr>
<tr>
<td>Positive tlgE level (100 kU/L), no. (%)</td>
<td>19 (26.5)</td>
<td>85 (55.9)</td>
<td>107 (64.5)</td>
<td>&lt;.001 †</td>
<td>&lt;.001 †</td>
<td>NS †</td>
</tr>
<tr>
<td>tlgE (log), geometric mean (SD)</td>
<td>38.30 (4.06)</td>
<td>153.13 (5.27)</td>
<td>163.68 (5.23)</td>
<td>&lt;.001*</td>
<td>&lt;.001 †</td>
<td>NS †</td>
</tr>
<tr>
<td>SE IgE positive (0.1 kU/L), no. (%)</td>
<td>9 (13.0)</td>
<td>62 (40.8)</td>
<td>99 (59.6)</td>
<td>&lt;.001 †</td>
<td>&lt;.001 †</td>
<td>&lt;.001 †</td>
</tr>
<tr>
<td>SE IgE (log), geometric mean (SD)</td>
<td>0.32 (2.18)</td>
<td>0.66 (2.86)</td>
<td>0.73 (3.26)</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDM IgE positive (0.35 kU/L), no. (%)</td>
<td>19 (27.9)</td>
<td>86 (57.7)</td>
<td>75 (48.4)</td>
<td>&lt;.001 †</td>
<td>&lt;.001 †</td>
<td>&lt;.005 †</td>
</tr>
<tr>
<td>HDM IgE (log), geometric mean (SD)</td>
<td>4.50 (5.67)</td>
<td>8.82 (5.85)</td>
<td>7.10 (5.89)</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP IgE positive (0.35 kU/L), no. (%)</td>
<td>17 (38.6)</td>
<td>69 (58.5)</td>
<td>72 (45.6)</td>
<td>&lt;.05 †</td>
<td>&lt;.05 †</td>
<td>NS †</td>
</tr>
<tr>
<td>GP IgE (log), geometric mean (SD)</td>
<td>5.26 (6.39)</td>
<td>6.87 (8.17)</td>
<td>4.30 (7.10)</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECP (log), geometric mean (SD)</td>
<td>6.84 (2.27)</td>
<td>12.79 (2.31)</td>
<td>15.05 (2.49)</td>
<td>&lt;.001*</td>
<td>&lt;.001 †</td>
<td>NS †</td>
</tr>
<tr>
<td>Oral steroid use, no. (%)</td>
<td>--</td>
<td>7 (5.2)</td>
<td>99 (67.8)</td>
<td>&lt;.001 †</td>
<td>NS †</td>
<td>&lt;.001 †</td>
</tr>
<tr>
<td>FEVi, mean (SD)</td>
<td>98.48 (10.94)</td>
<td>91.78 (13.97)</td>
<td>62.84 (19.80)</td>
<td>&lt;.001*</td>
<td>&lt;.001 †</td>
<td>&lt;.001 †</td>
</tr>
<tr>
<td>Hospitalized, no. (%)</td>
<td>--</td>
<td>13 (9.7)</td>
<td>83 (69.2)</td>
<td>&lt;.001 †</td>
<td>&lt;.05 †</td>
<td>&lt;.001 †</td>
</tr>
</tbody>
</table>

*-Kruskal Wallis test, † Mann-Whitney U test, fisher exact test.
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 represented by FEV1. Oral steroid use and hospitalizations were significantly increased in SE IgE positive but GP IgE negative subjects. GP IgE levels were associated with a higher FEV1 percent predicted value and SE IgE levels were associated with a lower FEV1, percent predicted value versus those seen in specific IgE-negative subjects.

**DISCUSSION**
This study identified that subjects with serum specific IgE antibodies to SEs have a significantly increased risk of asthma 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 study. 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 non-atopic. 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, independent of classical atopy.

The findings build on previous studies in asthmatic patients that have identified the increased prevalence of SE IgE positivity, asthmatic patients in comparison with that seen in non-asthmatic control subjects and meta-analysis evaluating the relevance of staphylococci and their enterotoxins to airways disease. Patients were recruited from a geographically distinct Egyptian site 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 HEM 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 risk of having non-severe and severe asthma versus control subjects and having severe versus non-severe 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 non-severe asthma. SE IgE was evident in asthmatic patients in both those with aeroallergen-specific IgE positivity and those who would classically be considered non atopic. It is well recognized that a substantial group of patients with severe asthma, although nonatopic, have increased tIgE levels. 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 non-allergic 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. Over findings might also provide an explanation for the apparent similarity of the pathology in patients with non-atopic and atopic asthma, with both being associated with airway eosinophil recruitment.
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. 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 to activate cells in human tissue to promote overexpression of IgG4 and IgE through B-cell activation and class-switching to induce T-cell activation that is poorly responsive to glucocorticoid regulation, to inhibit regulatory T-cell function, and to strongly amplify airway inflammation in combination with allergen exposure after sensitization. Furthermore, in nasal polyp tissue specific IgE antibodies have been demonstrated to be functional in degranulating mast cells, 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 inert cells on contact with multiple and diverse environmental or local tissue allergens, including staphylococcal enterotoxins, thereby supporting persistent mucosal inflammation in association with polyclonal activation of T cells.

There is some overlap between severe and non-severe asthma in terms of SE IgE concentration 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. 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 not 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 hyper responsiveness than in those who have normal SE IgE measures. 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 non-atopic (intrinsic) asthma. The link with tIgE levels suggests that this is mediated, at least in part, through the super antigenic 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.

REFERENCES


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