

Clinical and immunological determinants of severe/refractory asthma (SRA): association with Staphylococcal superantigen-specific IgE antibodies

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Keywords

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Abstract

Background: Demographic and immunological determinants of severe refractory asthma (SRA) are not well characterized. Because *Staphylococcus aureus* enterotoxins with superantigenic activity have been associated with upper and lower airway inflammation, we aimed to evaluate the association of sensitization to Staphylococcal enterotoxins with asthma severity and various asthma phenotypes.

Methods: The study included 109 patients with SRA diagnosed according to the American Thoracic Society Workshop 2000, and 101 patients with nonsevere asthma, followed for at least 12 months. Specific IgE to *Staphylococcus* enterotoxins and total IgE and eosinophil cationic protein concentrations were measured in serum with immunoassays.

Findings: A significant risk for severe asthma was associated with female gender [Odds Ratio (OR) = 2.04], history of wheezing in childhood (OR = 2.47), presence of hypersensitivity to aspirin (OR = 1.96) and with body mass index (OR = 3.08). The mean level of enterotoxin-specific IgE was 3-fold higher in patients with severe asthma when compared to patients with nonsevere asthma ($P = 0.01$). Serum-specific IgE to enterotoxins was significantly associated with low respiratory function parameters (FEV₁, FEV₁/FVC and MEF 25/75) and increased airway reversibility in response to albuterol. The presence of specific IgE to enterotoxin carried a significant risk for patients to have serum total IgE level above 100 kU/l (OR = 7.84).

Interpretation: Specific immunological response to enterotoxins is associated with clinical and immunological parameters of asthma severity, suggesting a role for Staphylococcal enterotoxins in the asthma pathogenesis.

A significant proportion of patients with asthma cannot be adequately controlled despite optimal anti-inflammatory treatment, and these asthmatics have been referred to as suffering from severe refractory or difficult to control disease (1). According to the American Thoracic Society (ATS) workshop definition, patients with severe refractory asthma (SRA) need oral corticosteroids or high doses of inhaled corticosteroids to remain under control and/or have persistent airway obstruction, high requirement for additional asthma treatment (including need for oral steroid bursts) and urgent care visits (2). These patients have high rate of missed school and work days, have increased health care utilization (including emergency room visits and hospitalizations) and poor quality of life presenting high economic burden for the society (3).

However, demographic, clinical and immunological determinants of severe/difficult to control asthma remain largely unknown (4–6). Ongoing Th2 type inflammation (with Th1 type immune response contribution in some cases), epithelial and smooth muscle abnormalities in the airways and an increased recruitment of inflammatory cells from bone marrow are constant features of SRA (1, 7, 8). In addition to genetic factors, IgE-mediated sensitization to common inhalant allergens contributes to the development of airway inflammation at least in the subpopulation of patients with SRA, but its influence on development of severe asthma phenotype remains unclear. Chronic/recurrent viral or bacterial infections have been implicated in the pathophysiology of severe asthma and more recently (9) *Staphylococcus aureus* (*S. aureus*) bacteria, which can be detected in association

with asthma, have the potential to release enterotoxins with superantigenic activity modifying not only B and T cells function but also activating inflammatory cells. Stimulatory role of superantigens in the development of mucosal inflammation in chronic rhinosinusitis and nasal polyposis has been documented (10), and *S. aureus* and its products have been implicated in the pathogenesis of allergic and inflammatory diseases including rhinitis and atopic dermatitis (11, 12). More recently, *S. aureus* enterotoxin B (SEB) was shown to favour Th2 type cytokines while disfavours T-regulatory cytokines release in nasal polyp tissue (13), and enterotoxin A seems to induce pro-asthmatic, T-cell-mediated changes in the airway smooth muscle when presented by airway smooth muscle to CD4⁺ lymphocytes (14). Furthermore, in earlier study involving a small group of patients, the occurrence of specific IgE antibodies to *S. aureus* enterotoxins (SEs) was also associated with asthma and related to its severity (15). The available evidence for the potential role of bacterial superantigens in the pathogenesis of asthmatic inflammation has been recently summarized (16).

In this study, we aimed to determine the potential involvement of an immunological, IgE-mediated response to *S. aureus* superantigens in the pathogenesis of difficult/severe asthma. We recruited a relatively large cohort of patients with asthma, followed them in one clinical centre for 12 months and then distinguished patients with severe refractory phenotype. Clinical, ventilatory and immunological characteristics of patients with severe asthma phenotype were then compared to non-severe asthmatics, and associations between serum levels of specific IgE to a mixture SEs with asthma severity traits and with various asthmatic phenotypes were analysed.

Methods

Patients

The study included 210 patients with bronchial asthma recruited from one clinic –Department of Immunology, Rheu-

matology and Allergy, Medical University Hospital in Lodz. Before being included to the study, all patients were followed up for at least 12 months; during this time, asthma control was periodically assessed, and pharmacological treatment was appropriately modified. Severe refractory asthma was diagnosed according to the definition of American Thoracic Society Workshop 2000 (2), and nonsevere asthma was diagnosed if a patient did not meet the above criteria. ATS2000 workshop requirements for diagnosis of SRA (SRA) requiring a combination of one of two major and two of seven minor criteria were fulfilled by 109 patients. Remaining 101 patients who did not meet the ATS2000 criteria had either moderate or mild asthma based on respiratory function measurement and requirement for asthma treatment as defined by The Global Initiative for Asthma 2002 guidelines (17).

In the SRA group, at least one major criterion was fulfilled by 100%, and single minor criteria were fulfilled by 22–99% patients. Daily use of controller drugs was the only minor criterion fulfilled by a significant number of patients with non-SRA (Table 1).

Aspirin hypersensitivity was diagnosed based on convincing history of adverse reaction to nonsteroidal anti-inflammatory drugs (NSAIDs) and confirmed by a bronchial challenge with lysine aspirin (18). Body mass index (BMI) was calculated according to formula: BMI = weight (kg)/height²(m²). A control group for specific IgE to SEs assessment included 45 healthy, nonatopic individuals (26 men and 19 women; mean age 31.05 ± 8.5) selected from a random sample of the city general population recruited for another study (19). All subjects gave their informed consent, and the study was approved by Local Ethic Committee.

Procedures

In all patients, skin prick tests were performed (Allergopharma, Reinbeck, Germany), and a wheal diameter of 3 mm or more in excess of the negative control was considered a positive result. The following allergens were included: *D. pteronyss-*

Table 1 Fulfilment of American thoracic society workshop criteria for severe refractory asthma in the study group (n = 210)

	Number of patients (%)	
	SRA (n = 109)	Non-SA (n = 101)
Major characteristics		
Continuous high-dose of ICS (i.e. >1200 ug budesonide/ daily)	109 (100%)	0
Continuous or near continuous oral GCS	71 (65.1%)	0
Minor characteristics		
Daily use of a controller drug (LABA or ALD)	92 (99.1%)	87 (86.13%)
Daily or nearly daily use of inhaled β_2 agonists	103 (94.5%)	0
Airway obstruction; FEV ₁ < 80%	90 (82.6%)	4 (3.96%)
1 urgent care visit/ year	29 (26.6%)	0
≥3 oral GCS bursts/ year	24 (22%)	0
Deterioration (FEV ₁ < 80%) with at least <25% reduction in GCS	Not assessed	Not assessed
History of a near fatal asthma event	34 (31.2%)	0

ICS, Inhaled Corticosteroids; GCS, Glucocorticosteroids; LABA, Long Acting β_2 Agonists; ALD, Antileukotriene Drugs; SRA, Severe/Refractory Asthma.

sinus, *D. farinae*, cat, dog, trees, grass mix, weeds, *Alternaria tenuis*.

Respiratory function (flow-volume curve: FEV₁, FEV₁/FVC, MEF 25–75) was measured with an automatic spirometer (ABC Pneumo 2000RS, Poland) when patients were without inhaled short acting β_2 agonists for at least 8 h and long acting β_2 agonists for at least 12 h. Airway reversibility was assessed 15 min after nebulization of 2.5 mg of albuterol (Sterineb Salamol, Norton, Poland).

The results were expressed as the best measurement and maximal % change from baseline. Total IgE, eosinophil cationic protein (ECP) and specific IgE to SEs mix including SEA, SEC and toxin shock syndrome toxin (TSST-1) were measured in serum with ImmunoCAP system (*Pharmacia Diagnostic, Sweden*). The lower limit of SEs mix IgE detection was 0.1 kU/l. Blood eosinophilia (as a differential count) was assessed after staining with Pappenheim's method.

Statistical analysis

Data are presented as mean $\pm$ SEMs (standard errors) or $\pm$ SD (standard deviation) as indicated. The data distribution was tested by Kolmogorov-Smirnow test. The following statistical methods were applied as appropriate and as indicated in the text: chi-square Person's test, exact Fisher test and analysis of variance (ANOVA and MANOVA). Models of logistic regression were applied to assess the risk of severe asthma. For analysis, total IgE values were transformed logarithmically and were presented as geometric mean and 95% CI. *P*-values <0.05 were considered to be significant.

Results

Patients with severe/refractory and nonsevere asthma

The mean age and the duration of asthma were significantly higher in patients with SRA when compared to nonsevere asthma (non-SA). More patients with SRA were women, and had a history of frequent childhood wheezing, aspirin hypersensitivity and/or smoking (Table 2). Patients with severe asthma had on average six times more asthma exacerbations per year confirming a lack of full control of asthma despite a significantly higher dose of inhaled glucocorticosteroids (GCS) and more frequent use of oral steroids. Only 2.8% of patients with SRA did not report a single asthma exacerbation during preceding year while 58% of patients with non-SA did not have such exacerbation ($P < 0.005$).

A significant risk for SRA was associated with female gender [OR = 2.04 (1.159–3.6); $P < 0.02$], history of wheezing in childhood [(OR = 2.47 (1.32–4.62); $P < 0.005$], presence of hypersensitivity to acetylsalicylic acid (ASA) and other NSAIDs (OR = 1.96; 1.05–3.65; $P < 0.035$) and with BMI $> 35\%$ (OR = 3.08; (1.739–5.442; $P < 0.001$) (Fig. 1).

Patients with SRA had on average lower respiratory function when compared to patients with nonsevere disease. Interestingly, mid expiratory flow (MEF 25–75%), which is reflecting function of small airways, was on average almost 2-fold lower in patients with severe asthma. Although the

Table 2 Comparison of clinical characteristics of patients with severe refractory and nonsevere asthma (mean $\pm$ SD)

	Severe refractory asthma (=109)	Nonsevere asthma (n = 101)	<i>P</i> -value
Age (years)	50.9 $\pm$ 13.8	38.4 $\pm$ 16.1	$<0.005^{\&}$
Female (%)	69.7	53.0	$<0.02^{\#}$
Atopy (%)	72	80	NS [#]
Wheezing in childhood	39.6	21.6	$<0.01^{\#}$
Duration of asthma (years)	20.9 $\pm$ 12.0	11.6 $\pm$ 10.1	$<0.005^{\&}$
Perennial rhinitis (%)	73.4	93	$<0.005^{\#}$
Aspirin hypersensitivity (%)	33.9	20.8	$<0.04^{\#}$
BMI ≥ 25 (%)	70.4%	43.6	$<0.005^{\#}$
Exacerbations/year	2.6 $\pm$ 1.44	0.47 $\pm$ 0.61	$<0.005^{\&}$
FEV ₁ (% of predicted)	72.4 $\pm$ 20.29	103.1 $\pm$ 14.6	$<0.005^{\&}$
FEV ₁ /FVC %	62.9 $\pm$ 1.2	78.8 $\pm$ 1.2	$<0.005^{\&}$
MEF 25–75 (% of predicted)	43.2 $\pm$ 23.3	85.3 $\pm$ 28.0	$<0.005^{\&}$
% increase in FEV ₁ after salbutamol	20.5 $\pm$ 14.4	8.7 $\pm$ 7.2	$<0.005^{\&}$

$\&$ one way ANOVA; $\#$ chi² test; BMI, Body Mass Index.

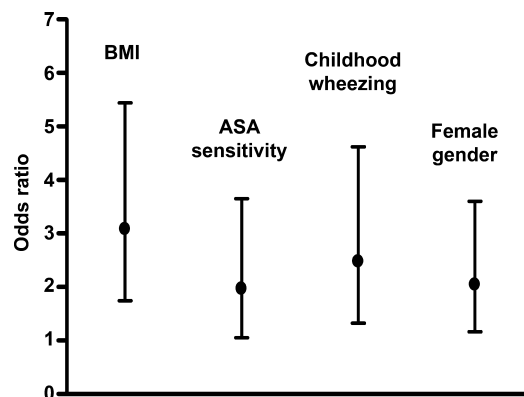


Figure 1 Risk factors for severe refractory asthma (Odds Ratios). BMI: Body Mass Index.

mean FEV₁% increased significantly after nebulization of 2.5 mg of salbutamol in both groups, on average, the increase was 2.3 times higher in patients with SRA when compared to non-SA. When individual responses to salbutamol were analysed, the postbronchodilator FEV₁ increase was found to be significant (at least 12% from baseline) in 71.2% of patients with SRA and only in 25.7% of patients with non-SA. There was no difference between groups with respect to prevalence of positive prick tests (atopy), diagnosis of nasal polyposis or number of polypectomies.

Patients with SRA had significantly higher total serum IgE and plasma ECP concentrations when compared to non-SA (Fig. 2A, B). Furthermore, ECP plasma levels were weakly associated with FEV₁ and with blood eosinophilia (Table 3).

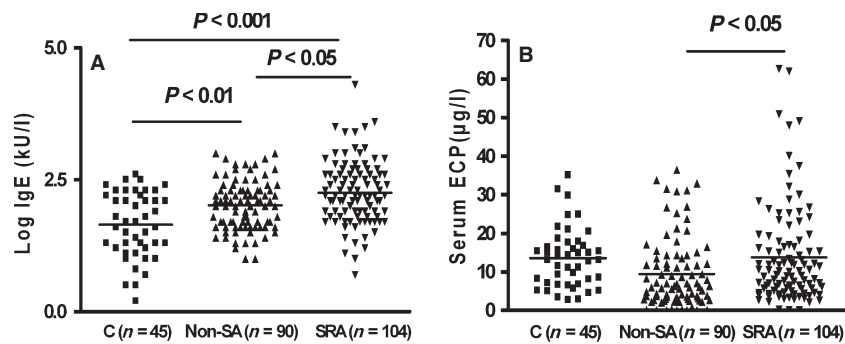


Figure 2 Total IgE (A) and eosinophil cationic protein (B) levels in serum of healthy subjects (controls), patients with nonsevere asthma (non-SA) and patients with severe refractory asthma. Data

are expressed in means after a Dunn's Multiple Comparison Test, and P -values lower than 0.05 are considered as significance.

Table 3 Associations of antibodies against SEs, total IgE and ECP in serum with clinical parameters (analysis of regression; R^2 – determination coefficients)

	Specific IgE to SEs		Total IgE		ECP	
	R^2	P -value	R^2	P -value	R^2	P -value
Age	0.022	<0.04	0.002	NS	0.012	NS
FEV ₁ % of predicted	0.071	<0.005	0.036	<0.01	0.025	<0.03
FEV ₁ /FVC	0.116	<0.005	0.039	<0.01	0.018	NS
MEF 25/75% of predicted	0.043	<0.005	0.01	NS	0.015	NS
Postbronchodilator increase in FEV ₁	0.100	<0.005	0.041	<0.01	0.001	NS

ECP, Eosinophil Cationic Protein.

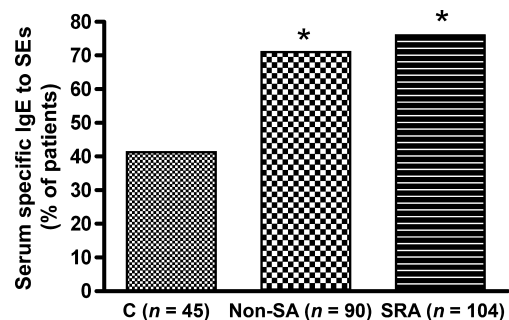


Figure 3 Percentage of subjects with detectable serum-specific IgE antibodies to SEs among healthy subjects (controls), patients with nonsevere asthma (non-SA) and severe refractory asthma; * P < 0.05 significantly different from controls (Pearson's chi-square test).

Determination of specific IgE to SEs was performed in 194 patients with asthma and in 29 control patients. The sensitization rate to SEs was significantly higher among patients with asthma (76.1% and 71.1% in SRA and non-SA respectively) when compared to healthy controls (41.3%) as shown in Fig. 3. Although the sensitization rate was not significantly higher in SRA when compared to non-SA, the presence of specific IgE in serum tended to increase the risk for severe asthma [OR = 1.94 (0.995–3.788) P = 0.052].

With regression analysis, considering age as confounding factor, the presence of IgE to SEs was significantly associated with the following asthma severity markers: low respiratory function parameters (FEV₁, FEV₁/FVC, and MEF 25/75), increased bronchial response to albuterol (Table 3) and higher serum total IgE level (R^2 = 0.29; P < 0.005) in the whole group of patients with asthma.

Furthermore, the mean level of specific IgE to SEs was 3-fold higher in patients with SRA when compared to non-SA patients (1.39 ± 0.30 vs 0.38 ± 0.07 ; P = 0.01). The mean concentration of specific IgE in both groups of asthmatics was significantly higher when compared to control subjects without asthma. (Fig. 4).

Asthmatic patients with and without serum-specific IgE to SEs

Among 194 asthmatics, 147 patients (i.e. 75.8%) had detectable specific IgE to SEs in serum with a mean level of 0.92 ± 0.17 kU/l. The mean age of patients with detectable serum-specific IgE to SEs was significantly lower (43.9 ± 1.33 years) when compared to patients without SEs immune response (49.3 ± 2.43 years; P = 0.04) but both groups did not differ with respect to any other demographic characteristics including gender, presence of atopy, history of aspirin sensitivity, nasal polyps and number of polypectomies. Exacerbation rates, mean dose of inhaled GCS, mean pulmonary function parameters (FEV₁, MEF 25–75) and bronchodilator response to albuterol (delta FEV₁%-l) were also similar in both subgroups of asthmatics.

The mean serum total IgE levels were significantly higher in SE (+) when compared to SE (–) patients [187 kU/l (156–224) vs 50 kU/l (38.6–65 kU/l); P = 0.001]. Total IgE level was associated with serum concentrations of sIgE to SEs (P < 0.005), and the presence of specific IgE to SEs carried a significant risk for patients to have serum total IgE levels above 100 kU/l [OR = 7.84 (95% CI 3.65–16.81)] P < 0.005.

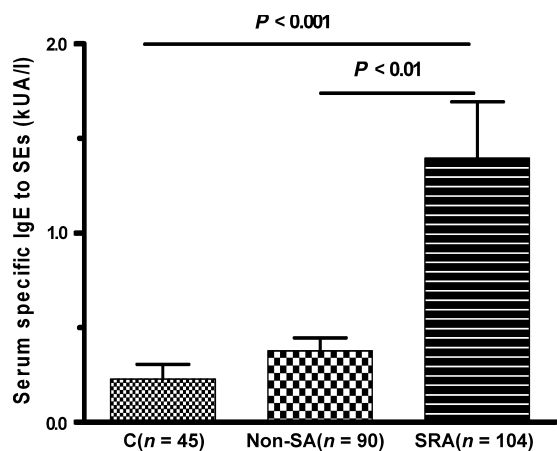


Figure 4 Specific IgE antibodies against SEs in serum of healthy subjects (controls), patients with nonsevere asthma (non-SA) and patients with severe refractory asthma. Data are expressed in means after a Dunn's Multiple Comparison Test, and *P*-values lower than 0.05 are considered as significance.

Patients with SRA and serum-specific IgE to SEs had significantly higher blood eosinophilia when compared to severe asthmatics without SEs sensitization (327 ± 30.5 vs 191.8 ± 32.9 cells/mm³; $P < 0.005$). However, serum concentration of IgE to SEs was associated (regression analysis) with blood eosinophilia only in patients with nonsevere asthma ($P = 0.01$).

The mean concentration and prevalence of specific IgE to SEs were not different if patients with asthma when stratified according to the presence of atopy, aspirin sensitivity or nasal polyps (Table 4).

Discussion

In our patients' population, as opposed to most previous studies, asthma severity was assessed after at least 12 months of follow-up and treatment, and patients were under constant control of asthma specialists. Difficult to control nature of

asthma in the study group was stressed by the fact that despite close medical supervision and high degree treatment with anti-inflammatory drugs, the mean number of exacerbations approached 2 per year. Our observations confirm limited effectiveness of available medications to prevent asthma exacerbations in a subpopulation of difficult to treat asthmatics (20).

In our patients population, a history of wheezing in childhood, female gender, hypersensitivity to aspirin or high BMI ($> 35\%$) carried a significant risk for difficult asthma. These risk factors are in agreement with some but not all previous studies, and the differences between these studies may reflect both genetic and environmental differences between the populations studied as well as the definitions of severe asthma used (4–6, 21).

In contrast to some other studies, our patients with severe and nonsevere asthma had similar and relatively high (above 70%) prevalence of atopy defined as the presence of at least one positive skin prick test to common inhalant allergens (5, 22, 23), indicating that allergic sensitization, either alone or through interaction with other environmental factors may contribute to asthma course regardless of the disease severity.

Patients with SRA had not only lower respiratory function parameters (FEV₁) reflecting obstruction of large airways, but in addition their mean mid expiratory flow rate (MEFR 25–75%), which is thought to reflect involvement of small airways (< 2 mm diameter), was on average almost two times lower than in patients with nonsevere asthma. This observation confirms that involvement of small airways is an important feature of severe difficult to control asthma and may contribute to poorer control of these patients when compared to those with nonsevere asthma (24). On the other hand, bronchial obstruction in severe refractory asthmatics was largely reversible with nebulized salbutamol. High prevalence and degree of the airway reversibility in severe asthmatics confirms previous observations that smooth muscle constriction is an important component of chronic bronchial obstruction in these patients.

Total IgE levels were significantly higher in severe asthmatics when compared to nonsevere asthmatics despite the fact that the prevalence of allergic sensitization was not dif-

Table 4 Comparison of serum anti-SE antibodies and immunological parameters in patients with various asthma phenotypes (mean values $\pm$ SEM)

	Number of patients	Specific IgE to SEs(kU/l)	Total IgE(kU/l)	ECP(μ g/l)	Blood eosinophilia (cells/mm ³)
Aspirin hypersensitive	58	1.0 ± 0.25	118.5 (82–170)	14.44 ± 1.66	$345.8 \pm 39.83^{\#}$
Aspirin tolerant	152	0.84 ± 0.19	142.6 (117–173)	11.46 ± 1.02	248.8 ± 16.5
Nasal Polyps	52	0.82 ± 0.25	94.6 (71–125) ^{&}	15.02 ± 1.8	$343.8 \pm 42.9^{\&\&}$
No nasal polyps	158	0.93 ± 0.19	154.8 (126–190)	11.44 ± 0.1	251.2 ± 16.2
Atopic	161	0.85 ± 0.18	154.8 (128–187) ^{&}	12.52 ± 1.05	275.2 ± 17.8
Nonatopic	49	1.02 ± 0.3	89.7 (62–130)	11.63 ± 1.52	283 ± 39.3

[#] $P < 0.01$ when compared to aspirin-tolerant group (one way ANOVA analysis).

[&] $P < 0.05$ when compared to non-nasal polyps group.

^{&&} $P < 0.01$ when compared to non-nasal polyps group.

ECP, Eosinophil Cationic Protein.

ferent between groups. Total IgE was associated not only with SRA phenotype but clearly with respiratory function parameters and bronchodilator responsiveness to salbutamol, suggesting that pathophysiological associations between serum total IgE levels and reversible airway obstruction exist, particularly among most patients with severe asthma (25).

The mechanisms underlying difficult to treat asthma are complex and have not been fully elucidated (1, 6). Although genetic predisposition to severe asthma phenotype seems to be important, influence of environmental and infectious (viruses and bacteria) factors may have crucial impact in the degree of asthma severity. *Staphylococcus aureus*-derived enterotoxins (SEs) are a group of molecules with superantigenic activity and potent stimulatory effect on T-cell, eosinophils, neutrophils and other inflammatory cells involved in asthmatic inflammation (9). Presence of specific IgE to SEs has been originally associated with the development of eosinophilic inflammation in nasal polyps but also with allergic rhinitis and atopic dermatitis (26–28).

Specific IgE antibodies to SEs may be present in sera of apparently healthy subjects; however, both colonization rates and antibody titres are significantly increased in patients with chronic upper airway inflammatory diseases (rhinosinusitis, nasal polyposis and allergic rhinitis) and are related to other markers of eosinophilic inflammation (ECP, IL-5 and eotaxin) (8, 12). In this study, we confirmed that patients with asthma have increased rate of sensitization to Staphylococcal enterotoxins when compared to healthy controls.

Weak association of the presence of sIgE to SEs with defined according to the ATS criteria severe asthma phenotype is balanced by strong associations of SEs with individual severe asthma features. Regression analysis confirmed that increased concentrations specific IgE to SEs were significantly associated with lower respiratory function parameters (reflecting obstruction in both large and small airways), which are good markers of asthma severity. The most striking observation was the 3-fold increase in serum level of anti-SEs antibodies in severe refractory asthmatics when compared to nonsevere asthmatic subjects indicating that not sensitization itself but rather the degree of immunological responsiveness to SEs antigens was reflective of more severe airway disease. It has been reported that asthma severity is associated with

increased total serum IgE, and treatment with anti-IgE antibodies seems to be particularly effective in the most severe asthma (29, 30). Our study confirmed the previously reported strong relationship between the titre of SEs-specific IgE and total serum IgE (24). Asthmatic patients with immune response to SEs had five times higher total IgE levels when compared to anti-SEs negative patients, and the presence of specific IgE to the enterotoxins carried a significant risk (OR = 7.84) for patients to have increased (above 100 kU/l) serum total IgE level. These data strongly suggest a pathophysiological role of immunological response to Staphylococcal enterotoxins in boosting IgE generation, offering a potential link between sensitization to SEs, asthma severity and asthma control.

Nasal polyposis and rhinosinusitis in patients with aspirin sensitivity are characterized by highly intense tissue eosinophilia, and earlier studies demonstrated increased anti-SEs-specific IgE in nasal polyps of these patients (25, 31, 32). In this study, the presence of aspirin sensitivity was associated with significant risk for having severe asthma, but similarly to the study of Lee et al. (33), the prevalence and concentration of anti-SEs IgE in serum of aspirin-sensitive asthmatics were not significantly increased when compared to aspirin-tolerant patients, despite higher blood eosinophilia in the former group. It may suggest that increased immune response to SE antigens may represent a local mechanism related to aspirin-sensitive rhinosinusitis/polyposis rather than bronchial asthma.

In conclusion, our study demonstrated that patients with severe/difficult asthma phenotype have distinct clinical and immunological characteristics. Sensitization to Staphylococcal enterotoxins and multiclonal IgE synthesis are associated with total IgE production and may play a role in the pathogenesis of asthma severity.

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References

- Chanez P, Wenzel SE, Anderson GP, Anto JM, Bel EH, Boulet LP et al. Severe asthma in adults: what are the important questions? *J Allergy Clin Immunol* 2007;**119**:1337–1348.
- Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. American Thoracic Society. *Am J Respir Crit Care Med* 2000;**162**: 2341–2351.
- Dockrell M, Partridge MR, Valovirta E. The limitations of severe asthma: the results of a European survey. *Allergy* 2007;**62**:134–141.
- 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**:470–477.
- Moore WC, Bleecker ER, Curran-Everett D, Erzurum 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**:405–413.
- Humbert M. The many faces of severe asthma. *Allergy* 2008;**63**:489–491.
- Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999;**160**:1001–1008.
- Makowska S, Grzegorzczak J, Cieslak M, Bienkiewicz B, Kowalski ML. Recruitment

- of CD34+ progenitor cells into peripheral blood and asthma severity. *Ann Allergy Asthma Immunol* 2008;**101**:402–406.
9. Wenzel SE, Busse WW, the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Severe asthma: lessons from the severe asthma research program. *J Allergy Clin Immunol* 2007;**119**:14–21.
 10. Bachert C, Zhang N, Patou J, van Zele T, Gevaert P. Role of staphylococcal superantigens in upper airway disease. *Curr Opin Allergy Clin Immunol* 2008;**8**:34–38.
 11. Rossi RE, Monasterolo G. Prevalence of serum IgE antibodies to the Staphylococcus aureus enterotoxins (SAE, SEB, SEC, SED, TSST-1) in patients with persistent allergic rhinitis. *Int Arch Allergy Immunol* 2004;**133**:261–266.
 12. Tomi NS, Kränke B, Aberer E. Staphylococcal toxins in patients with psoriasis, atopic dermatitis, and erythroderma, and in healthy control subjects. *J Am Acad Dermatol* 2005;**53**:67–72.
 13. Patou J, Gevaert P, Van Zele T, 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**:110–115.
 14. Veler H, Hu A, Fatma S, Grunstein JS, DeStephan CM, Campbell D et al. Superantigen presentation by airway smooth muscle to CD4+ T lymphocytes elicits reciprocal proasthmatic changes in airway function. *J Immunol* 2007;**178**:3627–3636.
 15. Bachert C, Gevaert P, Howarth P, Holtappels G, van Cauwenberge P, Johansson SG. IgE to Staphylococcus aureus enterotoxins in serum is related to severity of asthma. *J Allergy Clin Immunol* 2003;**111**:1131–1132.
 16. Barnes PJ. Intrinsic asthma: not so different from allergic asthma but driven by superantigens? *Clin Exp Allergy* 2009;**39**:1145–1151.
 17. The Global Initiative for Asthma (GINA 2002) <http://www.ginasthma.com>
 18. Nizankowska-Mogilnicka E, Bochenek G, Mastalerz L, Swierczyńska M, Picado C, Scadding G et al. EAACI/GA2LEN guideline: aspirin provocation tests for diagnosis of aspirin hypersensitivity. *Allergy* 2007;**62**:1111–1118.
 19. Shaheen S, Potts J, Gnatiuc L, Kowalski ML, Joos G, van Zele T et al. The relation between paracetamol use and asthma: a GA2LEN European case-control study. *Eur Respir J* 2008;**32**:1231–1236.
 20. Holgate ST, Polosa R. The mechanisms, diagnosis, and management of severe asthma in adults. *Lancet* 2006;**368**:780–793.
 21. Moore WC, Peters SP. Severe asthma: an overview. *J Allergy Clin Immunol* 2006;**117**:487–494.
 22. Sullivan SD, Wenzel SE, Bresnahan BW, Zheng B, Lee JH, Pritchard M et al. Association of control and risk of severe asthma-related events in severe or difficult-to-treat asthma patients. *Allergy* 2007;**62**:655–660.
 23. Haselkorn T, Borish L, Miller DP, Weiss ST, Wong DA. High prevalence of skin test positivity in severe or difficult-to-treat asthma. *J Asthma* 2006;**43**:745–752.
 24. Balzar S, Chu HW, Strand M, Wenzel S. Relationship of small airway chymase-positive mast cells and lung function in severe asthma. *Am J Respir Crit Care Med* 2005;**171**:431–439.
 25. Naqvi M, Choudhry S, Tsai HJ, Thyne S, Navarro D, Nazario S et al. Association between IgE levels and asthma severity among African American, Mexican, and Puerto Rican patients with asthma. *J Allergy Clin Immunol* 2007;**120**:137–143.
 26. 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**:607–614.
 27. Pérez-Novo CA, Kowalski ML, Kuna P, Ptasińska A, Holtappels 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**:255–260.
 28. Lee JH, Lin YT, Yang YH, Wang LC, Chiang BL. Increased levels of serum-specific immunoglobulin e to staphylococcal enterotoxin a and B in patients with allergic rhinitis and bronchial asthma. *Int Arch Allergy Immunol* 2005;**138**:305–311.
 29. Humbert M, Beasley R, Ayres J, Slavin R, Hébert J, Bousquet J et al. Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. *Allergy* 2005;**60**:309–316.
 30. Holgate S, Buhl R, Bousquet J, Smith N, Panahloo Z, Jimenez P. The use of omalizumab in the treatment of severe allergic asthma: a clinical experience update. *Respir Med* 2009;**103**:1098–1113.
 31. Suh YJ, Yoon SH, Sampson AP, Kim HJ, Kim SH, Nahm DH et al. Specific immunoglobulin E for staphylococcal enterotoxins in nasal polyps from patients with aspirin-intolerant asthma. *Clin Exp Allergy* 2004;**34**:1270–1275.
 32. Kowalski ML. Aspirin-sensitive rhinosinusitis and asthma. *Clin Allergy Immunol* 2007;**19**:147–175.
 33. Lee JY, Kim HM, Ye YM, Bahn JW, Suh CH, Nahm D et al. Role of staphylococcal superantigen-specific IgE antibodies in aspirin-intolerant asthma. *Allergy Asthma Proc* 2006;**27**:341–346.