

Specific serum IgE antibodies to bacterial antigens in allergic lung disease*

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Summary

A radio-allergosorbent test (RAST) to measure specific IgE antibodies in man to whole bacterial cells of *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae* was developed to investigate different well-defined lung diseases (chronic bronchitis, allergic bronchopulmonary aspergillosis (ABPA), bronchial asthma, allergic rhinitis, cystic fibrosis) and also in urticaria as compared with non-atopic blood donors. In addition, total IgE values and skin prick tests were assessed in these patients.

The ABPA group gave the highest specific IgE RAST scores to all three bacteria, whilst the chronic bronchitis and cystic fibrosis groups also gave raised RAST scores with *H. influenzae*.

There was a positive correlation between the patients' *Sta. aureus* and *Str. pneumoniae* immediate-type skin reactions and their RAST scores and total serum IgE concentrations, but there was only a low incidence of immediate-type skin test positivity to *H. influenzae*.

Introduction

It has been suggested that some allergic lung diseases can be caused or exacerbated by hypersensitivity reactions to bacteria. This allergic response may be due, at least in part, to IgE which has been implicated in some recent studies (Schopfer *et al.*, 1979; Walsh *et al.*, 1980; Pauwels, Verschraegen & Van der Straeten, 1980).

A RAST test was therefore developed to measure IgE antibodies specific to three bacteria commonly found in lung disease, namely a coagulase positive strain of *Staphylococcus aureus*, an alpha haemolytic strain of *Streptococcus pneumoniae* and a non-capsulated strain of *Haemophilus influenzae*. This test was assessed by correlations

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with the clinical diagnosis of the patients, the results of specific skin prick tests and their atopic status.

Materials and methods

Patients investigated

1. *Chronic bronchitis*. Thirty patients diagnosed as having chronic bronchitis, i.e. conformed to the definitions recommended by a Medical Research Council Working Party, 1965, were attending the Wandsworth Chest Clinic under Drs K. Citron and J. de Silva.

2. *Allergic bronchopulmonary aspergillosis (ABPA)*. This was defined as patients with asthma, transient lung shadows, pulmonary and blood eosinophilia, bronchial damage and who at one time or another had expectoration of plugs in which *Aspergillus* mycelium could be demonstrated. The twenty patients all had a positive immediate skin prick test and the majority had precipitating antibody to *A. fumigatus*.

3. *Bronchial asthma*. Twenty patients with airways obstruction which was reversible either spontaneously or by treatment.

4. *Allergic Rhinitis*. Twenty-two patients.

5. *Urticaria*. Twenty patients.

6. *Cystic Fibrosis*. Twenty-three patients, all with definitive diagnostic criteria, who were attending Dr Margaret Hodson's Cystic Fibrosis Clinic at the Brompton Hospital.

Patients in Groups 2, 3, 4 and 5 came from the Allergy Clinic, Brompton.

The control subjects, matched where possible for age and sex, were blood donors who had not been skin prick tested but whose serum total IgE levels were less than 200 iu/ml. These samples were kindly supplied by the South London Blood Transfusion Centre, London, SW17.

Atopic status

The patients in all but the chronic bronchitis disease group were skin prick tested with twenty-three common inhalant and food allergens (Bencard, U.K.), together with a negative control solution (Coca's: glycerol). The chronic bronchitis group were skin prick tested with five allergens (Bencard), viz. Group B3 tree pollens, Group B2 grass pollens, *D. pteronyssinus*, cat fur, *A. fumigatus*. The production of at least one weal greater than that produced by the negative control solution placed the subject in the atopic category.

Antigens

Bacterial allergens used in skin prick testing. Extracts prepared from bacterial allergens were used for skin prick testing and were prepared as follows:

Sta. aureus allergen extract. Growth from an overnight nutrient broth culture of *Sta. aureus* (NCTC No. 6571) was washed three times in isotonic saline, resuspended in distilled water and the bacteria ultrasonically disrupted at 20 Kc/sec for 2.5 min in an ice-cooled chamber. The cell sediment was then resuspended in isotonic saline, autoclaved at 10 lb/in² for 10 min, dialysed against distilled water for 48 hr and freeze-dried.

Str. pneumoniae allergenic fraction. The supernatant of an overnight 2% glucose

broth culture of *Str. pneumoniae* (NCTC No. 10319) was Seitz filtered and dialysed against repeated changes of distilled water at 4°C for 48 hr. It was then autoclaved at 10 lb/in² for 10 min and the product filtered and freeze-dried.

H. influenzae allergen extract containing the specific *H*₁ and *H*₂ groups. Growth from an overnight culture on XV agar plates (Turk & May, 1967) of a non-capsulated strain of *H. influenzae* (Brompton 909) was scraped off and washed and ultrasonically disrupted as for *Sta. aureus*. The species specific *H*₁ and *H*₂ antigens (Burns & May, 1967) were separated from the non-species specific *H*₃, *H*₄ and *H*₅ groups by absorbing the sonically disrupted allergenic extract with A25 DEAE Sephadex.

These extracts were used to prepare the skin prick test solutions by dissolving them in 50% Coca's solution (5 g sodium chloride, 2.75 g sodium bicarbonate and 4 g phenol made up to 1 l with distilled water) and 50% glycerol. Concentrations of 100 mg/ml were used for the *Sta. aureus* and *Str. pneumoniae* extracts and that of 10 mg/ml for the *H. influenzae* (*H*₁*H*₂) extract. After centrifugation the extract supernatants were passed through a 0.45-μMillipore filter.

Bacteria used in the RAST assays. Whole bacterial cells of the following strains were used for all the RAST assays.

Sta. aureus (NCTC No. 6571)

Str. pneumoniae (NCTC No. 10319)

H. influenzae (NCTC No. 4842)

Freeze-dried whole cells of these bacteria were supplied by the Microbiological Research Establishment at Porton, Wiltshire. The cultures had been heat-killed at 60°C for 90 min and washed twice with phosphate-buffered saline before freeze-drying.

Immobilization of bacterial antigen. The freeze-dried whole bacterial cells were immobilized by coupling to cyanogen bromide-activated Sepharose 4B (Pharmacia) as follows: 125 mg freeze-dried whole bacterial cells was added to 12.5 ml packed volume of the activated Sepharose 4B resuspended in 12.5 ml 0.2 M sodium bicarbonate containing 0.5 M sodium chloride (pH 8.5), mixed for 18 hr at 4°C and any remaining active groups were blocked by the addition of 1 M ethanolamine at pH 8 for 3 hr.

The suspensions were filtered to a moist cake on a sintered glass funnel No. 3 over reduced pressure and three washing cycles with acetate buffer pH 4 and borate buffer pH 8 were performed to remove non-covalently adsorbed protein. The immobilized antigen was stored at 4°C in phosphate-buffered saline containing 0.3% human serum albumin and 0.05% sodium azide.

Sepharose RAST assay. One hundred microlitre aliquots of the resulting immunoadsorbents, diluted 1:4 in incubation buffer, were agitated in polystyrene tubes (Seward GW4, London) with 50 μl neat test sera for 16 hr. All tests were made in duplicate and cord blood sera (ten), containing no demonstrable total IgE, were run in parallel as negative controls.

After three washes with phosphate-buffered saline, achieved by successive centrifugation (2000 rev/min for 8 min) and aspiration, specific bacterial IgE was detected by a further incubation with agitation for 16 hr with ¹²⁵I-labelled anti-IgE (50 μl containing 180 000 ct/min, pre-absorbed by agitation for 3 hr with half the label volume of solid phase 1:4). Following six washes with PBS 0.5% Tween to remove unbound radiolabelled antibody, the contents of the tubes were counted in a gamma counter. The number of radioactive counts bound to the solid phase is directly proportional to the concentration of specific bacterial IgE in the serum, i.e. RAST

score. The results are expressed as a ratio of the patient's RAST to cord blood RAST, *viz.*

$$\text{RAST score ratio} = \frac{\text{Patient's specific RAST score}}{\text{Cord blood specific RAST score}}$$

Inhibition studies

The specificity of the assay was established by the inhibition of binding of specific ^{125}I -anti-IgE with freeze-dried bacterial antigen.

Two hundred and fifty microlitres of sera previously giving a positive RAST score to *Str. pneumoniae* (five sera), *Sta. aureus* (eight sera) or *H. influenzae* (five sera) were absorbed with 1 mg, 3 mg, 10 mg and in some cases 20 mg specific freeze-dried antigen, and also with 10 mg of the other two freeze-dried antigens to test for cross-reactivity between the bacteria. Cord blood sera were treated similarly. The absorptions were agitated in tubes at room temperature for 24 hr and after centrifugation (2000 rev/min for 10 min) the supernatants were assayed in duplicate.

Percentage inhibition figures were calculated as follows:

$$\% \text{ Inhibition} = \frac{(\text{Neat serum RAST} - \text{neat cord RAST}) - (\text{Absorbed serum RAST} - \text{absorbed cord RAST})}{\text{Neat serum RAST} - \text{neat cord RAST}} \times 100$$

Determination of total IgE concentration in human serum. Total serum IgE estimations were also made on the sera and were determined in an assay employing anti-human IgE immobilized by coupling to cyanogen bromide activated Sepharose 4B. This method is based on that described by Ceska and Lundkvist (1972).

One millilitre rabbit anti-human IgE (Behring) was immobilized by coupling to 12.5 ml packed volume of activated Sepharose 4B. The assay was performed as for the IgE RAST. The number of radioactive counts bound to the solid phase are directly proportional to the concentration of total IgE in the serum. Absolute values were derived from a standard curve constructed for each assay with a suitable reference serum (Laboratory Standard) obtained by testing doubling dilutions from 1:1 to 1:512.

The laboratory standard was first established by running it in parallel in the assay with the WHO standard of known total IgE content six times.

Results

Table 1 shows data on numbers, age, atopic status and bacterial skin prick test reactivity.

Atopic status—common allergens. One or more positive skin prick test reactions were given by 10% chronic bronchitis, 100% ABPA, 85% bronchial asthma, 73% allergic rhinitis, 47% urticaria and 70% cystic fibrosis.

Bacterial skin prick tests. Immediate wealing type I skin reactions were elicited by the skin prick method against extracts of all three bacteria studied. The macroscopic appearance of these reactions was similar to that following testing with common inhalant allergens.

Thirty three of the 133 patients in the study had a positive skin prick test to one or more of the bacterial antigens. Four of these patients were from the non-atopic category.

Table 1. Data on study patients

Disease group	Group (n)	Average age (years)	Age range (years)	Atopic state (1 or > 1 SPT)	Skin prick test positivity		
					<i>Sta. aureus</i> extract	<i>Str. pneumoniae</i> extract	<i>H. influenzae</i> H ₁ H ₂ extract
Chronic bronchitis	30	66	55-80	3:30	2:30	2:30	1:30
ABPA	20	51	24-71	20:20	12:20	9:20	3:20
Bronchial asthma	20	37	22-73	17:20	2:19	2:19	0:19
Rhinitis	22	33	19-67	16:22	2:22	6:22	0:22
Urticaria	20	35	16-69	9:19	2:15	4:15	1:15
Cystic fibrosis	23	22	9-41	16:23	0:22	2:22	1:22
Blood Bank	20	36	19-60	—	—	—	—
Total					20:128	25:128	6:128
Percentage positive					15.6	19.5	5.0

Of patients overall, 19.5% gave positive skin prick tests to *Str. pneumoniae* extract, 15.6% were positive to *Sta. aureus* extract and only 5% were positive to the H₁H₂ fractions of *H. influenzae* extract.

RAST inhibition results

Figure 1 displays dose response curves of bacterial RAST scores with increasing amounts of absorbing antigen. With *Sta. aureus* such curves were obtained with all the positive RAST sera but not with the cord blood serum (Fig. 1a).

The *Str. pneumoniae* RAST inhibitions are similarly shown in Fig. 1b. With all but one serum, the specific antibody titration is reduced to cord blood value (100% inhibition) by absorption with 10 mg freeze-dried specific antigen.

The *H. influenzae* RAST inhibitions are shown in Fig. 1c and whilst dose response curves were again obtained, more antigen (20 mg) was required to reduce the *H. influenzae* RAST score to cord blood values and 100% inhibition was only achieved with one serum.

Figure 2 displays the percentage inhibition in histogram form of the other two bacterial antigens compared with that of the specific antigen. A certain degree of cross-reactivity is found to occur between the bacteria, for whilst the inhibition is always greatest with the specific antigen, a varying amount of RAST inhibition (0-57%) does occur with the other two bacteria, especially in the *Sta. aureus* and *H. influenzae* RAST systems.

Specific bacterial IgE RAST assays

The IgE RAST score ratios to the three bacteria studied are plotted in Figs 3-5. Results are shown for each of the six disease groups and the blood bank controls. The horizontal bars represent the arithmetic means for each group and χ^2 tests were applied to test for any statistically significant differences in RAST scores between the disease groups.

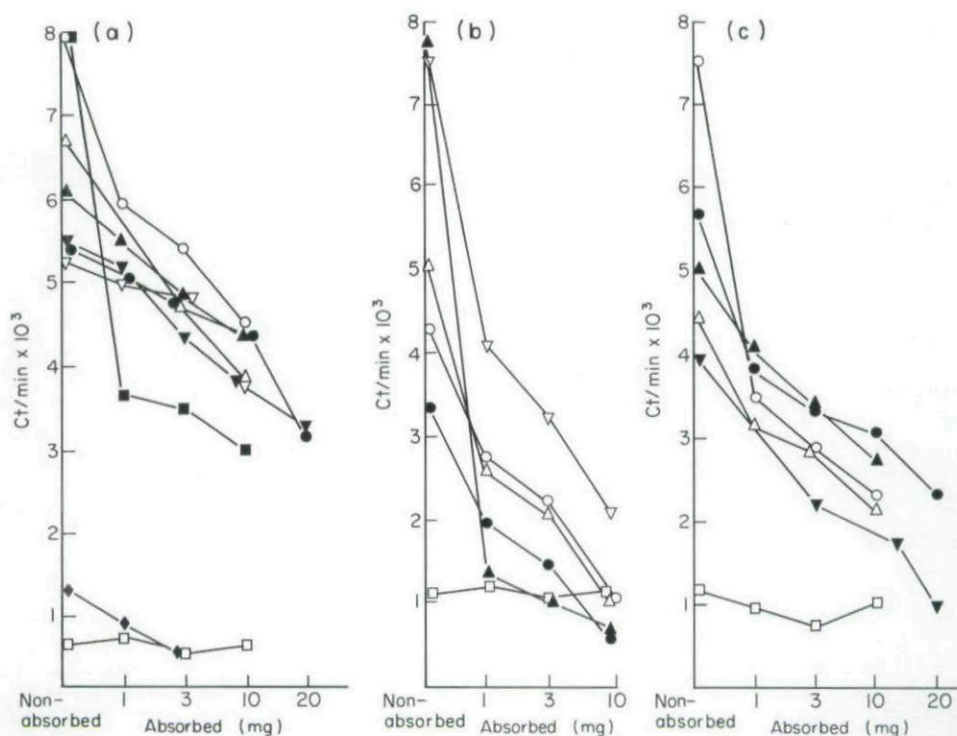


Fig. 1. IgE RAST Inhibitions; freeze-dried whole cells of (a) *Sta. aureus*, (b) *Str. pneumoniae* and (c) *H. influenzae*. □, Cord blood.

Sta. aureus IgE RAST scores (Fig. 3). The blood bank control group had a mean RAST score ratio of 1.0 and a range from 0.81 to 1.22, with one higher score of 1.49. The chronic bronchitis group scores (mean 0.8) had little spread and were significantly lower ($P < 0.001$) than the other disease groups. In the ABPA, (mean 1.4), asthma (mean 1.2), rhinitis (mean 1.0), urticaria (mean 1.1) and cystic fibrosis groups (mean 1.2), there was a greater range of RAST scores, some patients having demonstrable specific antibody to *Sta. aureus*. The patients' RAST scores in the ABPA group (mean 1.4) were significantly higher than those in all other groups ($P < 0.01$). On application of a median test the ABPA was greater than all the other disease groups ($P < 0.001$). There were no significant differences between the asthma, rhinitis, urticaria and cystic fibrosis groups.

Str. pneumoniae IgE RAST scores (Fig. 4). The RAST score ratios of the ABPA group (mean 1.7) were again higher than all other groups (χ^2 test, $P < 0.01$) and higher than all the disease groups (median test, $P < 0.001$). RAST score ratios were higher generally with *Str. pneumoniae* than with *Sta. aureus* with ratios > 1.4 occurring in all groups. Ratios ranged from 0.7 in the chronic bronchitis group to 5.03 in the ABPA group. The scores of the chronic bronchitis group (mean 1.0) were significantly lower than all the other disease groups (median test, $P < 0.001$). There were no significant differences between the asthma (mean 1.3), rhinitis (mean 1.3), urticaria (mean 1.3) and cystic fibrosis (mean 1.2) groups.

H. influenzae IgE RAST scores (Fig. 5). With this organism, the chronic bronchitis,

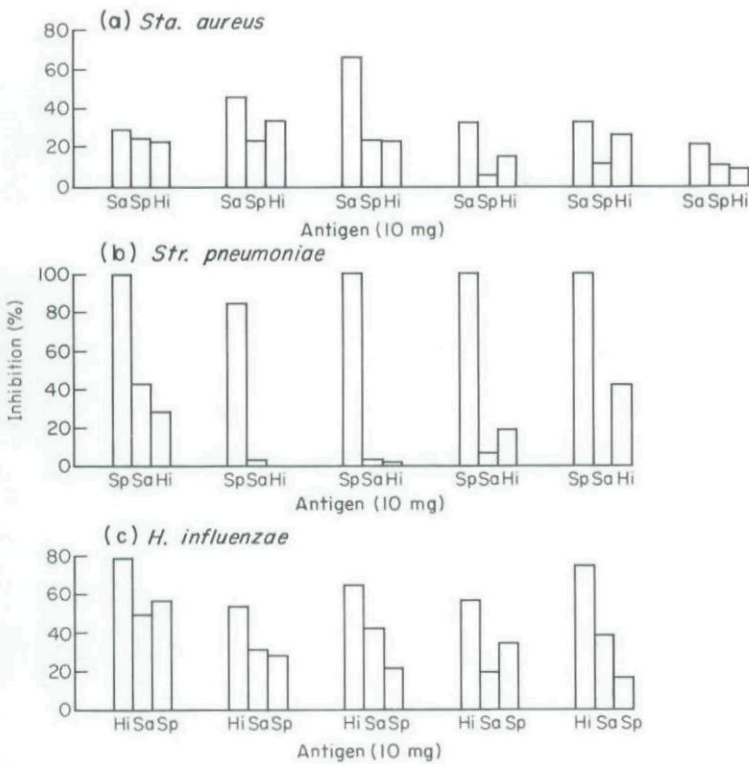


Fig. 2. IgE Sepharose RAST assays of cross-reaction antigen inhibition. Sa, *Sta. aureus*; Sp, *Str. pneumoniae*; Hi, *H. influenzae*.

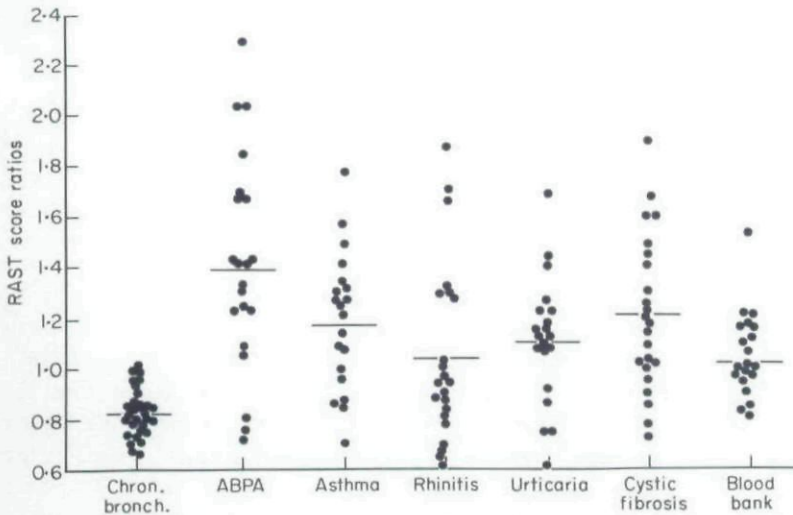


Fig. 3. IgE RAST scores of study patients to whole cells of *Sta. aureus*.

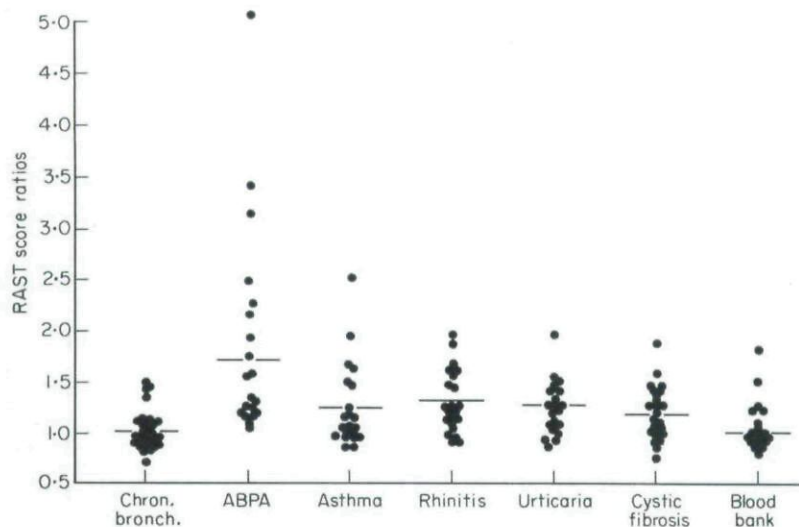


Fig. 4. IgE RAST scores of study patients to whole cells of *Str. pneumoniae*

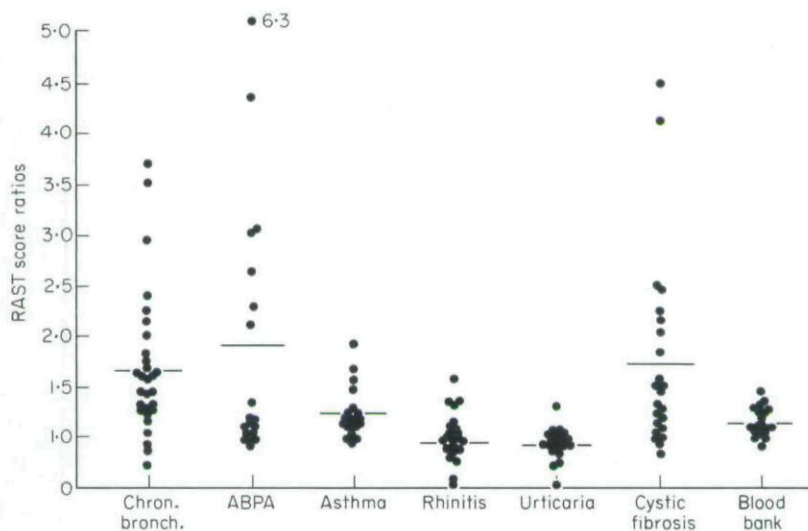


Fig. 5. IgE RAST scores of study patients to whole cells of *H. influenzae*

ABPA and cystic fibrosis groups all had significantly raised RAST scores. Those of the chronic bronchitis group (mean 1.7) were significantly higher than the asthma (mean 1.2), rhinitis (mean 0.9), and urticaria (mean 0.9) groups (median test, $P < 0.001$). Those of the ABPA (mean 1.9) and asthma groups were significantly higher than the rhinitis group ($P < 0.005$) and the urticaria group ($P < 0.001$). Those of the cystic fibrosis group (mean 1.7) were also significantly higher than these two groups at the 0.1% level.

Total IgE Measurements (Fig. 6). Figure 6 shows the total IgE levels for the seven study groups and the horizontal bars denote the geometric means of the groups.

Using the same assay, the total IgE levels of seventy-eight blood bank donor sera

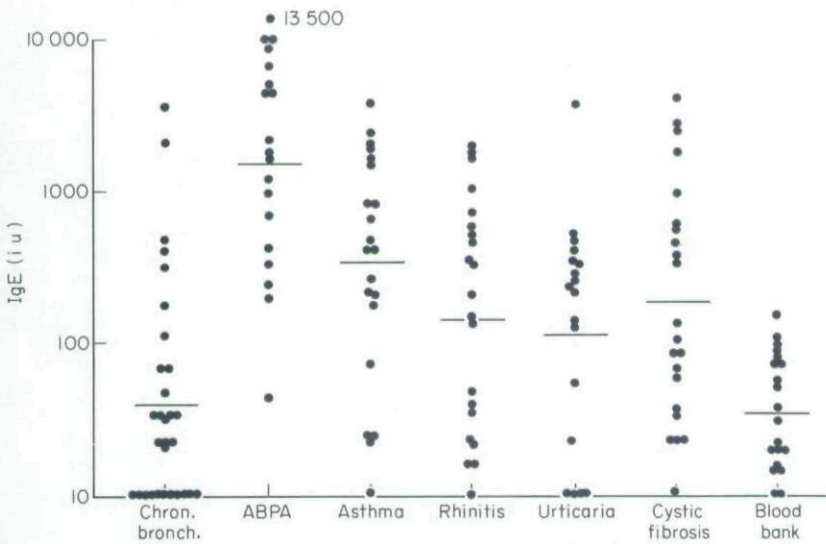


Fig. 6. Total IgE levels of study patients

had been estimated to range from 2–240 iu/ml (geometric mean 33.6 iu/ml). Taking 240 iu/ml as the upper limit of normal, none of the current blood bank study patients had a raised total IgE level. Four of the chronic bronchitis patients had raised levels (maximum 3700 iu) as did seventeen of the ABPA group (maximum 13 500 iu/ml); fifteen of the asthma patients (maximum 3800 iu), eleven of the rhinitis patients (maximum 1800 iu), ten of the urticaria patients (maximum 3700 iu) and ten of the cystic fibrosis patients (maximum 4000 iu).

On application of a median test to the results the mean of the total IgE of the blood bank sera was significantly lower than all the other disease groups except the chronic bronchitis group ($P < 0.001$), whilst the ABPA total IgE mean was significantly higher than all other groups ($P < 0.001$). There were no significant differences between the means of the remaining groups.

The number of patients in each disease group with RAST score ratios greater than 1.5 are shown in Table 2.

When individual patients were considered, only five patients (three in the ABPA group, one in the rhinitis group and one in the cystic fibrosis) had RAST score ratios greater than 1.5 to all three bacteria. Three patients (two ABPA, one rhinitis) had RAST score ratios of this level to both *Sta. aureus* and *Str. pneumoniae*. Three patients (two ABPA, one asthma) had scores > 1.5 to *Str. pneumoniae* and *H. influenzae* whilst four patients had scores > 1.5 to *Sta. aureus* and *H. influenzae* (one asthma, three cystic fibrosis). The total IgE levels of these patients ranged from 460 to 9900 iu/ml.

Correlation of skin prick tests with bacterial IgE RAST score ratios. The bacterial IgE RAST scores of all the patients in the study were considered in relation to their skin prick test positivity to the bacterial extracts. Data are presented in Table 3. Mean RAST scores with all three bacteria were higher in patients with positive specific skin prick tests, viz. with *Str. pneumoniae*, mean RAST score ratios for the skin test positive group were 1.52 compared with 1.21 in the skin test negative group. Similarly with *Sta. aureus* they were 1.29 as against 1.06 and with *H. influenzae* 2.03 as against 1.38.

Table 2. No. of patients with raised IgE RAST scores

Disease group	RAST scores					
	<i>Sta. aureus</i>		<i>Str. pneumoniae</i>		<i>H. influenzae</i>	
	> 1.5	> 2	> 1.5	> 2	> 1.5	> 2
Chronic bronchitis	0	0	0	0	14	6
ABPA	6	3	10	6	7	7
Asthma	2	0	4	1	2	0
Rhinitis	3	0	6	0	1	0
Urticaria	1	0	2	0	0	0
Cystic fibrosis	4	0	2	0	10	7
Blood bank	0	0	1	0	0	0

Table 3. Correlation of skin prick test positivity of study patients with their specific bacterial RAST score ratios

Antigen	Skin prick test (RAST score)				P†
	Positive		Negative		
	Mean*	n	Mean*	n	
<i>Str. pneumoniae</i>	1.52(±0.09)	25	1.21(±0.04)	103	<0.001
<i>Sta. aureus</i>	1.29(±0.08)	20	1.06(±0.03)	108	<0.02
<i>H. influenzae</i>	2.03(±0.67)	6	1.38(±0.06)	122	NS

*Mean (±s.e. mean)

†Mann-Whitney U test

On application of a Mann-Whitney U test significant correlations were found between specific positive skin prick tests and specific bacterial RAST scores to *Str. pneumoniae* and *Sta. aureus* at the 0.1 and 2% levels respectively, but not with *H. influenzae*.

Correlation of skin prick tests with total IgE levels. The total IgE levels of the patients in the study were also considered in relation to their skin prick test reactions to bacterial extracts. Data are presented in Table 4.

Patients with a positive skin prick test to *Str. pneumoniae*, *Sta. aureus* or *H. influenzae* had higher mean total IgE levels than patients with negative bacterial skin prick tests. By Mann-Whitney assessment a significant correlation was found between positive skin prick tests to *Str. pneumoniae* and *Sta. aureus* and total IgE at the 1 and 0.1% level respectively, but not with *H. influenzae*.

Discussion

In this study evidence favouring the involvement of type I allergic reactions has been given by the significant association between the presence of positive skin prick tests and raised levels of specific IgE antibody in the serum.

Table 4. Correlation of skin prick test positivity of study patients with their total IgE levels

Antigen	Skin prick test (iu/ml)				P†
	Positive		Negative		
	Mean*	n	Mean*	n	
<i>Str. pneumoniae</i>	2092(± 706)	24	794(± 176)	100	<0.01
<i>Sta. aureus</i>	2299(± 548)	20	778(± 203)	104	<0.001
<i>H. influenzae</i>	2568(± 1623)	6	941(± 187)	118	NS

*Mean (± s.e. mean)

†Mann-Whitney U test

The blood bank control group had the lowest geometric mean total IgE (35 iu/ml) and did not give raised specific RAST scores to any of the three bacteria studied. In contrast the ABPA group, whilst showing a wide variation in individual RAST scores, gave the highest specific mean RAST scores to all three bacteria and also had the highest geometric mean total IgE (1520 iu/ml).

It is interesting that the mean *H. influenzae* RAST score ratios in the chronic bronchitis and the cystic fibrosis group were also significantly higher than in the other disease groups, since *H. influenzae* is known to be the principal pathogen involved in chronic bronchitis (May, 1967) and with *Sta. aureus* and *Pseudomonas aeruginosa* is the commonest pathogen found in cystic fibrosis.

There was a positive correlation between the patients' *Sta. aureus* and *Str. pneumoniae* immediate-type skin reactions and their RAST scores and their total serum IgE concentrations. The low incidence of immediate-type skin test positivity to *H. influenzae* prevented comparable correlations with this organism and in future work, a higher concentration of antigen should be used for skin prick testing.

RAST is seen to give a more definitive answer in the measurement of the patient's allergic reaction to an allergen and the test can be adapted for quantifying other immunoglobulins. The specificity of the RAST systems is shown by the 100% inhibition obtained with *Str. pneumoniae* and *H. influenzae*. The less good inhibition with *Sta. aureus* (maximum 68%) may be due to the strain studied, in common with most pathogenic strains, containing Protein A, which has been shown to fix non-specifically to the Fc fragment of three of the four human IgG subclasses (Forsgren & Sjoquist, 1966). Additionally Johansson (1978) has reported that a high proportion of IgE from serum samples containing polyclonal IgE showed a high affinity for Protein A, possibly via the F(ab')₂ region. However, it cannot be said that *Sta. aureus* RAST scores were directly related to total IgE levels in this study since the correlation value *r* was only 0.422 for these two criteria.

These results, taken together, suggest that specific IgE antibodies to bacteria can be identified in the sera of patients from a number of respiratory diseases and especially ABPA. With further development of the technology the role of bacteria in atopic and allied disease may be more clearly defined.

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