

New onset wheezing in an older male population: Evidence of allergen sensitization in a longitudinal study

Results of the normative aging study

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Background: Thirty-nine male subjects, with new-onset wheezing, were selected from participants in the Department of Veterans Affairs Normative Aging Study and compared with 74 age-matched controls. Wheezing was defined by responses to a standardized and regularly administered questionnaire. The subjects with wheezing had a reduced FEV₁ compared with controls ($p = 0.005$), but most had values above 80% predicted. Current smoking was more common in subjects with wheezing (36.8% vs 8.11% in controls, $p < 0.001$). The mean age of both subjects and controls was 64 years.

Methods: Allergen-specific IgE antibodies were measured, starting with sera at the time of the most recent questionnaire, and on the average 3.1, 7.6, and 12.3 years before that, with use of stored serum samples.

Results: Total IgE did not differ significantly between the groups. IgE binding to dust mite antigen was detected in 13% to 15% of the subjects with wheezing compared with fewer than 7% of the controls over four time intervals ($p = 0.014$). IgE binding to cat and ragweed antigens did not differ significantly between groups. If current nonsmokers were analyzed separately, IgE binding to cat allergen was also slightly greater in subjects with wheezing compared with controls ($p = 0.054$). Sequential analysis of IgE antibody levels to mite antigen, over time, indicated that IgE antibody antedated the onset of wheezing.

Conclusions: New-onset wheezing in an older adult male population is significantly associated with allergic sensitization to dust mite. There was a borderline association with sensitization to cat in noncurrent smokers only. This supports the hypothesis that a subgroup may have allergic triggers to their symptoms. (*J ALLERGY CLIN IMMUNOL* 1993;91:752-7.)

Key words: Wheezing, aging, IgE antibody, mite allergen, cat allergen, smoking, longitudinal

Asthma can be defined as reversible obstructive airway disease. Asthma is associated with evidence of IgE antibody to inhalant allergens in both children and adults,^{1, 2} and it is probable that allergens play an

Abbreviations used

FVC:	Forced vital capacity
FEV ₁ :	Forced expiratory volume in 1 second
PBS:	Phosphate-buffered saline
PBS-T:	PBS containing 0.05% Tween 20
BSA:	Bovine serum albumin
PBS-T-BSA:	PBS-T containing 0.1% BSA

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important role in the pathogenesis of asthma. A number of cross-sectional and longitudinal studies have suggested that aging results in a general decline in total IgE and skin test reactivity.³⁻⁸ This coincides with the generally held opinion that onset of reversible obstructive disease in the older male population is predominantly nonallergic in nature.

The Veterans Affairs Normative Aging Study offered us an opportunity to examine the relationship

between new-onset respiratory symptoms and allergic sensitization in a sample of middle-aged and older men who have been prospectively followed up, since study entry, between 1961 and 1969. Total IgE and specific IgE antibody to dust mite, cat, and ragweed allergens were measured in a group of subjects with new-onset wheezing. These measurements were compared with those of a group of matched controls.

METHODS

Study population

The Normative Aging Study is a longitudinal study of aging established by the Veterans Administration in 1961.⁹ The study cohort consists of 2280 community-dwelling men from the Greater Boston area who were 21 to 80 years of age on enrollment in the study. Volunteers were screened, at entry, according to specific health criteria and were free of known chronic medical conditions, including asthma, chronic bronchitis, and chronic sinusitis.⁹ After entry, volunteers have reported every 3 to 5 years for a comprehensive examination including medical history, physical examinations, blood and urine tests, electrocardiography, chest radiography, and spirometry. Participation in this study has been approved by the Human Studies Subcommittee of the Research and Development Committee, Department of Veterans Affairs Outpatient Clinic, Boston, Mass.

Respiratory questionnaire and the definition of new-onset wheezing

At each examination since 1983 a questionnaire (ATS-DLD-78) was administered to each participant to derive information regarding smoking history, respiratory symptoms, and illnesses.¹⁰ Subjects designated as having "new-onset wheezing" had, on their most recent questionnaire, a positive response to one or more questions pertaining to wheezing. The preceding questionnaire had a negative response to these questions. The questions consisted of the following: (1) Do you wheeze occasionally, apart from colds? (2) Do you wheeze most days or nights? (3) Have you ever had an attack of wheezing that has made you feel short of breath? and (4) Have you been diagnosed as having asthma? Two controls with negative responses to these questions were matched to each subject with wheezing on the basis of age (exact year) and date of most recent questionnaire (closest match). For four of the 39 subjects with wheezing, only one matched control could be found; consequently our analyses are based on data from 74 subjects without a history of wheezing.

Pulmonary function testing

Pulmonary function testing was performed with use of an 8-liter water-filled survey recording spirometer and an Eagle II minicomputer (Warren E. Collins; Braintree, Mass.). We considered a tracing acceptable if effort appeared maximal to the observer and resulted in a smooth time-volume curve lasting at least 6 seconds or until flow was below 40 ml/sec for at least 0.5 seconds. The forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV₁) were derived from the spirometric tracing and corrected to body temperature and pressure saturated

with water vapor.¹⁰ FVC and FEV₁ values were converted into percent predicted values on the basis of the subject's age and height with use of a prediction equation derived from asymptomatic subjects in the Normative Aging Study who had never smoked.

Purified allergen

Partially purified *Fel d* I was prepared as previously described.¹¹ In brief, crude cat pelt extract was subjected to sequential fractionation on DE52 cellulose followed by Sephadex G100 (Sigma Chemical Co., St. Louis, Mo.) gel filtration. The active fraction had a molecular weight range of 25 to 40 kd.

Ragweed fraction D, containing the major allergenic activity of crude ragweed extract, was prepared as previously described.¹² In brief, sequential fractionation was carried out on DEAE-cellulose and Sephadex gel filtration to obtain a fraction containing protein of a molecular weight of 25 to 50 kd.

Partially purified dust mite allergen was prepared as follows. Crude mite culture eluates of *Dermatophagoides farinae* and *D. pteronyssinus* were obtained from T.A.E. Platts-Mills, MD, University of Virginia, Charlottesville, Va. Protein fractions were first obtained by precipitation in 50% saturated ammonium sulfate. After resuspension in saline solution and dialysis, the protein was subjected to gel filtration on Sephadex G100. Allergen-enriched fractions were pooled corresponding to a molecular weight range of 10 to 50 kd. Equal quantities of *D. farinae* and *D. pteronyssinus* derived fractions were pooled.

ELISA measurement of IgE antibody

A 25 µg/ml concentration of mite, ragweed, or cat antigen in coating buffer (0.2 mol/L bicarbonate buffer [0.1 mol/L NaCO₃ + 0.1 mol/L Na₂CO₃, pH 9.6] for mite and ragweed; 0.05 mol/L borate buffer [0.0125 mol/L H₃BO₃ + 0.0375 mol/L Na₂B₄O₇, 10 H₂O, pH 8.6] for cat antigen) was plated on Dynatech Immulon II (Dynatech Laboratories, Inc., Chantilly, Va.) U-bottom, 96-well plates (50 µl/well). The plates were incubated overnight at 4° C. The next day, the plates were washed four times with phosphate-buffered saline (PBS), pH 7.4 (Sigma) containing 0.05% Tween 20 (PBS-T). Unknown sera samples along with a serial dilution ladder of standardized pooled sera from three individuals (previously determined to contain large amounts of antidust mite IgE) or from another individual (which had high titers of anticat and antiragweed IgE) were diluted in PBS with 0.1% bovine serum albumin (BSA) (Boehringer Mannheim Corp., Indianapolis, Ind.) and 0.05% Tween 20 (PBS-T-BSA), plated in triplicate and incubated overnight at 4° C. The plates were washed four times with PBS-T and plated with 1:250 dilution of biotinylated goat antihuman IgE (TAGO, Inc., Burlingame, Calif.) in PBS-T-BSA and incubated overnight at 4° C. After they were washed four times with PBS-T, the plates were coated with a dilution of horseradish peroxidase-conjugated avidin (TAGO) in PBS-T-BSA and incubated overnight at 4° C. The next day, the plates were allowed to warm up for 1 hour at room temperature, followed by an incubation for 5 minutes at 37° C. The plates were washed three times with PBS-T and one time with PBS and incubated with the

TABLE I. Subject characteristics

Characteristic	Subjects with wheezing (n = 39)	Controls (n = 74)	p Value
Age in years mean (SD)	64.2 (6.6)	64.3 (6.4)	0.959
Smoking status			
Current smokers (%)	36.8	8.1	<0.001*
Former smokers (%)	39.5	48.6	
Never smokers (%)	23.7	43.2	
Pulmonary function			
All subjects			
FEV ₁ , % predicted, mean (SD)	91.4 (19.5)	101.3 (16.2)	0.005†
FVC, % predicted, mean (SD)	83.4 (16.0)	90.4 (12.5)	0.012†
Current smokers			
FEV ₁ , % predicted, mean (SD)	88.6 (10.9)	82.2 (4.7)	0.184
FVC, % predicted, mean (SD)	84.1 (11.9)	84.2 (7.6)	0.988†
Current nonsmokers			
FEV ₁ , % predicted, mean (SD)	93.0 (23.2)	103.0 (15.7)	0.058†
FVC, % predicted, mean (SD)	83.1 (18.2)	91.0 (12.7)	0.058†

*Chi square.

†t test.

TABLE II. IgE binding to allergens, percent positive*

	Subjects with wheezing (n = 39)				Controls (n = 74)				p Value
	Time points				Time points				
	1	2	3	4	1	2	3	4	
Dust mite	15.4 (7.7)	15.4 (7.7)	12.9 (10.3)	12.8 (7.7)	6.8 (0)	2.7 (0)	4.0 (0)	2.7 (0)	0.014
Cat	10.2 (5.1)	7.7 (0)	2.6 (0)	2.6 (0)	1.4 (1.4)	1.4 (0)	2.7 (0)	2.7 (0)	0.221
Ragweed	15.4 (7.7)	15.4 (7.7)	10.3 (0)	2.6 (0)	8.1 (8.1)	9.4 (4.0)	5.4 (1.4)	4.0 (0)	0.448

*Greater than 3 SD above the mean value for the control group at the fourth time point.

†Chi-square test.

(The percents strongly positive are in parentheses, that is, greater than 6 SD above the mean value for the control group at the fourth time point).

color developing reagent containing 0.5 mg/ml orthophenylenediamine and 0.006% H₂O₂ in 0.2 mol/L potassium phosphate buffer (0.1 mol/L K₂HPO₄ + 0.1 mol/L KH₂PO₄, pH 7.0) at RT in a dark box (90 minutes for mite, cat, and total IgE; 120 minutes for ragweed). The optical densities of the wells were read at 410 nm on a Dynatech MR 5000 microplate reader equipped with a statistical data analysis package. Optical densities of the wells containing the standards were converted into a standard curve of concentrations with use of semilogarithmic regression analysis. The data reduction package also computes the prediction error associated with each point (typical values are <15%) on the regression curve. The concentrations (in ar-

bitrary units) were calculated from the standard curve. Detection limits were determined as follows. The lowest concentration point to be included in the linear part of the regression curve was chosen to be a minimum of 6 SD above the lower knee of the sigmoidal curve where the optical density values become asymptotic, indicative of background values. Typically, the lowest point included in the linear standard curve was between 6 and 10 SD above this lower cutoff limit. Also, this criterion always coincided with the prediction error being less than 15% at each of the six consecutive values included on the standard curve. Typical coefficient of correlation value, with use of six consecutive points on the standard curve, was $r^2 = 0.985$ or

better. Intraassay and interassay variations were less than 20%. Each of the assays was checked for specificity by means of known negative control sera.

To define the minimum value in units that was significantly positive in this assay, the IgE antibody measurements of the control group at the fourth time interval were averaged. Three standard deviations above this mean was taken as significantly positive. IgE antibody measurements for each of the three antigens were averaged separately. The mean of the control group for each antigen fell well above the lower end of the linear portion of the standard curve.

ELISA measurement of total IgE

The protocol for this assay was similar to those for the IgE antibody assays described above. Purified antihuman IgE specific for the epsilon chain (5 µg/ml) in 0.05 mol/L borate buffer was initially applied to the plates.¹¹ Dilutions of a standard serum with known IgE content were used to generate a standard curve against which the unknown sera were compared.

Statistical analysis

Relationships were examined by independent *t* tests and chi-square tests. All analyses were performed with the SAS statistical software package (SAS Institute Inc., Cary, N.C.).

RESULTS

Subject characteristics

Subject characteristics are summarized in Table I. Thirty-nine subjects with wheezing and 74 controls were selected. No subjects were selected on the basis of a positive response to question 4 alone (Have you been diagnosed as having asthma?). Therefore wheezing was the defining characterization of the subject group. Of the responses to the three questions that pertained to wheezing, 15 subjects responded only to question 1 (see Methods), 10 subjects responded only to question 3, 12 subjects responded to question 1 and 2, and two subjects responded to all three questions. As a result of matching, the mean age of each group was 64 years. There was a significant difference in smoking history between the subjects with wheezing and controls. Subjects with wheezing were more likely to be current smokers and to have greater total pack years than controls. Pulmonary function testing indicated that the subjects with wheezing had a somewhat reduced FEV₁ and FVC, compared with controls (Table I). In an analysis not shown, 33% of subjects with wheezing had an FEV₁ less than 77% of predicted (5th percentile of asymptomatic never-smokers). Analysis of pulmonary function stratified by current smoking status failed to show significant differences between subjects and controls (Table I).

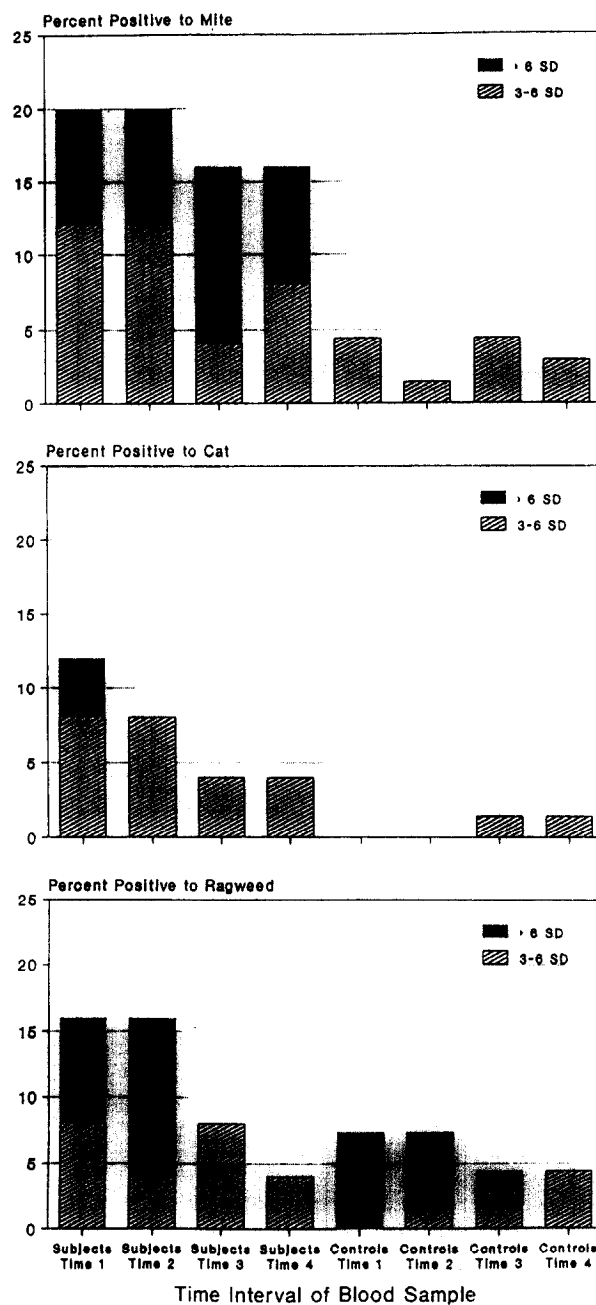


FIG. 1. IgE antibody in noncurrent smokers. IgE antibody to mite (upper panel), cat (middle panel), and ragweed (lower panel) was measured at four time points in the subjects with wheezing ($n = 25$) and controls ($n = 68$). Two levels of positivity are indicated: 3 to 6 SD (striped) and greater than 6 SD (black) above the mean for the control group at the fourth time point. With use of a chi-square test that takes into account the four time points and the two levels of positivity, significant differences between subjects with wheezing and controls were seen for IgE antibody to mite ($p = 0.015$) and cat ($p = 0.054$).

IgE ANTIBODY TO MITE LONGITUDINAL CHANGES OVER TIME

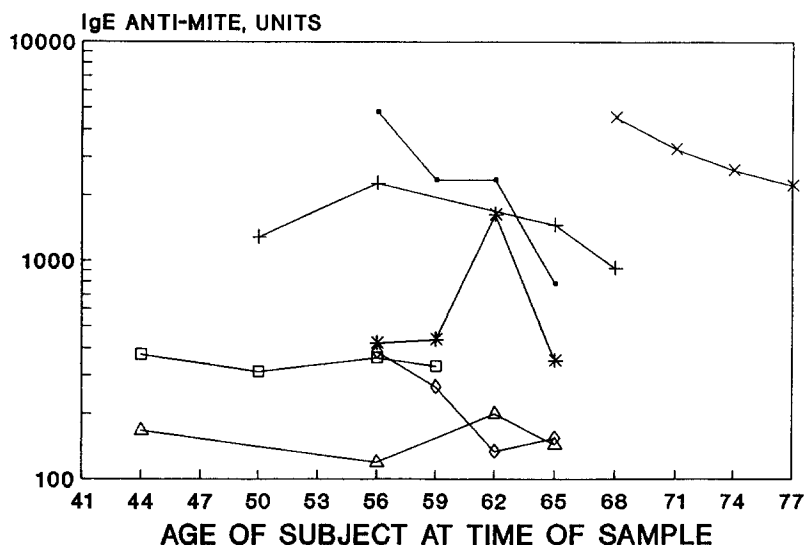


FIG. 2. IgE antibody to mite. Longitudinal changes in IgE antibody to mite are illustrated in seven subjects with wheezing. Although IgE antibody can drop substantially with age in some subjects, high levels can persist over many years in others. Levels above 150 units are considered significantly elevated (i.e., 3 SD above the mean for the control group at the fourth time point).

Total IgE measurement

Blood samples were studied in all of the subjects at four time points. The fourth time point was the time of the most recent questionnaire (and of wheezing symptoms). The first, second, and third time points were on the average 12.3, 7.6, and 3.1 years before the fourth time point, respectively. Mean total IgE levels for subjects with wheezing and control subjects did not vary significantly over time. Mean (SD) total IgE for the subjects with wheezing and control subjects at the fourth time point was 34.4 (33.8) and 26.1 (27.9), respectively. This was not a significant difference.

IgE antibody to allergens

Table II summarizes the results of IgE antibody binding to the three antigens in subjects with wheezing and controls at the four time points. Differences between subjects and controls were significant for mite only with use of a chi-square test that took into account the two levels of sensitivity at the four time points. Sensitization to mite occurred in 13% to 15% of the subjects with wheezing at all four time intervals. Of particular interest was the strongly positive level of IgE antibody that occurred in 8% to 10% of the subjects with wheezing. There were trends of increased binding to both cat and ragweed in the subjects with wheezing as compared with controls at the first two

time points (not statistically significant). It is also apparent from an inspection of the data that there was no marked change in degree of sensitization to mite with time.

Because a recent report suggested that allergic sensitization was more evident in older asthmatic subjects who were current nonsmokers,¹³ we analyzed the results for current nonsmokers. These data are illustrated in Fig. 1 for 25 subjects with wheezing (mean age, 66 years) and 68 controls (mean age, 65 years). The results are similar except that the IgE binding to mite, in subjects with wheezing, increased to a total of 20%. In addition, IgE binding to cat allergen in the subjects with wheezing versus controls approached statistical significance ($p = 0.054$).

Levels of IgE antibody to mite, over time, in seven wheezing subjects with one or more IgE antibody measurements above 150 units/ml are illustrated in Fig. 2. It is apparent that over 10- to 20-year intervals, new sensitization to mite did not occur in any of these subjects.

DISCUSSION

We have shown that in a population of men with a mean age of 64 years, the new onset of wheezing symptoms is associated with increased IgE antibody binding to mite antigen. If noncurrent smokers are analyzed separately, IgE binding to cat antigen is also

associated weakly with wheezing. IgE antibody to ragweed antigen, which is an important allergen in our area, is not associated with wheezing, although there is a trend toward increased binding in the subjects with wheezing.

The study subjects were drawn from a population that had health screening on enrollment in the 1960s to exclude those with major medical disease, including asthma. It is possible that mild allergic asthma was present years before the study entry.

The use of wheezing as a criterion for selection in this age group might cause the inclusion of subjects with chronic bronchitis and emphysema as well as asthma. The subjects with wheezing had a higher frequency of smoking (Table I) and also a higher prevalence of cough (34%) and regular sputum production (52%) compared with controls (5% cough, 10% sputum). The occurrence of cough and sputum production does not definitely distinguish asthma from chronic obstructive lung disease. The finding that only 33% of the 39 subjects in whom new wheezing developed had abnormal pulmonary function, suggests that most of these subjects' symptoms may have been due to asthma rather than chronic obstructive lung disease. The presence of wheezing may be associated with allergic sensitization in both asthma and chronic obstructive lung disease.

Sensitization to mite in older subjects with wheezing does not, of course, demonstrate that mites cause the wheezing in that age group. Wheezing might be associated with a nonspecific tendency to produce IgE antibody to certain ubiquitous antigens that may not play a role in the causality of pulmonary symptoms. Several findings suggest that something more than a nonspecific relationship exists. Total levels of IgE were not significantly higher in subjects with wheezing, suggesting that absence of a nonspecific IgE forming diathesis. Another population study did show a relationship between total IgE and asthma in adults.¹⁴ Indoor allergens such as mite and cat antigens have been associated with asthma in a recent study.¹⁵

Subjects with elevated antimite-specific IgE antibody did not show an increase in IgE antibody before the onset of wheezing. This supports the hypothesis that the immune sensitivity to antigens precedes symptoms by an extended period of time. This has been observed for seasonal hay fever in young adults.¹⁶

The occurrence of late-onset wheezing needs further documentation. Late-onset wheezing in subjects with preexisting IgE antibody to environmental allergens might be (1) part of an evolving process of the

disease, (2) caused by increased environmental exposure, or (3) associated with an increase in respiratory tissue reactivity induced by other factors such as a virus infection. The causative role of specific allergens in late-onset wheezing could, in future investigations, be further established by correlating symptoms and allergic sensitization with environmental levels of allergens.

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