

Staphylococcus aureus sensitization and allergic disease in early childhood: Population-based birth cohort study

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Background: *Staphylococcus aureus*-secreted enterotoxins (SEs) may be involved in the pathophysiology of atopic diseases.

Objective: We investigated the role of SEs in allergic diseases during early childhood (using the mixture of SE-specific IgEs [SE-mix] as a marker).

Methods: Children (N = 510) were followed from birth to age 5 years (repeated questionnaires, IgE to inhalant and food allergens, lung function [spirometry, plethysmography], airway reactivity [dry air challenge]). We measured SE-mix specific IgE (SE-A, SE-C, toxic shock syndrome toxin 1) by using fluorescence immunoassay.

Results: We found no association between rhinitis and SE-mix sensitization. Children with eczema were more frequently SE-mix-sensitized than children without (17.4% vs 8.3%; $P = .02$). SE-mix sensitization rate increased significantly with increasing eczema severity (no eczema, mild, moderate/severe: 8.3%, 14.8%, 42.9%; $P = .003$) and remained independently associated with eczema in a multivariate model adjusting for total IgE (adjusted odds ratio, 2.19; 95% CI, 1.05-4.56; $P = .04$). SE-mix sensitization was associated with current wheeze in the univariate but not the multivariate model. Among wheeze phenotypes, persistent wheezers were most commonly sensitized to SE-mix (never, transient, late-onset, persistent: 8.5%, 3.8%, 7.7%, 17.6%; $P = .05$). Among wheezers, those SE-mix-sensitized had significantly higher airway reactivity compared with those nonsensitized (mean FEV₁ change, mL [95% CI]: -59 [-121, 3] vs 19 [-10.2, 48.9]; $P = .04$), with little difference after adjusting for atopy.

Conclusion: We found differences in SE-mix IgE antibodies between healthy 5-year-old children and children with eczema and wheeze. The proportion of patients sensitized to SE-mix increases with increasing disease severity.

Clinical implications: Staphylococcal enterotoxins are potential modifiers of childhood wheeze and eczema. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: *Staphylococcus aureus*, superantigens, early life, wheeze, eczema

Staphylococcus aureus is a human pathogen responsible for a variety of clinical diseases. There is an increased interest in strains secreting enterotoxins and their potential role in the pathophysiology of atopic diseases. *S aureus*-secreted enterotoxins (SEs) are a large protein family composed of SEs A to Q and toxic shock syndrome toxin 1 (TSST-1), which may act as superantigens. Once bound to the T-cell receptor, these superantigens may activate 20% of the naive T-cell population (whereas conventional antigens stimulate only 0.01%¹). They bypass the common antigen presentation mechanisms and act by cross-linking the MHC class II molecule on antigen-presenting cells with a variable V β region of the T-cell receptor. Both murine models and human studies have demonstrated T_H2-type cytokine secretion and polyclonal IgE synthesis in response to SE stimulation.²⁻⁴

Skin of patients with eczema is frequently colonized by enterotoxin-excreting *S aureus* strains,⁵⁻⁷ which can cause higher disease activity compared with nontoxigenic strains.⁵ Direct application of SE-B to skin elicits local inflammatory response in both healthy subjects and subjects with eczema.⁸

Adults with allergic rhinitis are 4 times more commonly sensitized to SEs than healthy controls (25% vs 6.3%).⁹ Moreover, rhinitis severity increases in SE-sensitized compared with nonsensitized patients.^{10,11} SE sensitization in patients with mite allergy with rhinitis may contribute to a higher mite-specific IgE response.¹⁰

There is a growing evidence that SEs may be implicated in the pathogenesis of asthma.^{12,13} Adult patients with asthma have higher specific IgE levels to mixture of SE-specific IgEs (SE-mix) than controls, and the level of SE-mix-specific IgE correlates with severity of symptoms.¹² Patients with poorly controlled asthma had higher expression of V β + 8T cells in bronchoalveolar lavage fluid compared with patients with well controlled asthma.¹⁴ Patients with allergic asthma have higher SE-A and SE-B specific IgE compared with patients who have only eczema or rhinitis.¹³ Finally, SEs aggravate sinonasal polyp disease, characterized by a polyclonal IgE

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Abbreviations used

MAAS: Manchester Asthma and Allergy Study
 SE: *Staphylococcus aureus*-secreted enterotoxin
 SE-mix: Mixture of SE-specific IgEs
 sR_{aw}: Specific airway resistance
 TSST-1: Toxic shock syndrome toxin 1

formation, a pronounced eosinophilic inflammation, and an increased likelihood for comorbid asthma.¹⁵

These findings confirm that staphylococcal enterotoxins have a role in pathogenesis of allergic and airway diseases. However, the majority of data on the relationship between SEs and atopic disease has been obtained in studies conducted in adults, whereas there is very little information on their association with allergic disease in childhood. Within the context of a population-based birth cohort study (Manchester Asthma and Allergy Study [MAAS]), we investigated the potential role of SEs in atopic disease during early childhood by using SE-specific IgE as a marker.

METHODS**Study population**

The MAAS is an unselected population-based birth cohort described in detail elsewhere.^{16,17} Briefly, subjects were recruited from the antenatal clinics within the first trimester of the pregnancy when all pregnant women were screened for eligibility. Both parents completed a questionnaire and skin testing to common inhalant allergens. Multiple births were excluded. Children were followed prospectively and attended review clinics at ages 3 and 5 years (within 4 weeks of birthday). The study was approved by the Local Research Ethics Committee. Written informed consent was obtained from subjects' families, and children gave their assent.

Outcomes

A validated questionnaire¹⁸ was interviewer-administered to collect information on parentally reported symptoms, physician-diagnosed illnesses, and treatments received.

Rhinitis was defined as a runny or blocked nose when the child did not have a cold or chest infection. Questions included the presence of symptoms in the past 12 months (current rhinitis) and during lifetime (rhinitis ever).

Eczema was defined as an itchy rash coming and going for at least 6 months. The diagnosis was confirmed by physical examination at the 5-year follow-up.

Children were defined as having current wheeze if they had wheezing in the previous 12 months at age 5 years. In addition, we assigned children into different wheeze phenotypes according to history of wheeze at the 2 follow-ups¹⁹: no wheezing: no wheeze during the first 3 years of life, no wheezing ever by age 5 years; transient early wheeze: wheezing during the first 3 years, no wheezing in the previous 12 months at age 5 years; late-onset wheezing: no wheeze during the first 3 years, wheezing in the previous 12 months at age 5 years; and persistent wheezing: wheezing during the first three years, wheezing in the previous 12 months at age 5 years.

Specific airway resistance (sR_{aw}) was measured at age 5 years by using plethysmography as previously described.^{17,20,21} Three measurements of sR_{aw} were performed, and each was calculated from

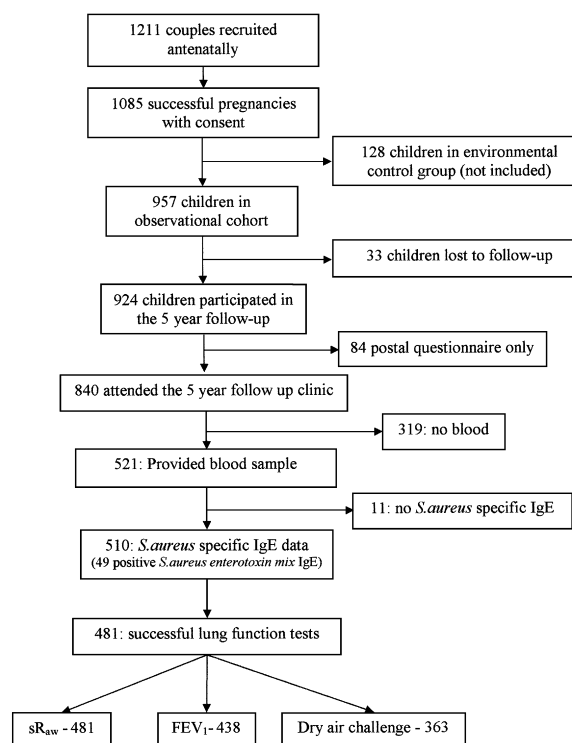


FIG 1. Study profile.

the means of 5 consecutively measured technically acceptable loops. The median of these 3 measurements of effective sR_{aw} was used in the analysis. Children were asymptomatic at the time of lung function assessment.

Dynamic lung volumes and expiratory flow were measured using incentive animation software. The test was repeated at intervals of 30 seconds until 3 technically acceptable traces were obtained and the highest FEV₁ was recorded.

Airway reactivity was assessed by eucapnic voluntary hyperventilation challenge.²⁰ Subjects hyperventilated gas containing 21% O₂, 5% CO₂, and remainder N₂ with a water content of less than 10 mg/L for 6 minutes at a ventilation rate of 75% of maximum voluntary ventilation. The lowest FEV₁ value measured at 2, 5, or 10 minutes after challenge was recorded, and the response was expressed as change in FEV₁.

Sensitization was ascertained by the measurement of specific IgE to mite, cat, dog, grasses, milk, egg and peanut (ImmunoCAP; Phadia, Uppsala, Sweden) in serum collected at age 5 years. Sensitization was defined as allergen-specific IgE > 0.35 kU_A/L.

IgE to staphylococcal enterotoxins

The serum samples were assayed for specific IgE to staphylococcal enterotoxins. Specific IgE was measured using a fluorescence immunoassay (UniCap 100; Phadia) according to the manufacturer's recommendations. A mix of SEs consisting of SE-A, SE-C, and TSST-1 (SE-mix) was determined as described before¹²; sensitization was defined as specific IgE level > 0.35 kU_A/L.

Statistical methods

Statistical analysis was performed by using SPSS for Windows version 13.0 (SPSS Inc, Chicago, Ill). Appropriate univariate tests (*t* test and χ^2 test) were used to investigate the differences between children with and without SE-mix sensitization. Further analysis was

TABLE I. SE-mix sensitization rate among children and atopic disease outcomes

	Whole population			Atopic children only		
	SE-mix sensitization		<i>P</i> value	SE-mix sensitization		<i>P</i> value
	<i>n</i>	Percent (<i>n</i>)		<i>n</i>	Percent (<i>n</i>)	
Rhinitis ever, yes	153	9.8% (15)	.9	76	14.5% (11)	.3
No	349	9.5% (33)		100	21% (21)	
Rhinitis current, yes	143	9.8% (14)	.8	70	14.3% (10)	.3
No	357	9.2% (33)		106	20.8% (22)	
Eczema ever, yes	223	11.7% (26)	.2	104	20.2% (21)	.4
No	280	7.9% (22)		72	15.3% (11)	
Eczema current, yes	153	12.4% (19)	.3	77	22.1% (17)	.2
No	348	8.0% (28)		98	14.3% (14)	
Doctor diagnosed eczema, yes	193	11.4% (22)	.3	92	20.7% (19)	.4
No	309	8.4% (26)		83	15.7% (13)	
Eczema at examination, yes	69	17.4% (12)	.02	36	30.6% (11)	.03
No	432	8.3% (36)		139	15.1% (21)	
Eczema score*						
No eczema	432	8.3% (36)	.003	139	15.1% (21)	.02
Mild	61	14.8% (9)		29	27.6% (8)	
Moderate/severe	7	42.9% (3)		6	50% (3)	
Wheeze ever, yes	196	12.8% (25)	.05	85	23.5% (20)	.08
No	307	7.5% (23)		91	13.2% (12)	
Wheeze current, yes	111	14.4% (16)	.05	57	22.8% (13)	.3
No	392	8.2% (32)		119	16% (19)	
Wheeze phenotypes*						
Never wheezed	248	8.5% (21)	.05	72	13.9% (10)	.09
Transient wheeze	105	3.8% (4)		31	9.7% (3)	
Late-onset wheeze	26	7.7% (2)		11	9.1% (1)	
Persistent wheeze	68	17.6% (12)		36	27.8% (10)	
Asthma ever, yes	101	14.9% (15)	.04	46	28.3% (13)	.04
No	401	8.2% (33)		129	14.7% (19)	

*Linear-by-linear association.

performed by using logistic regression. sR_{aw} measurements were subject to a $\log_e$ -transformation; the results are presented as geometric means and 95% CIs.

RESULTS

Participant flow

Study profile is shown in Fig 1. Of 1211 couples who initially agreed to take part, 1085 had a successful full-term pregnancy (>36 weeks gestation; child did not need intensive care) and gave consent to a further follow-up. Of those, 128 were prenatally randomized to environmental control and excluded from this analysis; 957 were followed in the observational cohort. Of the children in the observational cohort, 840 (87.8%) attended the 5-year follow-up (a further 84 returned postal questionnaires). A total of 521 children (62%) provided a blood sample; a sufficient sample for SE-mix IgE determination was available in 510. Children excluded did not differ from those included in terms of family history, parental smoking, maternal age, socioeconomic status, gestational age, birth weight, history of wheeze, and atopic status.

Overall, 49 of 510 children (9.6%) had SE-mix IgE > 0.35 kU_A/L. Boys were more frequently SE-mix-sensitized than girls (39/283 [13.8%] vs 10/227 [4.4%]; $P <$

.001). Among 495 children who had full IgE data for both SE-mix and common inhalant allergens, atopic children were nearly 4 times more commonly SE-mix-sensitive than nonatopic children (19.2% vs 5.0%; $P <$.0001).

Specific IgE SE-mix and clinically expressed symptoms suggestive of allergic disease

Analyses on the relationship between SE-mix sensitization and clinically expressed allergic diseases were conducted among the whole study population and atopic children only; data are summarized in Table I.

Rhinitis. There was no association between rhinitis ever or current rhinitis and SE-mix sensitization, either in a whole population or among atopic children only.

Eczema. There was no significant difference in SE-mix sensitization between children with physician-diagnosed eczema ever or current eczema compared with those without. However, SE-mix IgE was more commonly detected among children who had eczema confirmed at physical examination compared with those without signs of eczema (17.4% vs 8.3%; $P = .02$). Moreover, the proportion of children sensitized to SE-mix increased significantly with increasing eczema severity (no eczema, mild, moderate/severe: 8.3%, 14.8%, 42.9%; $P = .003$;

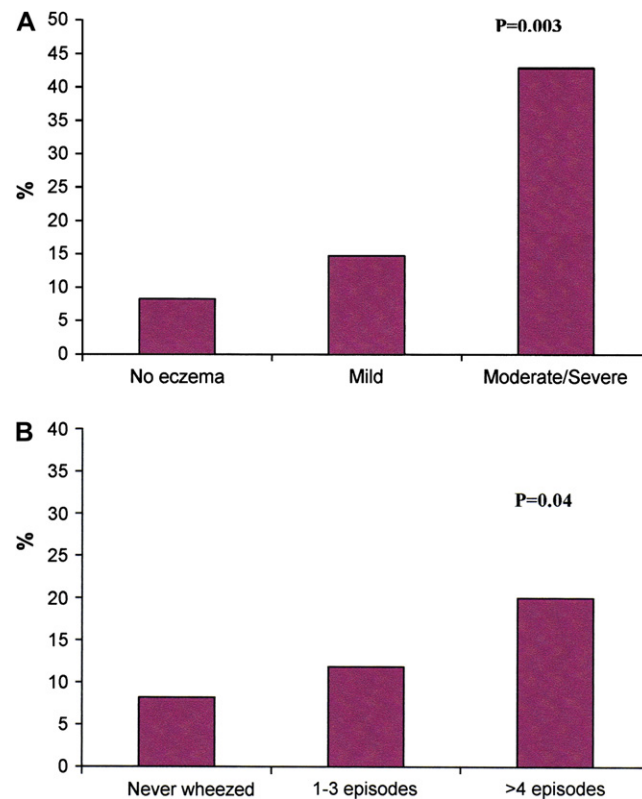


FIG 2. A, SE-mix sensitization and eczema severity in the whole population. B, SE-mix sensitization and the frequency of wheezy episodes within the previous 12 months in the whole population.

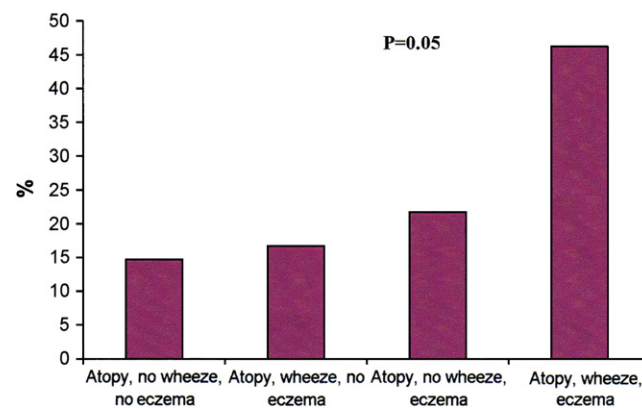


FIG 3. SE-mix sensitization, current wheeze, and eczema among atopic children.

Fig 2, A). Similar associations were observed among atopic children only (eczema vs no eczema: 30.6% vs 15.1%, $P = .03$; no eczema, mild, moderate/severe eczema: 15.1%, 27.6%, 50%, $P = .02$).

Further analysis of the association between SE-mix sensitization and eczema confirmed at physical examination was performed by using logistic regression. In the univariate model, SE-mix sensitization was a significant associate of eczema (odds ratio, 2.31; 95% CI, 1.14-4.71; $P = .02$). In a multiple logistic regression analysis,

adjusting for maternal eczema, paternal eczema, eczema among siblings, and the level of total serum IgE, SE-mix sensitization remained significantly and independently associated with confirmed eczema, with the strength of the association being practically unchanged (aOR, 2.19; 95% CI, 1.05-4.56; $P = .04$). In a further multivariate model, we explored the interaction between the SE-mix sensitization and total IgE; this interaction was the only significant and independent association with eczema (aOR, 1.18; 95% CI, 1.03-1.35; $P = .02$).

Wheeze. Among 503 children with complete data on wheeze ever, significantly more children who had wheezed ever were sensitized to SE-mix compared with those who had never wheezed (12.8% vs 7.5%; $P = .05$). Similarly, current wheezers were more frequently sensitized to SE-mix (14.4% vs 8.2%, wheezers vs nonwheezers; $P = .05$), as were the children with a physician-diagnosed asthma (14.9% vs 8.2%; $P = .04$).

Amongst atopic children ($n = 176$), those with wheeze ever tended to have higher sensitization rate to SE-mix compared with never-wheezers, but this failed to reach statistical significance (23.5 % vs 13.2%; $P = .08$). Similar trends were observed for the other wheeze outcomes (Table I).

In the univariate logistic regression analysis, SE-mix sensitization was a significant associate of current wheeze (OR, 1.90; 95% CI, 1.00-3.60; $P = .05$). However, in a multivariate model, adjusting for maternal asthma, paternal asthma, child's eczema, and the total serum IgE, there was no significant and independent association between SE-mix sensitization and wheeze ($P = .35$); in this model, a significant association with current wheeze was total serum IgE, with the probability of current wheeze increasing 1.21-fold (95% CI, 1.06-1.39; $P = .004$) per logarithmic unit increase in the IgE levels.

SE-mix sensitization rate appeared to increase with increasing wheeze frequency (no wheeze during last year, 1-3 attacks/y, 4 or more attacks/y: 8.2%, 11.8%, 20%, respectively; $P = .04$; Fig 2, B).

Among atopic children, those who had both wheeze and eczema were significantly more commonly SE-mix-sensitized compared with those with neither or only 1 of these features ($P = .05$; Fig 3).

Wheeze phenotypes. Using never-wheezers as a reference, SE-mix sensitization rate was significantly more prevalent among the persistent wheezers, but not late-onset or transient early wheezers (never, early transient, late-onset, persistent: 8.5%, 3.8%, 7.7%, 17.6%, respectively; $P = .05$). This association strengthened in the multiple ANOVA model adjusted for confirmed eczema ($F = 8.22$; $P = .004$), with the other significant and independent associates being confirmed eczema ($F = 6.36$; $P = .01$) and the interaction between the SE-mix sensitization rate and confirmed eczema ($F = 6.02$; $P = .02$).

Specific IgE to SE-mix, lung function, and airway reactivity

Data on sR_{aw} and FEV_1 at age 5 years were available for 481 and 438 children, respectively. There was no significant difference in the baseline lung function (both sR_{aw} and FEV_1) or airway reactivity between subjects sensitized to SE-mix compared with those who were not sensitized (Table II).

However, SE-mix sensitization among current wheezers was associated with significantly higher airway reactivity (sensitized vs nonsensitized wheezers, mean change in FEV_1 post dry air challenge in mL [95% CI]: -59 [$-121, 3$] vs 19 [$-10.2, 48.9$], $P = .04$; FEV_1 % of

TABLE II. Lung function and airway reactivity results among SE-mix-sensitized and nonsensitized children

Outcome (n)	Mean	95% CI	P value
All children			
sR_{aw} , kPa/s (481)			
Not sensitized (435)	0.15	0.13, 0.16	.1
Sensitized (46)	0.19	0.14, 0.24	
FEV_1 (438)			
Not sensitized (397)	1.04	1.03, 1.06	.9
Sensitized (41)	1.04	0.98, 1.10	
BHR, FEV_1 change in mL (363)			
Not sensitized (329)	30.0	17.2, 42.8	.08
Sensitized (34)	-7.9	-61.6, 45.5	
BHR, FEV_1 % baseline change (363)			
Not sensitized (329)	3.59	2.32, 4.87	.2
Sensitized (34)	0.56	-4.61, 5.75	
Current wheezers			
sR_{aw} , kPa/s (105)			
Not sensitized (91)	0.23	0.1, 0.27	.4
Sensitized (14)	0.28	0.15, 0.41	
BHR, FEV_1 change in mL (73)			
Not sensitized (62)	19.4	-10.2, 48.9	.04
Sensitized (11)	-59.1	-121.1, 3.0	
BHR, FEV_1 % baseline change (73)			
Not sensitized (62)	2.48	0.41, 5.38	.03
Sensitized (11)	-5.51	-11.05, 0.15	
Atopic current wheezers			
sR_{aw} , kPa/s (55)			
Not sensitized (43)	0.22	0.14, 0.28	.7
Sensitized (12)	0.24	0.10, 0.37	
BHR, FEV_1 change in mL (40)			
Not sensitized (31)	21.3	-26.4, 69.0	.09
Sensitized (9)	-58.9	-120.6, 2.8	
BHR, FEV_1 baseline % change (40)			
Not sensitized (31)	3.03	-1.55, 7.61	.06
Sensitized (9)	-5.61	-10.75, 0.46	

BHR, Bronchial hyperreactivity.

change postchallenge mean in % [95% CI]: -5.5 [$-11, 0.2$] vs 2.5 [$0.4, 5.4$], $P = .03$; Fig 4). A similar but non-significant trend was observed among atopic wheezers ($P = .09$; Table II).

DISCUSSION

This is the first study to suggest the relationship between sensitization to SEs and the presence and severity of eczema and wheeze in preschool children. Furthermore, SE-mix-sensitized children with wheeze had significantly higher airway reactivity compared with wheezers not sensitized to SE-mix.

Limitations

Although retention in this population-based observational birth cohort is excellent, we analyzed data from just more than half of the subjects (mostly because the child refused venepuncture). However, we emphasize that there

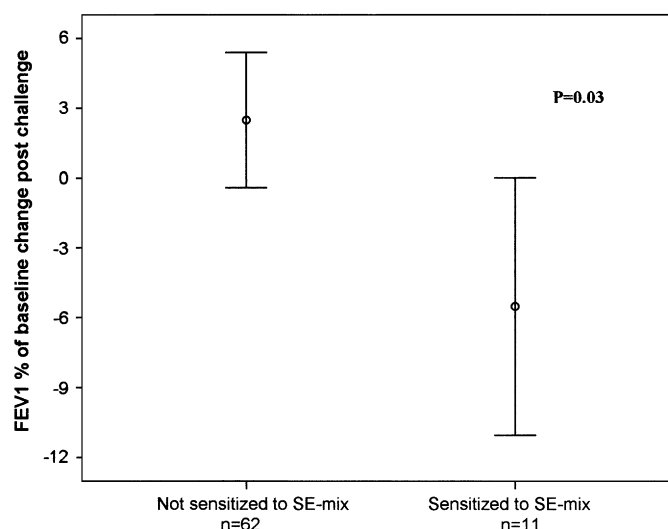


FIG 4. Bronchial hyperreactivity defined as postchallenge FEV₁ change in percentage among SE-mix-sensitized and nonsensitized wheezers (means and 95% CIs).

was no difference between children excluded or included in the analysis in any relevant parameter, suggesting that it is unlikely that the response rate to venepuncture has introduced bias. It is worth noting that the prevalence of allergic sensitization among the parents of the children is similar to that of young adults in the United Kingdom, suggesting the subjects are representative of the general population. Similar to the previous studies in adults, we used specific IgE antibodies directed against a mix of classic SEs as markers of the past or present immune reaction to staphylococcal superantigens and have not attempted to describe the basic functional mechanisms.

Comparison with other studies

A relationship between SEs and asthma was proposed by adult studies. Bachert et al¹² have recently reported that subjects who had severe asthma were at least 4 times more likely to be SE-mix-sensitized than controls without asthma. Furthermore, SE-mix sensitization rate was higher among patients with severe compared with mild asthma, suggesting a possible role of SEs in the severity of lower airway disease. It has been reported previously that in nasal polyps, an upper airway disease closely related to nonatopic asthma, eosinophil-related inflammation markers such as eosinophil cationic protein, IL-5, and cys-leukotrienes are significantly upregulated in SE-mix IgE positive versus negative disease.²² In a mouse model in which features of allergic asthma were induced by ovalbumin sensitization, both nasal and bronchial SE-B enhanced the allergen-induced bronchial inflammation, as reflected by more eosinophilic inflammation in the airway lumen and in bronchial tissue. Aggravation of experimental asthma correlated with higher expression of mRNA for IL-5, IL-4, and eotaxin-1, and with the upregulation of total IgE in serum.²³ Furthermore, it has recently been suggested that SE-B may effectively inhibit the regulatory T-cell's ability to suppress T-effector cell proliferation

via cell contact interaction,²⁴ explaining the relative deficit in controlling inflammation.

High SE-mix specific IgE levels that have been described in patients with chronic obstructive pulmonary disease decreased after hospitalization and appropriate treatment.²⁵ The decrease in SE-mix specific IgE levels in patients with chronic obstructive pulmonary disease was associated with improvement in disease severity, manifested by the increase in FEV₁. As indicated, experimental animal models may provide useful information of the possible mechanisms by which SEs affect the lower airways. Mice sensitized by intranasally applied SEs exhibit airway inflammation caused by inflammatory cell influx and cytokine release.²⁶ Correspondingly, bronchoalveolar lavage fluid of patients with severe asthma contains significantly higher levels of activated T cells compared with healthy controls.¹³

The current study found that eczema was significantly related to SE-mix sensitization among children at age 5 years. Although we found no association between SE-mix sensitization and parentally reported eczema, this sensitization was significantly more common among children who had eczema confirmed at physical examination compared with those without eczema, with the strength of this association unchanged after adjusting for total IgE levels and family history of eczema. This suggests that SEs may play an important role in the active skin disease and is further supported by the fact that SE-mix sensitization increased significantly with increasing eczema severity. This observation is in agreement with a previous study by Breuer et al²⁷ that found a direct correlation between Scoring Atopic Dermatitis scores and positive SE-B specific IgE levels, as well as a beneficial effect of antibiotic treatment on eczema severity.²⁸ Novak et al²⁹ reported that 53% of patients with intrinsic eczema were sensitized to various microbial components such as SE-A, SE-B, SE-C, SE-D, *Pityrosporum ovale*, or *Candida albicans*, indicating that in the absence of common inhalant and

food allergens, these microbial components may trigger an allergic immune response.

We have extended the observation of the association between SE-mix sensitization and the severity of allergic disease to the lower airway symptoms in young children. The proportion of children sensitized to SE-mix increased with increasing frequency of wheeze. Furthermore, among preschool wheeze phenotypes, persistent wheezers were most commonly sensitized to SE-mix, even after adjusting for the presence of eczema.

The association between SE-mix sensitization and the severity of the lower airway symptoms is further emphasized by our finding on the objective measure of airway reactivity. Although we did not detect any difference in the baseline lung function between SE-mix-sensitized and nonsensitized children, among children with current wheeze, those sensitized to SE-mix had significantly more severe airway reactivity than nonsensitized children.

CONCLUSION

We have demonstrated an increased sensitization rate to SEs in 5-year-old children with eczema and wheeze. Furthermore, the proportion of patients sensitized to SE-mix increases with increasing symptoms severity. Our data may suggest that SEs should be considered disease modifiers. Although mounting experimental evidence supports the hypothesis that the observed associations may be causative, our data cannot confirm or refute this notion, and the clinical relevance of the staphylococcal superantigens in the development of airway and skin disease needs further study.

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