

Pneumococcus-specific immunoglobulin E in cigarette smokers

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Summary

A relationship between elevated serum immunoglobulin E levels and smoking has been demonstrated in epidemiological studies. Allergy skin test data suggest that the excess immunoglobulin E of smokers is not specific for aeroallergens. It is possible that the excess immunoglobulin E is specific for microorganisms that often infect the lower respiratory tract of smokers. To investigate this possibility we utilized a radio-allergosorbent test assay for detecting serum immunoglobulin E specific for *Streptococcus pneumoniae*, an organism commonly isolated from the respiratory tract of smokers with chronic bronchitis. We assayed sera of thirty smokers and thirty non-smokers for immunoglobulin E specific for *Streptococcus pneumoniae*. Individual sera were considered positive for pneumococcus-specific immunoglobulin E if the binding was at least twice the non-specific binding at the total immunoglobulin E concentration of the particular serum. Eleven of the thirty sera of smokers and two of the thirty non-smokers were positive for pneumococcus-specific immunoglobulin E. By chi-square analysis of these data, the prevalence of pneumococcus-specific immunoglobulin E was significantly greater in the smoking group compared with the non-smoking group ($P < 0.02$). These results suggest that the excess immunoglobulin E of smokers is, at least in part, specific for microorganisms that infect the airways.

Introduction

A relationship between elevated serum immunoglobulin E (IgE) levels and smoking has recently been demonstrated in an epidemiological study of respiratory disease in Tucson, Arizona [1]. The association between smoking and increased IgE levels was especially significant in the older age groups and was unrelated to allergy skin test reactivity. This association has been confirmed by Warren *et al.* in an epidemiological

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study of 1768 subjects drawn from a rural population [2]. Gerrard *et al.* had previously reported that serum IgE levels were significantly higher among ninety smokers compared to eighty-seven non-smokers [3], but the atopic state of their subjects was unknown. Although these studies have demonstrated that smokers have higher serum IgE levels than non-smokers, the antigenic specificity of this excess immunoglobulin E is unknown. In the two epidemiological studies cited above [1, 2], analysis of allergy skin test data for local aeroallergens suggested that the excess IgE of smokers was not specific for aeroallergens. If the excess IgE of smokers is not aeroallergen IgE, it is possible that the excess IgE is specific for microorganisms that often infect the lower respiratory tract of smokers. To investigate this possibility we developed a modified radioallergosorbent test (RAST) assay for detecting serum IgE specific for *Streptococcus pneumoniae*, an organism commonly isolated from the respiratory tract of smokers with chronic bronchitis [4]. Using this assay, we studied smokers and non-smokers for IgE specific for *S. pneumoniae*.

Materials and methods

Study sera were selected from those previously collected from subjects enrolled in a longitudinal epidemiologic study of respiratory disease. The study population consisted of a random stratified cluster sample of more than 1600 white non-Mexican-American households in Tucson, Arizona. Details of the selection of the population and study methods have been reported [5]. Questionnaire data including smoking history, respiratory symptoms, and respiratory diagnoses were available for these subjects. In addition, total serum IgE levels had been determined on the serum samples by the Phadebas paper radioimmunosorbent test (PRIST) method [6], and skin-prick test data for aeroallergens common to the Tucson area were available [7]. Study criteria were subject age greater than 35 years, no history of bronchial asthma, and total serum IgE greater than 100 IU/ml. Smokers were defined as subjects who presently smoked cigarettes or had stopped smoking within the past 10 years. Non-smokers were subjects who had never smoked cigarettes regularly for more than 1 year. Sera were selected for thirty smokers and thirty non-smokers.

A RAST assay modified from that reported by Berger *et al.* [8] was employed for determination of IgE specific for *S. pneumoniae*. *S. pneumoniae* (type 1) were obtained from the American Type Culture Collection (Rockville, MD). The bacteria were cultured in serial overnight passes in trypticase-soy broth at 37°C and incubated at 56°C for 30 min to prevent autolysis. Bacteria were harvested by centrifugation, washed with 0.0175 M phosphate in 0.15 M saline (PBS), pH 7.4 and stored in suspension at -70°C.

Staphylococcus aureus organisms used in the adsorption experiments were prepared in an identical manner. At the time of IgE binding studies, suspensions of pneumococci were diluted in PBS to a protein concentration of 1 mg/ml as determined by a biuret procedure [9]. ¹²⁵I-anti-human IgE produced in rabbits was purchased from Pharmacia Inc. (Piscataway, NJ). One ml aliquots of pneumococci were placed in 12 × 75 mm plastic tubes, washed twice with PBS, and centrifuged at 2500 × g for 10 min. The pellet of bacteria and 0.1 ml of serum were mixed vigorously and then incubated at room temperature for 3 hr. After this incubation, the pellets were washed three times with PBS and then incubated with 0.05 ml ¹²⁵I-anti-human IgE at room temperature overnight. The pellets were again washed three times and the bound radioactivity determined using a Packard auto-gamma spectrometer. The results were

expressed as the percentage of the total ^{125}I added that remained bound to the pellets. All determinations were performed in duplicate. The variability in the binding values for each serum sample ranged from 0.0 to 0.5% of total activity with a mean value of 0.2%. Values for controls in which PBS was used instead of serum were subtracted to correct for background binding of ^{125}I -anti-human IgE. Background binding averaged 4.3% of total activity and had a within assay variability of 0.1–0.2%. To establish whether IgE exhibited any non-specific binding in this assay, dilutions of an IgE myeloma serum PS (kindly provided by Dr K. Ishizaka, Johns Hopkins University, Baltimore, MD) were made in IgE-free serum (Pharmacia, Piscataway, NJ) and were assayed. A linear increase in binding with increasing IgE concentration was found. In order to identify the amount of non-specific binding with increasing IgE concentrations, we included appropriate dilutions of the myeloma serum in the assay corresponding to the range of total IgE levels of the sera being assayed. Individual sera were considered positive for pneumococcus-specific IgE if the binding was at least twice the non-specific binding at the IgE concentration of the particular serum. This level of twice the non-specific binding for positive sera was chosen because the variability of non-specific binding for the IgE myeloma was equal to or less than this level for all determinations. Forty-six binding determinations representing eight dilutions of the IgE myeloma were performed on four separate occasions. The prevalence of sera positive for pneumococcus-specific IgE in the two groups was compared by chi-square analysis.

The adsorption experiments were performed using pellets containing eight times more organisms than used in the binding assays. One ml of the serum was mixed vigorously with the pellet of organisms, incubated at room temperature for 45 min, centrifuged at $2500 \times g$ for 15 min and the supernatant carefully removed. The adsorption procedure was performed three times, and the final supernatant received an additional 20 min centrifugation at $2500 \times g$.

To obtain a quantitative estimate of the pneumococcus-specific IgE for sera positive in the RAST assay, we calibrated a serum that showed a high binding value in the assay. An aliquot of this reference serum was adsorbed with the *S. pneumoniae* organism used in the RAST assay, employing the adsorption procedure described above. Total serum IgE levels were determined for the pre-adsorbed and post-adsorbed reference serum by the PRIST method, and the difference was considered to be the amount of the pneumococcus-specific IgE in the serum. A control serum with a comparable total IgE value was processed in a similar manner to assure the specificity of the adsorption procedure. Dilutions of the calibrated serum were included in the RAST assay to establish a standard binding curve from which the amount of pneumococcus-specific IgE in each positive serum could be determined. Although the largest dilution corresponded to 1.0 IU/ml pneumococcal-specific IgE, the threshold of the assay was less than that, since specific binding for 1.0 IU/ml was 2% (total activity minus non-specific binding), a value which is four times the maximum variability of the assay.

Results

Serum samples from thirty smokers and thirty non-smokers were assayed for pneumococcus-specific IgE. The mean age in the group of smokers was 53 years (range 36–70 years) compared with 58 years (range 40–80 years) in the non-smoking subjects. Total serum IgE values in the smoking subjects ranged from 139 to 2496 IU/ml with a

geometric mean of 513 IU/ml. In the non-smoking group, total serum IgE ranged from 102 IU/ml to 2874 IU/ml with a geometric mean of 224 IU/ml. Student's *t*-test analyses of these data showed that the two groups were not different with regard to age but that smokers had significantly higher IgE values ($P < 0.01$). Although the smokers had higher serum IgE values, significantly more non-smokers (26 of 30) than smokers (17 of 30) had positive skin tests for local aeroallergens (chi square, $P < 0.05$). Three of the smokers had physician-diagnosed chronic bronchitis or emphysema. None of the non-smokers had a respiratory diagnosis. The mean percent predicted forced expiratory volume in 1 sec (FEV₁) was higher for the non-smokers (100.7%) than the smokers (87.5%). This was a significant difference (*t*-test, $P < 0.01$).

In Fig. 1, the RAST binding values for pneumococcus-specific IgE in the sixty sera (thirty smokers and thirty non-smokers) are shown in relation to total serum IgE level for each serum. The non-specific IgE binding (solid line) was determined by simultaneously assaying serial dilutions of an IgE myeloma serum. Binding values for eleven of the thirty sera of smokers and two of the thirty non-smokers were positive for pneumococcus-specific IgE using the criterion of greater than twice the level of nonspecific binding at the corresponding total IgE level (dashed line). By chi-square analysis of these data, the prevalence of pneumococcus-specific IgE was significantly greater in the smoking group compared with the non-smoking group ($P < 0.02$). Although both of the non-smokers positive for pneumococcus-specific IgE had

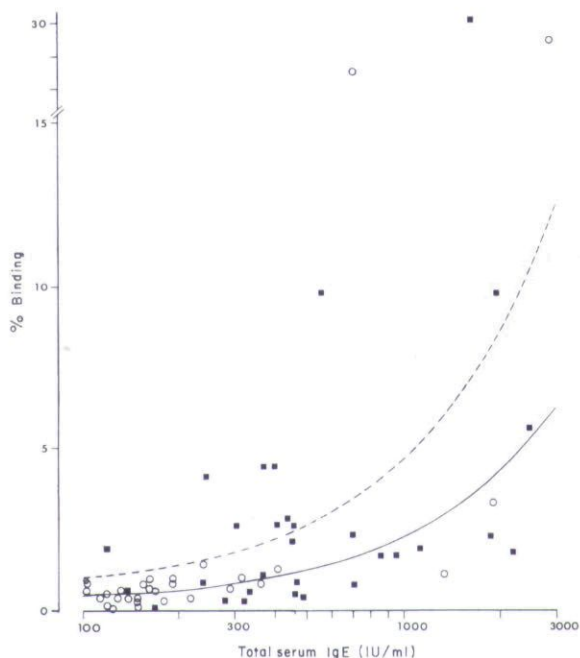


Fig 1. RAST binding values for pneumococcus-specific IgE in the thirty smokers (closed squares) and thirty non-smokers (open circle) are shown in relation to the total serum IgE for each serum. The non-specific binding is shown by the solid line. Although the increase in non-specific binding with increasing total IgE is linear, the line is curved as a result of using a logarithmic IgE scale on the abscissa to provide a better visualization of IgE values. Sera with binding values greater than twice the level of non-specific binding (dashed line) were considered positive.

Table 1. Age, smoking status, total serum IgE, and estimated pneumococcus-specific IgE for the thirteen subjects positive in the pneumococcus RAST assay

Subject no.	Age	Smoking status*	Total serum IgE (IU/ml)	Estimated pneumococcus-specific IgE (IU/ml)
5	49	S	1940	4
10	58	S	438	1
11	68	S	1629	59
12	45	S	121	1
14	63	S	459	1
15	37	S	400	2
18	64	S	558	6
20	56	S	373	2
23	59	S	406	1
25	62	S	305	1
28	52	S	245	2
33	56	NS	700	17
41	80	NS	2874	18

* S = smokers; NS = Non-smokers

positive allergy skin tests for local aeroallergens, only four (subject nos 5, 11, 12, 14) of the eleven smokers positive in the assay had positive allergy skin tests. Comparison of the eleven smokers positive for pneumococcus-specific IgE with the nineteen negative smokers showed no difference with regard to respiratory symptoms, respiratory diagnoses, allergy skin tests, or percent predicted FEV₁.

The quantitative amount of pneumococcus-specific IgE in each of the thirteen positive sera was estimated from the standard binding curve of the calibrated serum. Table 1 shows the age, smoking status, total serum IgE and estimated amount of pneumococcus-specific IgE for each of the subjects with a positive assay result. The estimated amount of pneumococcus-specific IgE ranged from 1 to 59 IU/ml.

We investigated the specificity of the IgE binding in the RAST assay by adsorbing selected positive and negative sera with *S. pneumoniae* or *S. aureus* organisms. The reference serum for the study and the serum from subject no. 15 were adsorbed with *S. pneumoniae*. The pre-adsorption value for the reference serum showed 35.6% specific binding which decreased to 0.2% below background binding after adsorption. For the serum of subject no. 15 the pre-adsorption value was 7.9% specific binding and 0.4% specific binding after adsorption. Five sera were adsorbed with *S. aureus*. Pneumococcal binding values for these sera after adsorption with *S. aureus* were unchanged compared with the pre-adsorption values.

Discussion

This investigation was undertaken because studies have demonstrated that smokers have higher serum IgE levels than non-smokers [1-3]. Two findings in the Tucson study of respiratory disease suggest that an effect of smoking actually causes the increase in serum IgE levels [2, 10]. First, IgE values in smokers were significantly related to pack

years of smoking. Also, in ex-smokers IgE levels tended to decline with time since quitting cigarettes. The finding that smokers had comparatively low rates of positive skin tests to local aeroallergens suggests that there are also qualitative differences between the excess IgE of smokers and that of atopic subjects [1, 10]. Also among non-atopic smokers, high IgE levels were not significantly related to complaints of 'allergic' rhinitis, suggesting that the excess IgE is not specific for anything inhaled through the nose [10]. These findings suggest that excess IgE in smokers is directed at antigens other than aeroallergens.

Studies by other investigators have demonstrated that tobacco extracts and cigarette smoke contain components capable of stimulating an immune response in laboratory animals [11, 12]. Lehrer *et al.* tested the sera of sixteen smokers, four non-smokers, and seven 'smoke-sensitive' subjects for IgE antibody to tobacco leaf and smoke antigens [13]. They could not demonstrate serum IgE to smoke extract in any subject. In a few subjects, IgE to leaf antigens was detected, but no correlation was found between the presence of these antibodies and smoking or clinical smoke sensitivity. Results of studies performed on sera of 105 selected smoking and non-smoking subjects in the Tucson respiratory disease study for the presence of IgE to tobacco leaf and smoke extracts were also negative [10]. These data suggest that the excess IgE of smokers is not specific for a component of tobacco leaf or cigarette smoke.

IgE specific for microorganisms has been found by investigators who studied the sera of patients with the hyperimmunoglobulin E syndrome [8, 14]. These studies demonstrated serum IgE specific for *S. aureus* and *Candida albicans*, microorganisms that chronically infect patients with the hyperimmunoglobulin E syndrome. Sera of patients with various lung diseases have also been investigated for IgE specific for microorganisms. Shen *et al.* demonstrated IgE specific for *Pseudomonas aeruginosa* in the sera of cystic fibrosis patients that were colonized with this bacterium [15]. Pauwels *et al.* studied the sera of 190 adult asthmatic patients and found IgE specific for *Hemophilus influenza* in 12% and *S. pneumoniae* in 19% of the patients [16]. Tee & Pepys investigated the sera of 135 patients with various lung diseases including chronic bronchitis, allergic bronchopulmonary aspergillosis, bronchial asthma, and cystic fibrosis for IgE specific for *S. pneumoniae*, *S. aureus* and *H. influenzae* [17]. The patients with allergic bronchopulmonary aspergillosis had an increased prevalence of IgE to all three bacteria, while the chronic bronchitis and cystic fibrosis groups had IgE specific for *H. influenzae*. IgE specific for *S. pneumoniae* was not demonstrated in the chronic bronchitis patients. The cigarette smoking history of these patients was not reported.

The pathogenic significance of the antimicrobial IgE was not addressed in the studies cited above. However, in the Tucson study of respiratory disease, a relationship was found between IgE level and respiratory symptoms, such as chronic cough, that are not usually considered allergic [10]. Perhaps most importantly, reduced ventilatory function, when accompanied by chronic cough, chronic sputum production, or attacks of shortness of breath with wheeze, also related to increased levels of serum IgE [18]. In contrast, skin test reactivity to common aeroallergens showed no overall relationship to FEV₁. Furthermore, after accounting for total serum IgE, positive allergy skin tests tended to be associated with high rather than low FEV₁ levels. These findings suggest that some type of IgE, which is not specific for aeroallergens but which is associated with smoking, may be important in the pathogenesis of chronic ventilatory impairment.

In the present study, we employed a solid-phase radioimmunoassay to demonstrate that the prevalence of IgE specific for *S. pneumoniae* is significantly greater in smokers compared to non-smokers. Specificity of the assay for pneumococcal antigens was established by performing adsorption studies. A small amount of non-specific binding was detectable with an IgE myeloma serum and this non-specific binding increased linearly with increasing amounts of the IgE protein. The similarities in non-specific binding of the myeloma with many of the serum samples from individual subjects over the full range of total IgE values support the appropriateness of using a myeloma serum to control for non-specific binding. After compensating for non-specific IgE binding, we quantitated the amount of specific IgE binding present in the positive sera. Our results suggest that the excess IgE of smokers is, at least in part, specific for microorganisms that infect the airways. While this could represent an important mechanism for developing chronic airways obstruction, a pathogenic role for anti-microbial IgE has not yet been established.

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