

Relationship between the results of skin, conjunctival and bronchial tests and RAST with *Candida albicans* in patients with asthma

K. AKIYAMA, Y. YUI*, T. SHIDA* and T. MIYAMOTO

Department of Medicine and Physical Therapy, Faculty of Medicine, University of Tokyo, Tokyo, Japan, and *Allergy Centre, Sagamihara National Hospital, Sagamihara, Japan

(Received 15 August 1979; resubmitted 1 May 1980; accepted for publication 5 December 1980)

Summary

The results of the skin, conjunctival and inhalation tests and RAST with *Candida* antigen were compared with each other in patients with bronchial asthma. The correlation coefficient between the skin test and RAST was 0.50 ($P < 0.01$). The correlation coefficient between the skin test, RAST and the inhalation tests was 0.36 ($P < 0.01$) and 0.57 ($P < 0.01$). The positive agreement of the inhalation test to the skin test, RAST and the conjunctival test was 77, 86 and 93% respectively. In this study the conjunctival test was thought to be the best method in place of the inhalation test. There was no significant relation between the serum IgE concentration and the endpoint of the skin titration testing, and a low correlation was noticed between the serum IgE concentration and RAST counts ($r = 0.36$).

Introduction

Candida albicans has been thought to be one of the important causative allergens in bronchial asthma (Keeney, 1951; Itkin & Dennis, 1966). Many observers have reported immediate and late responses in the skin test and the provocation inhalation test with extracts of *C. albicans* (Herxheimer, 1952; Itkin & Dennis, 1966; Pepys *et al.*, 1968). The relationships between protein and polysaccharide fractions of *Candida* antigen and the types of reaction have been reported by some investigators (Pepys *et al.*, 1968; Kabe *et al.*, 1971; Longbottom *et al.*, 1976). Comparisons of the diagnostic methods for atopic asthma including RAST have been reported by many investigators against pollen, house dust and house-dust mite (Miyamoto, 1960; Aas, 1970; Aas & Johansson, 1971; Miyamoto *et al.*, 1974; Apold *et al.*, 1974; Berg & Johansson, 1974; Bryant, Burns & Lazarus, 1975; Collins-Williams & Bremner, 1976; Yui *et al.*, 1976; Stenius-Aarniala, Malmberg & Holopainen, 1978). However, these authors did not report on *Candida* antigen. Consequently, the immediate reactions of the skin test, the

Correspondence: Dr Kazuo Akiyama, Department of Medicine and Physical Therapy, Faculty of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo, Japan.

inhalation test, the conjunctival test and RAST with *Candida* antigen were compared with each other.

Materials and methods

Patients

One hundred and forty-nine asthmatic patients (sixty-five males and eight-four females), between 18 and 60 years of age, were the subjects of this study. They were examined with intradermal, conjunctival and provocative inhalation tests with *C. albicans* antigen. The sera were stored at -20°C until used for RAST and total IgE determination. None of the patients was receiving hyposensitization treatment with *C. albicans* at the time of testing. They were treated mainly with bronchodilators.

Skin testing

Intradermal skin testing was performed with thirty-five allergens, which included pollens, dusts, moulds and foods, as a routine. The allergens used in this study were obtained from Torii Pharmaceutical Company (Tokyo, Japan). The patients showing a positive reaction to *Candida* (1:10 000 w/v) were selected and then tested with serial tenfold dilutions of *Candida* extract (initial concentration 1:10 000) to determine the threshold of the skin reactivity. The skin test was performed by i.c. injection of 0.02 ml of the solution in the forearms. Bronchodilator drugs and antihistamines were discontinued for at least 12 hr before the test. When the erythema was more than 20×20 mm or the wheal was more than 9×9 mm within 15 min, the reaction was read as positive. The maximum dilution that elicited a positive skin reaction was considered to be the end-point. No skin reaction at 1:10 000 dilution was considered negative.

Conjunctival testing

A conjunctival test was performed on 113 patients. Lyophilized *Candida* antigen was diluted 1:200 for the test. One drop of antigen solution was instilled into the inferior recess of the lids. The appearance of itching, erythema of the conjunctiva and/or lacrimation within 5 min was considered to be a positive reaction. The test result was considered to be negative if none of these symptoms appeared.

Provocative inhalation testing

A provocation inhalation test was performed on seventy-one patients. The bronchodilators and antihistamines were discontinued for 12 hr before the test. In the test, 0.5 ml of control solution (saline) was first inhaled for 3 min, and if no reaction occurred, 0.5 ml of the allergen solution was inhaled for 3 min by means of a Vaponefrin nebulizer with compressed air at a flow rate of 3.5 l/min. FEV₁ was measured 15 min after inhalation by means of a Vitalor, and the best reading used. The provocation inhalation test was considered positive if the FEV₁ fell by 15% or more. The inhalation test was commenced with a concentration of 1:32 000 *Candida* antigen solution and stepped up with twofold more potent solutions until the greatest concentration (1:1000) of the extract was reached, or until significant reduction occurred. The test result was considered negative if no reduction in FEV₁ occurred following inhalation of the greatest concentration (1:1000) of the extract. The endpoint of the provocative inhalation test was determined as the maximum dilution with positive reaction.

RAST

Solid phase antigen. Cellulose paper discs (Toyo Roshi Kaisha Ltd, Tokyo) were activated with CNBr and coupled to the Candida antigen according to the method of Ceska *et al.* (1972). About 20 mg of lyophilized Candida extract in 10 ml of phosphate buffered saline were used for coupling to 100 paper discs. The allergen-coupled paper discs were stored in RAST incubation buffer (phosphate buffer 0.05 M, NaCl 0.9%, BSA 0.3%, NaN_3 0.05% and Tween 20 0.1% pH 7.4) at 4°C until use.

Incubation with sera. Fifty μl of 1:10 sera of patients were incubated at room temperature with one paper disc overnight, and washed with RAST incubation buffer 3 times.

Incubation with radiolabelled anti IgE. ^{125}I -labelled anti-IgE was obtained from Pharmacia Diagnostics AB (Uppsala, Sweden). Fifty μl of ^{125}I -anti IgE, which was adjusted to about 20 000 ct/min in 50 μl , was added to each tube containing the sera with paper disc and incubated overnight at room temperature and then washed with RAST buffer three times. The radioactivity combined with paper disc was measured with a gamma counter (Aloca Autowell Gamma System TDL-5).

Result. The results of RAST was expressed as the percentage of total counts of radiolabelled anti-IgE added. The result was considered positive when the count of the patient was more than 1.5 times that of healthy control subjects with negative skin test with Candida antigen.

Serum IgE level

Serum IgE level was measured by the RIST method (Pharmacia Diagnostics AB, Uppsala, Sweden).

Results

Skin test vs RAST

The relationship between the skin test and RAST was compared in 122 patients. The correlation coefficient was 0.50 ($P < 0.01$) as shown in Table 1. Positive RAST results were noted in fifty-eight of 100 patients with positive skin test. Negative RAST results were noted in nineteen of twenty-two patients with negative skin test. Agreement was 63%.

Skin test vs inhalation test

The relationship between the skin test and the inhalation test was compared in

Table 1. The relationships between the results of the skin test and RAST with *Candida albicans* extract

	Endpoint of skin test				
	$<10^{-4}$	10^{-4}	10^{-5}	10^{-6}	10^{-7}
RAST	1.32	3.58	3.40	6.53	15.57
(percentage counts)	± 0.72	± 3.14	± 4.39	± 6.08	± 6.65

$r = 0.50$; $P < 0.001$.

Table 2. The relationship between the results of skin test and the provocative inhalation tests with *Candida albicans* extracts

	End-point of skin test			
	$< 10^{-4}$	10^{-5}	10^{-6}	10^{-7}
Threshold of provocative inhalation test ($\times 10^{-3}$)	2.88 ± 1.76	2.76 ± 1.51	3.78 ± 2.02	5.29 ± 1.50

$r = 0.36$; $P < 0.01$.

Table 3. Agreement between the provocative inhalation test and the skin, RAST and the conjunctival test with *Candida albicans* extracts

		Patients (n)	Inhalation test		Agreement (%)
			Negative	Positive	
Skin test	{ Negative		ND	ND	
	{ Positive	71	16	55	77
RAST	{ Negative	20	8	12	40
	{ Positive	50	7	43	86
	Total	70	15	55	73
Conjunctival test	{ Negative	19	9	10	47
	{ Positive	41	3	38	93
	Total	60	12	48	78

Table 4. The relationships between the results of RAST and provocative inhalation test with *Candida albicans* extracts

		Threshold of provocative inhalation test ($\times 10^{-3}$)						
RAST	1.93	3.64	4.45	12.00	7.43	13.75	14.83	
(% counts)	± 1.03	± 2.62	± 3.39	± 8.45	± 5.63	± 12.37	± 9.81	

$r = 0.57$; $P < 0.01$.

seventy-two patients with positive skin test. The correlation coefficient was 0.36 ($P < 0.01$) as shown in Table 2. Fifty-five patients with positive skin test reacted positively to the inhalation test (77% for positive agreement) (Table 3).

Inhalation test vs RAST

The relationship between the inhalation test and RAST was compared in seventy patients. The correlation coefficient was 0.57 ($P < 0.01$) as shown in Table 4. Forty-three of the fifty patients with positive RAST reacted positively to the inhalation test (86% for positive agreement). Eight of the twenty patients with negative RAST did not react to the inhalation test (40% for negative agreement). The overall agreement was 73% (Table 3).

Conjunctival test vs skin and inhalation tests and RAST

Figure 1 shows the relationships between the conjunctival test and the skin test (112 patients), inhalation test (sixty patients) and RAST (eighty-seven patients). There was no significant difference between the end-point of the skin test and the reactivity in the conjunctival test, but there were significant differences between RAST, the inhalation test and the reactivity in the conjunctival test ($P < 0.001$). The agreement was 51% (conjunctival-skin), 78% (conjunctival-inhalation) and 82% (conjunctival-RAST).

Serum IgE levels vs skin and conjunctival tests and RAST

The relationships between the serum IgE levels and the results of the skin test (Fig. 2), the conjunctival test (Table 5) and RAST (Fig. 3, Table 5) were low.

Discussion

Mould has been thought to be one of the most important causative allergens of bronchial asthma. Keeney (1951) reported the first case of bronchitis and bronchial asthma due to *C. albicans*. Since then attention has been paid to *C. albicans* antigens especially as a causative agent of types 3 or 4 asthma. Pepys *et al.* (1968) demonstrated that precipitins against mannan antigens of *C. albicans* group A were common in asthma and especially in asthmatic patients with pulmonary eosinophilia. Kabe *et al.*

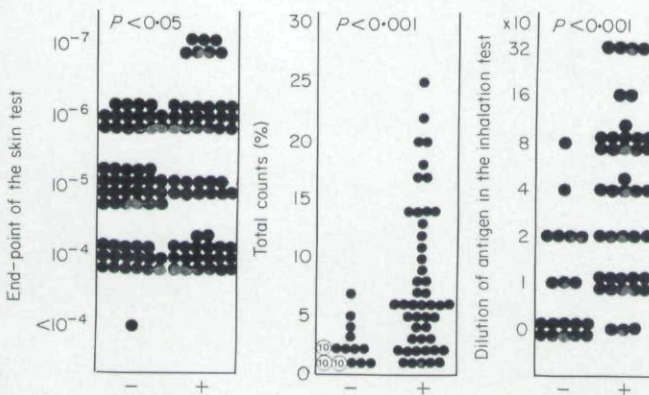


Fig. 1. The relationships between the results of the conjunctival test and the skin test, RAST and the provocative inhalation test with *Candida albicans* extracts. (—): negative conjunctival test, (+): positive conjunctival test.

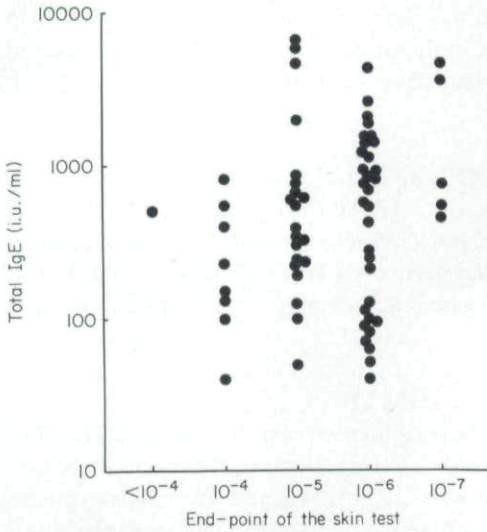


Fig. 2. The relationships between the total IgE levels (RAST) and the results of the skin test with *Candida albicans* extracts.

(1971) demonstrated that polysaccharide fraction provoked an immediate or delayed reaction or both. Longbottom *et al.* (1976) reported that passive transfer tests in the monkey gave the immediate reactions to the purified cytoplasmic protein mediated by heat-labile long-term sensitizing antibody, and to the polysaccharide, mannan, mediated by heat-stable short-term sensitizing antibody. On the other hand, *Candida* is known as an infectious agent of superficial or deep-seated infections in patients with malignant diseases treated with antimicrobial or immunosuppressive drugs. Precipitin antibodies against *C. albicans* were found in sera from patients with systemic or visceral *Candida* infection by Chew & Theus (1967). Many observers have reported the

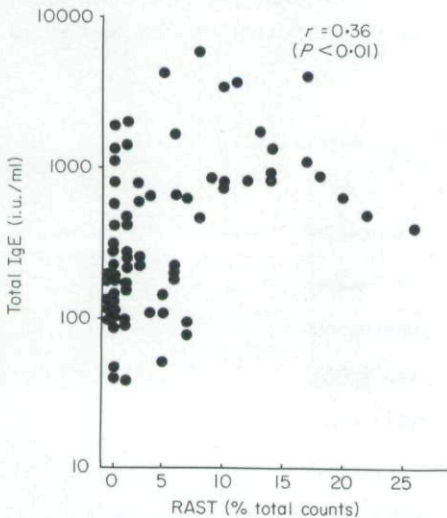


Fig. 3. The relationships between the total IgE levels (RAST) and the specific IgE antibodies against *Candida albicans* extracts.

Table 5. Agreement between the total IgE levels and RAST, the conjunctival test with *Candida albicans* extracts

		Total IgE (i.u./ml)	Patients (n)	Negative	Positive	Rate of positive reaction (%)
RAST	> 500		43	25	18	42
	500-1000		16	2	14	88
	< 1000		16	2	14	88
	> 500		39	24	24	39
Conjunctival test	500-1000		14	2	12	86
	< 1000		15	4	11	73

presence of agglutinating antibodies to *C. albicans* in normal subjects and an increasing frequency with age of positive skin test reactions to *C. albicans*. Taking these reports into consideration, it is not so easy to determine that *C. albicans* is the true causative agent of bronchial asthma. We could find only a few reports about type I allergy to *C. albicans*. RAST is a useful method to detect specific IgE antibodies *in vitro*. Many reports are established about close relationships between RAST and other methods in the cases of pollen, house dust and house-dust mite (Wide, Bennich & Johansson, 1967; Stenius & Wide, 1969; Apold *et al.*, 1974; Berg & Johansson, 1974; Yui *et al.*, 1976). Some observers (Collins-Williams & Bremner, 1976) reported that RAST against Alternaria or Aspergillus antigens correlated fairly well with the skin or the inhalation test, but the data of RAST against Candida were not reported. RAST against mould antigens is thought to be not so useful in detecting IgE antibodies because the major antigenic components of moulds are believed to be polysaccharides that are difficult to conjugate with the carrier activated with CNBr. So modifications of the carrier have been designed by some investigators (Yui *et al.*, 1976). But in this study RAST against *C. albicans* was performed by the conventional method with the sera of 122 subjects with positive skin test to *C. albicans*. The highest counts were 25% of the total counts and the mean value of the control subjects with negative skin test was under 2% of the total counts. The correlation coefficient between RAST and skin test was 0.50 ($P < 0.01$) and agreement was 63%. We have some data about Aspergillus and Alternaria: in the case of Aspergillus (fifty-eight subjects) highest counts were 33% of total, $r = 0.55$ ($P < 0.01$), and agreement was 63%; in the case of Alternaria (fifty-four subjects) the highest counts were 32% of the total, $r = 0.61$ ($P < 0.01$) and agreement was 63%. These data are the same as those of Bryant *et al.* (1975) regarding the correlation coefficient ($r = 0.51-0.56$) and show a higher positive agreement than those of Collins-Williams (1976). We think, therefore that RAST against crude mould antigens detected specific IgE antibodies against crude Candida antigens and can be used for clinical purposes. The inhalation test is a direct method to determine the causative allergen of bronchial asthma. But, it is not easy to make this test for every subject. Especially in the case of mould, the frequent appearance of late reactions make it difficult to do the test. So other easier and more convenient methods than the inhalation test are desirable. In this study, the skin test, RAST, and conjunctival test were compared with the inhalation test. The correlation between RAST and the

inhalation test was higher than that between the skin test and the inhalation test ($r=0.57:0.36$). This agrees with other data published (Apold *et al.*, 1974; Berg & Johansson, 1974; Bryant, Burns & Lazarus, 1975). The positive agreements of the skin test, RAST, and the conjunctival test with the inhalation test were 77, 86 and 93% respectively. Although Itkin & Dennis (1966), demonstrated that the agreement of the inhalation test with *C. albicans* with the conjunctival test was 54% (and it was not recommended to substitute the conjunctival test for the inhalation test), from these data the conjunctival test is thought to be the best method in place of the inhalation test—the second was RAST and the last was the skin test. Table 6 shows the relationships among the four different diagnostic methods. Positive agreement of three methods (RAST, skin test, conjunctival test) is the most reliable compared with positive reaction in the inhalation test (95%). Therefore, to replace the inhalation test all three methods are recommended. Some relation has been reported between the serum IgE concentration and the end-point of the skin titration testing with mite. In this study there was no significant relation between them. But a low relation was noticed between the serum IgE concentration and RAST counts ($r=0.36$). Apold *et al.* (1974) demonstrated that the specific IgE antibodies titre was higher in a group with elevated total IgE than in a group with normal IgE levels. The same tendency was also noted in our study (Table 5).

The appearance of a late or dual reaction following the skin test and the inhalation test, and the fact that the allergen components of *C. albicans*, such as polysaccharide and proteins, are related to these reactions, has been noticed recently in the case of *Candida* antigen (Pepys *et al.*, 1968; Kabe *et al.*, 1971). But, in this study we did not

Table 6. The relationships among four different diagnostic methods

Inhalation test	Skin test	RAST	Conjunctival test	<i>n</i>
+	+	+	+	35
		+	—	3
		—	+	3
		—	—	6
	+	+		4
		—	n.d.	3
		+	+	2
		+	—	4
		—	+	1
		—	—	6
		+		1
		—	n.d.	1
		n.d.	n.d.	2

+, Positive results; —, negative result; n.d. not done.

check this reaction nor its relation to the components. Further study is needed to clarify this point.

References

- AAS, K. (1970) Bronchial provocation tests in asthma. *Archives of Diseases in Childhood*, **45**, 221.
- AAS, K. & JOHANSSON, S.G.O. (1971) The radioallergosorbent test in the *in vitro* diagnosis of multiple reagenic allergy. *Journal of Allergy and Clinical Immunology*, **48**, 134.
- APOLD, J., HAVNEN, J., HVATUM, M., OSEID, S. & AAS, K. (1974) The radioallergosorbent test (RAST) in the diagnosis of reagenic allergy. *Clinical Allergy*, **4**, 401.
- BERG, T.L.O. & JOHANSSON, S.G.O. (1974) Allergy diagnosis with the radioallergosorbent test. *Journal of Allergy and Clinical Immunology*, **54**, 209.
- BRYANT, D.H., BURNS, M.W. & LAZARUS, L. (1975) The correlation between skin tests, bronchial provocation tests and the serum level of IgE specific for common allergens in patients with asthma. *Clinical Allergy*, **5**, 145.
- CESKA, M., ERIKSSON, R. & VARGA, J.M. (1972) Radioimmunosorbent assay of allergens. *Journal of Allergy and Clinical Immunology*, **49**, 1.
- CHEW, W.H. & THEUS, T.L. (1968) Candida precipitins. *Journal of Immunology*, **98**, 220.
- COLLINS-WILLIAMS, C. & BREMNER, K. (1976) Comparison of skin tests and RAST in the diagnosis of atopic hypersensitivity. *Annals of Allergy*, **36**, 161.
- HERXHEIMER, H. (1952) The late bronchial reaction in induced asthma. *International Archives of Allergy and Applied Immunology*, **3**, 328.
- ITKIN, I.H. & DENNIS, M. (1966) Bronchial hypersensitivity to extract of *Candida albicans*. *Journal of Allergy*, **37**, 187.
- KABE, J., AOKI, Y., ISHIZAKI, T., MIYAMOTO, T., NAKAZAWA, H. & TOMARU, M. (1971) Relationship of dermal and pulmonary sensitivity to extracts of *Candida albicans*. *American Review of Respiratory Disease*, **104**, 348.
- KEENEY, E.L. (1951) Candida asthma. *Annals of Internal Medicine*, **34**, 223.
- Longbottom, J.L., Brighton, W.D., Eady, G. & Pepys, J. (1976) Antibodies mediating type I skin test reaction to polysaccharide and protein antigens of *Candida albicans*. *Clinical Allergy*, **6**, 41.
- MIYAMOTO, T. (1960) The correlation between skin test, conjunctival test and bronchial provocation test in patients with asthma. *Japanese Journal of Allergy*, **9**, 968.
- MIYAMOTO, T., JOHANSSON, S.G.O., ITO, K. & HORIUCHI, Y. (1974) Atopic allergy in Japanese subjects: studies primarily with radioallergosorbent test. *Journal of Allergy and Clinical Immunology*, **53**, 9.
- PEPYS, J., FAUX, J.A., Longbottom, J.L., McCarthy, D.S. & HARGREAVE, F.E. (1968) *Candida albicans* precipitins in respiratory disease in man. *Journal of Allergy*, **41**, 35.
- STENIUS, B. & WIDE, L. (1969) Reagenic antibody (IgE), skin and provocation tests to *Dermatophagoides culinae* and house dust in respiratory allergy. *Lancet*, **ii**, 455.
- WIDE, L., BENNICH, H. & JOHANSSON, S.G.O. (1967) Diagnosis of allergy by an *in-vitro* test for allergen antibodies. *Lancet*, **ii**, 105.
- YUI, Y., MIYAMOTO, Y., NISHIMURA, H., SHIMIZU, S. & SHIDA, T. (1976) Atopic allergy diagnosis by use of radioallergosorbent test (RAST). *Japanese Journal of Allergy*, **25**, 493.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.