

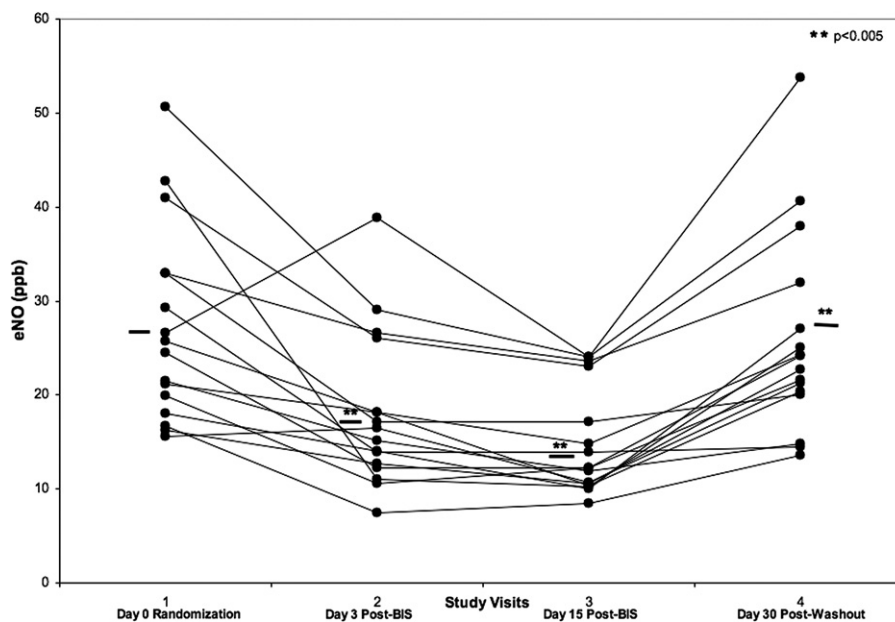


Figure 1. Exhaled nitric oxide (eNO) measurements in parts per billion in each asthmatic child at baseline, after budesonide inhalation suspension (BIS) (2 separate visits), and after washout. ** $P < .005$.

after objective control has been attained in young patients with atopic asthma. The future study of eNO to possibly identify breakthrough inflammatory control before changes in spirometry in young patients with atopic asthma would be ideal.

MIGUEL J. LANZ, MD
ANGELA P. BAUTISTA, BA
NATALIA M. PEYROU, MS
STEFANIA PRENDES, BS

AAADRS Clinical Research Center
Coral Gables, Florida
Miami Children's Hospital
Miami, Florida
mjlanz@pol.net

1. Moeller A, Diefenbacher C, Lehmann A, et al. Exhaled nitric oxide distinguishes between subgroups of preschool children with respiratory symptoms. *J Allergy Clin Immunol*. 2008;121:705–709.
2. Taylor DR. Nitric oxide as a clinical guide for asthma management. *J Allergy Clin Immunol*. 2006;117:259–262.
3. Basu K, Nair A, Williamson PA, et al. Airway and systemic effects of soluble and suspension formulations of nebulized budesonide in asthmatic children. *Ann Allergy*. 2009;103:436–441.
4. ATS/ERS recommendations for standardized procedures for the online and offline measurements of exhaled lower respiratory nitric oxide and nasal nitric oxide. *Am J Respir Crit Care Med*. 2005;171:912–930.
5. Buchvald F, Baraldi E, Carraro S, et al. Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years. *J Allergy Clin Immunol*. 2005;115:1130–1136.
6. Koopman M, Arets HGM, Uiterwaal CS, et al. Comparing 6 and 10 sec exhalation time in exhaled nitric oxide measurements in children. *Pediatr Pulmonol*. 2009;44:340–344.
7. Malmberg LP, Petays T, Hahtela T, et al. Exhaled nitric oxide in healthy nonatopic school-age children: Determinants and height-adjusted reference values. *Pediatr Pulmonol*. 2006;41:635–642.
8. Beck-Ripp J, Griesse M, Arenz S, Köring C, Pasqualoni B, Bufler P. Changes of exhaled nitric oxide during steroid treatment of childhood asthma. *Eur Respir J*. 2002;19:1015–1019.

PATTERN OF ALLERGEN-SPECIFIC IgE SENSITIZATION RELATIVE TO TOTAL SERUM IgE CONCENTRATION IN YOUNG ADULTS

Many studies^{1–3} have shown that total serum IgE concentrations are associated with the likelihood of having detectable allergen specific IgE. However, the relationship of sensitization to various allergens (ie, perennial, seasonal, and food) relative to total serum IgE concentration has not been studied. Our specific question was whether a relationship exists between increasing total IgE concentrations and the development of sensitization to different types of allergens. To examine this question, we analyzed the number of positive allergen specific IgE results for 10 common allergens for each fifth percentile of total IgE in young adults from a relatively large, geographically based, unselected birth cohort.

The selection of the study population has been previously described.^{4,5} Briefly, participants in this study were selected by location of residence and not by risk of allergic disease. Participants were enrolled as infants born between 1987 and 1989. The cohort was from the northern suburbs of Detroit, Michigan. For this study, 675 of the original 831 eligible individuals were contacted and completed a survey between 18 and 21 years of age. Total serum and allergen specific IgE concentrations were assayed in blood samples from 570 participants using the Pharmacia UniCAP system (Phadia, Portage, Michigan). Allergen specific IgE to dust mite (*Dermatophagoides farinae*), cat, dog, ragweed, *Alternaria*, timothy grass,

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peanut, cow milk, hen egg, and shrimp was measured. The laboratory performing the IgE testing was Clinical Laboratory Improvement Amendments certified, and regular quality assurance checks were performed, including repeating 1% of all samples. The coefficient of variation between runs averaged 5.9% for all allergens. As seen in Table 1, serum samples were not adequate to assay all allergens in all samples; therefore, the total number of samples tested varied by allergen. Allergen specific IgE concentrations of 0.35 IU/mL or higher were considered positive. Total serum IgE concentrations were stratified into percentiles (ie, 5th, 10th, 15th ... 100th percentile) based on the IgE concentrations in all participants, and the number of positive allergen specific IgE results for each allergen was determined for each 5th percentile. The institutional human subject review boards of both participating institutions approved all aspects of this study.

The median total IgE concentration was 40.75 IU/mL and the geometric mean $\pm$ SD was 38.4 $\pm$ 46.8 IU/mL for the 570 participants who had an adequate sample. Of the 675 participants, 320 were male and 630 were white. Among participants with specific IgE data, 93.3% were white compared with 92.5% of all participants ($P = .69$). Similarly, 47.6% of those with IgE data were male compared with 46.2% of those without these data ($P = .79$).

We observed interesting patterns of sensitization for perennial, seasonal, and food allergens occurring at different relative total IgE concentrations (Table 1). Specific IgE to dust mite and *Alternaria* were first seen in the lowest 10th percentile of total

serum IgE concentrations. In comparison, a positive result did not occur for ragweed until the 20th percentile and for timothy grass until the 25th percentile. Except for 2 responses to egg, positive responses to food allergens were not found until total serum IgE reached the 40th percentile. Specifically, up to the 40th percentile, only 2 of 49 positive specific IgE tests (4.1%) to all allergens were related to foods, whereas overall, 113 (14.1%) positive tests were specific for foods, and most (77.0%) positive food specific IgE test results were not found until total IgE was above the 60th percentile.

The main finding from this study is that sensitization to perennial, seasonal, and food allergens can be found at different relative total IgE levels. Specific IgE to perennial allergens is detectable at lower concentrations of total serum IgE compared with seasonal and food allergens (except egg). The rationale for this apparent sequence of sensitization in this cohort of young adults is not clear. Regarding the inhalant allergens, we speculate that the constant/long-term exposure to perennial allergens may explain why sensitization was seen at relatively lower total IgE concentrations compared with seasonal allergens. The age of this cohort may be the reason sensitization to food allergens was present at relatively higher total IgE concentrations because it is rare for "new" food sensitization to occur at this age or it may be that food allergen sensitization is waning at this age. In conclusion, the data from this cohort suggest that a pattern of allergen specific IgE sensitization occurs in relation to total IgE concentration.

Table 1. Total Serum IgE Concentrations Stratified Into Percentiles and the Number of Positive Allergen Specific IgE Test Results at Each Percentile Interval of Total IgE

IgE Percentile (IU/mL) ^a	Positive Allergen Specific IgE Test Results, No. (%) ^b									
	<i>Dermatophagoides farinae</i>	Cat	Dog	Ragweed	<i>Alternaria</i>	Timothy grass	Peanut	Cow milk	Hen egg	Shrimp
5 (2.68)	0	0	0	0	0	0	0	0	1 (5.6)	0
10 (5.64)	2 (1.2)	0	0	0	1 (0.9)	0	0	0	1 (5.6)	0
15 (8.65)	3 (1.8)	1 (0.9)	1 (1.0)	0	2 (1.8)	0	0	0	1 (5.6)	0
20 (11.5)	5 (3.1)	2 (1.7)	1 (1.0)	1 (0.6)	3 (2.7)	0	0	0	1 (5.6)	0
25 (13.8)	7 (1.3)	5 (4.4)	2 (2.0)	3 (1.8)	6 (5.4)	1 (0.7)	0	0	1 (5.6)	0
30 (17.5)	11 (6.8)	5 (4.4)	3 (2.9)	6 (3.7)	11 (9.9)	2 (1.4)	0	0	2 (11.1)	0
35 (21.5)	13 (8.0)	6 (5.2)	3 (2.9)	8 (4.9)	13 (11.7)	4 (2.8)	0	0	2 (11.1)	0
40 (27.9)	14 (8.6)	7 (6.1)	5 (4.9)	13 (7.9)	14 (12.6)	7 (4.8)	1 (2.1)	0	2 (11.1)	1 (3.6)
45 (33.0)	16 (9.8)	7 (6.1)	5 (4.9)	14 (8.5)	18 (16.2)	10 (6.9)	2 (4.3)	0	6 (33.3)	1 (3.6)
50 (40.6)	22 (13.5)	10 (8.7)	8 (7.8)	22 (13.4)	21 (18.9)	13 (9.0)	2 (4.3)	2 (9.5)	6 (33.3)	1 (3.6)
55 (49.1)	32 (19.6)	13 (11.3)	9 (8.8)	27 (16.5)	25 (22.5)	21 (14.5)	3 (6.4)	3 (14.3)	7 (38.9)	3 (10.7)
60 (55.2)	41 (25.2)	20 (17.4)	16 (15.7)	37 (22.6)	32 (28.8)	30 (20.7)	4 (8.5)	6 (28.6)	10 (55.6)	6 (21.4)
100 ($\geq$ 55.3)	163 (100)	115 (100)	102 (100)	164 (100)	111 (100)	145 (100)	47 (100)	21 (100)	17 (100)	28 (100)
Percentage of all positive tests ^c	28.6	20.2	17.9	28.8	19.5	25.4	8.2	4.9	3.9	6.9
Total No. of individuals tested ^d	570	570	570	570	570	570	570	427	439	405

^a Percentile of total serum IgE and, in parentheses, the concentration defining the percentile.

^b Positive results for allergen at each designated percentile of total serum IgE concentration.

^c The percentage of all positive test results represented by that particular allergen.

^d The total number of individuals tested for each allergen varies because the volumes of serum obtained were not sufficient to complete all tests for all individuals.

KARTHIK R. KRISHNAN, MD*
EDWARD L. PETERSON, PhD†
GANESA WEGIENKA, PhD†
SUZANNE HAVSTAD, MS†
CHRISTINE COLE JOHNSON, PhD†
EDWARD M. ZORATTI, MD‡
DENNIS R. OWNBY, MD*

*Section of Allergy and Immunology

Medical College of Georgia

Augusta, Georgia

†Department of Biostatistics and Research Epidemiology

Henry Ford Hospital

Detroit, Michigan

‡Division of Allergy

Department of Internal Medicine

Henry Ford Hospital

Detroit, Michigan

krk742@gmail.com

1. Omenaas E, Bakke P, Elsayed S, Hanoa R, Gulsvik A. Total and specific serum IgE levels in adults: relationship to sex, age and environmental factors. *Clin Exp Allergy*. 1994;24:530–539.
2. Oryszczyn MP, Annesi I, Neukirch F, Dore MF, Kauffmann F. Longitudinal observations of serum IgE and skin prick test response. *Am J Respir Crit Care Med*. 1995;151:663–668.
3. Hollander A, Doeke G, Heederik D. Cat and dog allergy and total IgE as risk factors of laboratory animal allergy. *J Allergy Clin Immunol*. 1996;98:545–554.
4. Misiak RT, Wegienka G, Havstad S, Ownby DR, Johnson CC, Zoratti EM. Specific allergic sensitization in parents and their 18-year-old offspring in the Suburban Detroit Childhood Allergy Study. *J Allergy Clin Immunol*. 2009;123:1401–1406.
5. Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA* 2002;288:963–972.

INCREASED AFFECTED SKIN GENE EXPRESSION AND SERUM LEVELS OF THYMIC STROMAL LYMPHOPOIETIN IN ATOPIC DERMATITIS

Atopic dermatitis (AD) is a chronic, highly pruritic inflammatory skin disease that has increased considerably in industrialized countries.¹ It is characterized by allergic inflammation in the skin,² and it has a strong association with other atopic disorders, such as asthma and allergic rhinitis (AR).¹

Thymic stromal lymphopoietin (TSLP), a cytokine that is closely related to interleukin 7 (IL-7),³ is mostly produced by epithelial cells and keratinocytes in response to infection, trauma, and inflammation. It mediates T_H2 responses through activation of dendritic cells, and it has emerged as an important regulator of allergic inflammation.⁴ Also, TSLP activates mast cells to release several cytokines, such as IL-5, IL-6, and IL-13, but only in the presence of IL-1 and tumor necrosis factor.⁴ One article³ reported increased TSLP protein expression in lesional skin of patients with AD.

We investigated gene expression of TSLP in lesional skin biopsy samples and serum TSLP levels in 9 patients with AD (3 males and

6 females; mean [SD] age, 41.33 [21.40] years) compared with 9 controls (3 males and 6 females; mean [SD] age, 42.8 [18.1] years).

All 10 mm punch skin biopsy samples were obtained from patients who had mild, localized, chronic AD (abdomen, arm, back, hand, and neck; there was no apparent correlation between any of these sites and TSLP levels) and from controls (back). No participant had received any anti-inflammatory medication for 15 days before the biopsy. Contact dermatitis, asteatotic eczema, and other types of dermatitis were excluded. The medical ethics committee of the University of Athens Medical School approved this protocol. All participants gave their written informed consent according to the Declaration of Helsinki principles. Patients were free of other major medical conditions except AR as noted.

All biopsy samples were placed in RNeasy lysis buffer (Qiagen, Crawfordsville, IN) and were stored at –20°C. Total RNA from skin biopsy samples was extracted using the TRIzol isolation method (Invitrogen, Carlsbad, CA). Complementary DNA (cDNA) was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Quantitative polymerase chain reaction (PCR) was performed using Taqman probes (Applied Biosystems, Foster City, CA) on a 7300 Real-Time PCR System (Applied Biosystems). For quantitative PCR, the cDNA was reverse transcribed from 300 ng of RNA from each sample. Relative messenger RNA levels were normalized to the glyceraldehyde-3-phosphate dehydrogenase endogenous control.

Data were analyzed using the nonparametric Mann-Whitney *U* test because the values did not conform to a normal distribution. Relative TSLP gene expression was significantly increased in lesional AD skin samples (median, 4.93; range, 2.27–23.99) compared with controls (median, 0.84; range, 0.49–1.85; *P* < .001) (Fig. 1A).

Blood samples were obtained the day before the biopsy, and the serum sample was isolated, aliquoted, and stored at –20°C until use. Levels of TSLP were determined using enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). There was a significant increase in TSLP levels in patients with AD (median, 130.5 pg/mL; range, 0–2,246 pg/mL) compared with controls (median, 0 pg/mL; range, 0–62.5 pg/mL; *P* = .006) (Fig. 1B). The 3 female patients with the highest serum levels (2,246.6, 859.4, and 768.2 pg/mL) also had AR confirmed by skin prick testing.

There was neither a positive nor a negative correlation between skin TSLP gene expression and serum levels in any participants. Local skin TSLP gene expression and production may be one of the earliest steps in the inflammatory process, preceding systemic increases in TSLP levels. These results contrast with those of another human study⁵ reporting that TSLP serum levels were not increased in AD (*n* = 46; average age = 26.5 years), but are in agreement with increased TSLP serum levels in children with atopic (*n* = 75) and nonatopic (*n* = 70) eczema compared with controls (*n* = 87).⁶ All of the present patients had mild, localized chronic AD and, therefore, do not permit an attempt to correlate with severity of disease. Besides, this study was not powered for such an analysis.

The highest serum values corresponded to patients with AD and concomitant AR confirmed using skin prick testing. This finding supports the hypothesis that increased serum TSLP levels may also precede other atopic diseases, such as AR and asthma. In fact, a study⁷ showed that skin-derived TSLP contributed to the aggravation of asthma in mice. The role of TSLP in human asthma is supported by another study⁸ that showed that bronchial TSLP expression was increased in asthmatic patients (*n* = 20) compared with in controls (*n* = 15) and correlated with disease severity. Based on the apparent association between increased serum TSLP levels and AR, investigation of atopic patients

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