

Clinical evaluation of a new *in-vitro* assay for specific IgE, the immuno CAP system

ANNETTE LEIMGRUBER, B. MOSIMANN, M. CLAEYS, M. SEPPEY, Y. JACCARD, V. AUBERT, R. PEITREQUIN, MARIE-PIERRE NISOLI and A. PÉCOUD

Division of Immunology and Allergy, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Summary

The Pharmacia CAP system is a new assay for specific IgE characterized by a new solid phase (hydrophilic polymer encased in a capsule). The CAP results were compared to those of the Phadebas IgE RAST and skin-prick tests (SPT) performed in 145 subjects suffering from rhinitis and/or asthma with eight common inhalant allergens (total number of tests = 1160). Concording CAP/RAST results were found in 91% of the tests. The CAP was positive in 78% and the RAST in 65% of the positive SPT. Conversely, the CAP was negative in 90.6% and the RAST in 96.4% of the negative SPT. A pattern 'negative SPT, negative RAST and positive CAP' was found in 56 tests (40 subjects): in four such subjects, the CAP positivity was confirmed by a positive IgE crossed radio-immunoelectrophoresis. Three borderline positive results were found among 240 negative controls (serum from cord blood or non-atopics). These data indicate that compared with SPT the CAP system is (a) more sensitive than the Pharmacia RAST and (b) does not seem less specific.

Clinical and Experimental Allergy, Vol. 21, pp. 127–131. Submitted 20 December 1989; revised 25 May 1990; accepted 28 August 1990.

Introduction

In 1967, Wide *et al.* [1] described the radio-allergosorbent test (RAST), the first *in-vitro* assay to detect allergen-specific IgE in serum of allergic patients. Since then a variety of assays have been produced: technical modifications have been applied to the solid phase used to render the allergens insoluble (paper disc, polystyrene tube wall), to the tracer (radio- or enzyme-labelled, monoclonal or polyclonal anti-IgE), or the substrates (spectrophotometric or fluorometric reading) [2–6]. The RAST has been shown generally to be less sensitive compared to the results of skin testing, being positive in about 60–70% of the positive skin-prick tests (SPT) and this has not been conclusively changed by the introduction of newer methods [7]. Recently, a new assay has been developed, the Pharmacia CAP system. We have compared the results of this test to those of the conventional Phadebas IgE RAST and to those of SPT, with a special emphasis on the sensitivity and the specificity of this new assay.

Correspondence: Dr A. Pécoud, Division of Immunology and Allergy, CHUV, CH 1011 Lausanne, Switzerland.

Subjects and methods

Subjects

Sera from the following subjects were tested.

- (a) One hundred and forty-five patients (83 males; mean age: 33 yr, range: 14–65) referred to our allergy outpatient clinic by their primary care physician for investigation of rhinitis (132) and/or asthma (65). In all a careful history was taken by a trained allergist. None had taken any drug which could interfere with the results of skin testing during the month prior to the investigation and none had used astemizole for 2 months.
- (b) Ten 'non-atopic volunteers', defined as adults who did not suffer from any past or present disease, being SPT and Phadebas IgE RAST negative for the following 15 extracts: *Dermatophagoides pteronyssinus* (Dpt) and *D. farinae*, cat, horse, dog, *Alternaria tenuis*, *Cladosporium herbarum*, pollen of *Dactylis glomerata*, a mixture in equal parts of four grass pollens (rye, timothy, velvet grass and cultivated rye), birch, hazel, oak, mugwort and ribwort (Pharmalgen, Pharmacia, Uppsala, Sweden). In all, total

IgE was lower than 80 kU/l (Phadezym IgE PRIST, Pharmacia).

(c) Twenty samples of cord serum, collected at birth from unselected newborns.

Allergens tested

All sera mentioned above were tested by both Phadebas IgE RAST and the CAP system to detect IgE specific for the following eight allergens: Dpt, cat, dog, horse, a grass pollen (*D. glomerata*), birch, ribwort and mugwort.

Skin prick tests

SPT were performed on the volar surface of both forearms by means of a disposable prick tester (Stallerpointe, Stallergene, Joinville-le-Point, France). A standardized allergen extract was used at the highest concentration available [7]: 10 000 BU/ml (for Dpt and animal danders) and 100 000 BU/ml (for pollens) of a standardized allergen extract (Pharmalgen, Pharmacia); SPT were not performed in duplicate. The reactions were graded according to the following scale: a test was negative if the reaction was similar to that produced by the vehicle solution (negative control) and 3+ positive when the magnitude of the weal was identical to that produced by 10 mg/ml histamine (positive control). The diameters of the weals obtained with all positive histamine controls were ≥ 4 mm. No negative control induced any sizeable weal (absence of dermographic reaction). In this study, all SPT with a negative or a 1+ result were considered negative, whereas results 2+ (3 mm) or above were considered positive.

Determination of specific IgE in serum

The conventional Phadebas IgE RAST was performed by following the manufacturers instructions exactly (single determination). Briefly, a cellulose disc to which the allergen is covalently bound is incubated with the patient's serum. After washing, radiolabelled goat anti-human IgE is added and, after a second wash, radioactivity is measured by a gamma counter. Results are expressed in PRU/ml and graded from class 0 (<0.35 PRU/ml) to class 4 (>17.5 PRU/ml). In the present study, all classes 1–4 results (≥ 0.35 PRU/ml) were considered positive.

The CAP was performed blind in the laboratories of Pharmacia Diagnostics AB, Uppsala (single determination). The technical characteristics of the assay are described elsewhere [8–10]. Briefly, the test uses a new type of solid phase, a flexible hydrophilic carrier polymer, CNBr-activated, encased in a capsule ('ImmunoCAP'). The allergen-bound IgE is detected by a mixture of

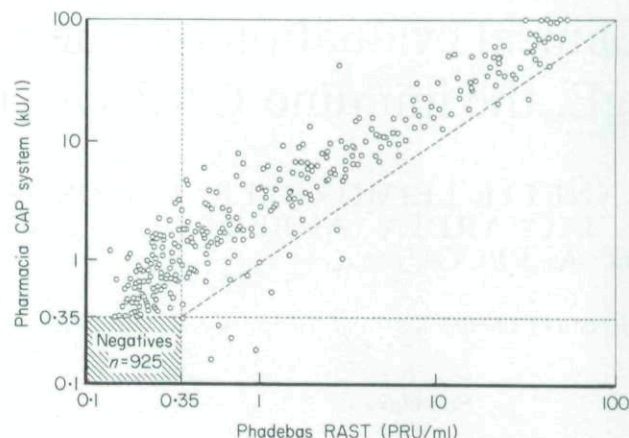


Fig. 1. Correlation between the results of RAST and CAP (145 sera tested for eight allergens: 1160 tests).

polyclonal and monoclonal antibodies directed against the Fc portion of IgE, either radiolabelled (^{125}I) or enzyme-labelled (β -galactosidase). The assay is calibrated against the WHO Standard for IgE, between 0.35 and 100 kU/l. The cut-off (positive–negative) and the class system remain the same as for the Phadebas RAST.

Detection of allergen-specific IgE by crossed immuno-electrophoresis and crossed radio-immuno-electrophoresis (CIE–CRIE)

The CIE–CRIE technique was used according to the method described by Lowenstein [11]. The allergen extract (cat: 30 μg and Dpt: 150 μg protein of Pharmalgen extract) was first subjected to electrophoresis and then migrated in the second dimension in a gel containing rabbit anti-cat and DPt (gift from R. Einarsson, Uppsala), respectively. After incubation with the patient's serum, precipitate-bound IgE were detected by incubation with [^{125}I] anti-IgE (Pharmacia) followed by autoradiography. Sera from non-atopic subjects were used as negative controls.

Results

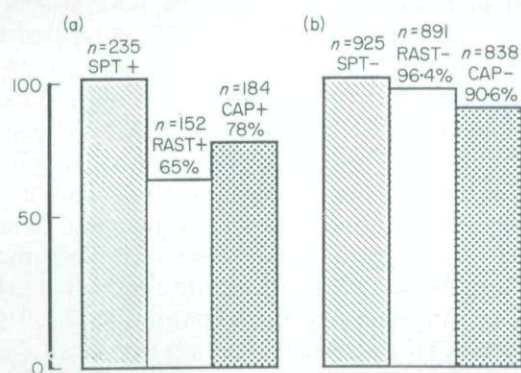
Among the 145 subjects, 43 (32 with rhinitis only, 11 with asthma) were found not to be allergic, all tests available being negative (SPT, RAST, CAP). All others had at least one test indicative of allergy.

The comparisons between CAP and RAST results of all subjects are shown in Fig. 1 and Table 1. Figure 1 shows that for each level of positivity the intensity of the signal was superior with CAP than with RAST. In Table 1, results of the concordant and discordant results are detailed: 91% of the discordant results consisted of a positive CAP and negative RAST.

Table 1. Comparison of RAST and CAP results on the same serum

Allergen	Concordant results		Discordant results	
	RAST-/CAP-	RAST+/CAP+	RAST+/CAP-	RAST-/CAP+
Dpt	104	23	0	18
Cat	109	31	0	5
Horse	121	14	1	9
Dog	107	15	2	21
Grass	86	55	0	4
Birch	114	24	0	7
Mugwort	111	19	2	13
Ribwort	107	18	0	20
Total	859	199	5	97
Percentage	74	17.2	0.4	8.4
	91.2%		8.8%	

Analysis of the concordant and discordant pairs (RAST- and CAP-positive if ≥ 0.35 PRU/l and ≥ 0.35 KU/l, respectively).

**Fig. 2.** Sensitivity (a) and specificity (b) of RAST and CAP compared to SPT.

Compared to the results of positive SPT, the CAP was more often positive (78%) than the RAST (65%) (Fig. 2a); among the 152 positive RAST, 147 were also positive with CAP. As shown in Table 2, this higher number of positive results with CAP was mainly obtained with Dpt, dog, horse, birch and ribwort.

Conversely, there were 925 negative SPT: in 891 (96.4%) of them the RAST was negative and in 838 (90.6%) the CAP was negative (Fig. 2b). Positive RAST and CAP corresponding to a negative SPT were found with all allergens. Among the 34 positive RAST, 31 were also positive with CAP.

The pattern 'negative SPT, negative RAST and positive CAP' was found in 56 tests (out of a total of 1160 tests

Table 2. Comparison of RAST and CAP results to positive SPT

Allergen	No. of positive SPT	No. of corresponding	
		Positive RAST (%)	Positive CAP (%)
Dpt	35	19 (54)	25 (71)
Cat	41	26 (63)	28 (68)
Dog	17	8 (47)	12 (71)
Horse	19	8 (42)	14 (74)
<i>D. glomerata</i>	54	47 (87)	49 (91)
Birch	23	17 (74)	20 (87)
Mugwort	24	17 (71)	18 (75)
Ribwort	22	10 (45)	18 (82)
Total	235	152 (65)	184 (78)

performed, i.e. 4.8%) and in 40 out of 145 subjects (27%). Among these positive CAP, 35 were found in class 1 and 21 in class 2. These 56 results were found with dog (13), ribwort (11), Dpt (10), mugwort (nine), horse (five), birch (four), cat (three) and *D. glomerata* (one). Ninety-one per cent of these positive CAP (51/56) were found in atopic subjects, defined as being SPT- and RAST-positive for at least one other allergen. In 23 of these 40 subjects the history was compatible with hypersensitivity to the allergen detected by CAP only.

In one such subject who suffered from chronic rhinitis, exacerbated at home where he was exposed to three cats,

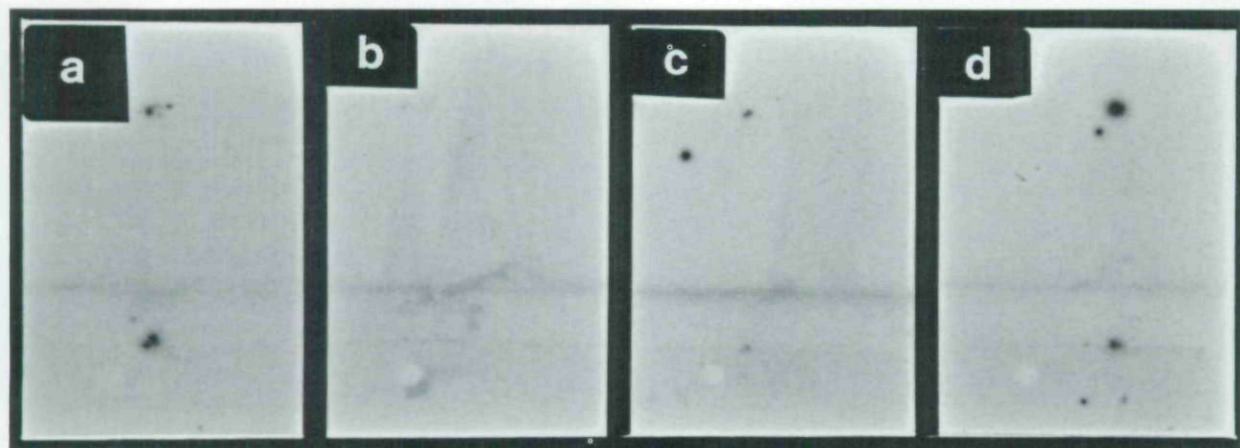


Fig. 3. CRIE pattern of four subjects with negative SPT, negative RAST and positive CAP, for cat (a) and for Dpt (b-d).

SPT and RAST were negative, whereas CAP was positive: the IgE CRIE of this subject disclosed a faint but clear-cut line of IgE bound to cat albumin (Fig. 3a). In three other subjects suffering from chronic non-seasonal rhinitis/asthma and who had negative SPT, negative RAST and positive CAP for Dpt, the IgE CRIE disclosed typical lines of IgE bound to Dpt proteins (Fig. 3b-d).

No precipitate was seen on the CRIE when these tests were performed in the same technical conditions but with serum obtained from non-atopic volunteers.

All CAP tests performed in 20 cord sera (160 tests) were negative; three (1.25%) out of 80 tests performed in serum from 10 non-allergic adult subjects were borderline positive: two for grass (0.5 and 1.7 kU/l) and one for ribwort (0.6 kU/l).

Discussion

The allergic investigation of respiratory diseases (rhinitis or asthma) is based on the association of a careful history and the detection of allergen-specific IgE either in the skin (SPT) or in serum by an *in-vitro* assay. Numerous studies have investigated the relative advantages and limits of each method [12-16]; it is commonly accepted that combining these methods provides information which is as accurate as provocation tests of the target organ with the allergen [17].

In the present study, we have considered the results of SPT, RAST and CAP as either positive or negative because, for the clinician, the fact that the result is positive or negative is more important than the degree of the skin reaction or the amount of specific IgE detected. In the recently developed CAP system, the new flexible hydrophilic polymer encased in a capsule can bind about three times as much proteic allergens compared to the conventional cellulose discs; improved detection of the allergen-

bound IgE was obtained using a mixture of both polyclonal and monoclonal IgE [8-10]. We designed a study to determine whether these modifications had improved the clinical results of the new assay, compared to the conventional RAST and to the results of SPT.

Our data show first that the new CAP system gives more positive results than the RAST; compared to the positive SPT results, the CAP was positive in 78% whereas the RAST was positive in 65% of the tests. This RAST performance is in agreement with the results of many studies performed with this method [12-17].

The fact that CAP provided more positive results raised doubts about the specificity of the new test. When we looked for the positive results obtained when the SPT were negative (so called 'false positive compared to SPT') we found a higher proportion of positive results: 9% with CAP versus 4% with RAST. This was true for all allergens tested.

We tried therefore to determine whether these apparently 'false positive' results were technical errors or indicated the true presence of low amounts of circulating specific IgE. Our results suggest that these positive results were true positives. First, the vast majority (91%) of these positive results were found in atopic subjects. Moreover, 31 of the 87 'false positive' CAP results were also positive in RAST: the fact that two *in-vitro* assays performed independently were positive suggests that both assays detected the presence of IgE in serum and that the SPT could be a 'false negative'. Then, when we looked more precisely into the 56 CAP-positive isolated, we found, in two-thirds of the subjects that the case history was compatible with hypersensitivity to the allergen detected by CAP only.

The number of subjects with negative SPT, negative RAST and positive CAP was surprisingly high (40 out of 145 subjects). We cannot conclude from the subjects that

the CAP was more sensitive than SPT because this obviously depends on the strength of the extract used for SPT. Four of the allergens (Dpt, cat, dog, horse) contained 10 000 BU/ml, i.e. the highest concentration commercially available. These four allergens accounted for 31 of the 40 subjects with negative SPT, negative RAST and positive CAP. SPT performed with more concentrated extracts could have given different results.

In addition, when we applied one method of different design (CIE-CRIE) to verify the presence of specific IgE in four of these sera, randomly selected, we obtained positive results with the four sera. With the CRIE technique, the presence of a precipitate line for cat albumin and for Dpt proteins, typically found in the well established CRIE pattern of these two allergens, undoubtedly indicates the presence of specific IgE for cat and Dpt allergens [18]. The IgE binding was specific because results obtained with sera from non-allergic controls were negative. Finally, indirect evidence that the observed increase in sensitivity of the CAP was not related to a decrease in specificity was derived from the determination of CAP in control sera, such as cord serum, which should not contain specific IgE [19,20]; indeed, no positive result was found among the 160 tests. Also, in sera obtained from 10 non-atopic volunteers, only three borderline results out of 80 tests were found positive.

We conclude that the new CAP system gives a higher number of positive results than the RAST (increased sensitivity) and that the specificity of the CAP (versus SPT) is good within the limits of our study (limited number of subjects and allergens studied). Further studies, using provocation tests, are needed to appreciate differences of specificity between CAP and RAST more precisely.

References

- Wide L, Bennich H, Johansson SGO. Diagnosis of allergy by an in-vitro test for allergen antibodies. *Lancet* 1967; ii:1105-7.
- Schröder H, Kober A, Yman L. New developments in specific IgE antibody measurement-Phadezym RAST. *Allergol Immunopathol* 1981; (suppl IX):46-50.
- Seltzer JM, Halpern GM, Tsay YG. Correlation of allergy test results obtained by the FAST, RAST and prick-puncture methods. *Ann Allergy* 1985; 54:25-30.
- Gilles JCG, Mareschal JCP, Saint Remy JMR. Latex allergosorbent test (LAST): a new immunoassay for specific IgE with latex particles. *J Allergy Clin Immunol* 1988; 82:35-9.
- Brown CR, Higgins KW, Frazer K, *et al.* Simultaneous determination of total IgE and allergen-specific IgE in serum by the MAST chemiluminescent assay system. *Clin Chem* 1985; 31:1500-5.
- Engvall E, Perlmann P. Enzyme-linked immunosorbent assay, ELISA III. Quantitation of specific antibodies by enzyme labelled anti-immunoglobulin in antigen-coated tubes. *J Immunology* 1972; 109:129-35.
- Bernstein IL. Proceeding on the task force on guideline for standardizing old and new technologies used for the diagnosis and treatment of allergic diseases. *J Allergy Clin Immunol* 1988; 82(suppl 3):499-504.
- Axén RE, Drevin H, Kober A, Yman L. A new laboratory diagnostic system applied to allergy testing. *New Eng Reg Allergy Proc* 1988; 9:503(abstr).
- Axén RE, Kaj GL, Björkman R. Method and device for biospecific affinity reactions. United States Patent 1987; 4:708,932.
- Björkman R. A device for handling porous matrixes and an analysis apparatus comprising the same. European Patent 1988; 0143097 B1.
- Löwenstein H, Lind P, Weeke B. Identification and clinical significance of allergenic molecules of cat origin. *Allergy* 1985; 40:430-41.
- Berg T, Johansson SGO. Allergy diagnosis with the radio-allergosorbent test. A comparison with the results of skin and provocation tests in an unselected group of children with asthma and hay fever. *J Allergy Clin Immunol* 1974; 54:209-21.
- Pécoud A, Bonstein HS, Frei PC. Value of the case history in the diagnosis of allergic state and the detection of allergens. *Clin Allergy* 1983; 13:141-7.
- Knaus KA, Adkinson NF Jr. Clinical significance of IgE. In: Middleton E Jr, Reed CE, Ellis EF, eds. *Allergy: principles and practice*, 2nd Edn. St Louis: CV Mosby Co, 1983:673-88.
- van der Zee JS, de Groot H, van Swieten P, Jansen HM, Aalberse RC. Discrepancies between the skin test and IgE antibody assays: study of histamine release, complement activation in vitro, and occurrence of allergen-specific IgG. *J Allergy Clin Immunol* 1988; 82:270-81.
- Pécoud A, Peitrequin R, Fasel J, Frei PC. Comparison of two assays for the determination of specific IgE in serum of atopic and non atopic subjects: the allergenetics FAST and the Phadezym RAST. *Allergy* 1986; 41:243-9.
- Eriksson NE, Ahlstedt S, Belin L. Diagnosis of reaginic allergy with house dust, animal dander and pollen allergens in adult patients. I. A comparison between RAST, skin tests and provocation tests. *Int Arch Allergy Appl Immunol* 1976; 52:335-46.
- Anderson MC, Baer H. Allergenicity active components of cat allergen extracts. *J Immunology* 1981; 127:972-5.
- Bazal M, Orgel HA, Hamburger RN. IgE levels in normal infants and mothers and an inheritance hypothesis. *J Immunol* 1971; 107:794-801.
- Michel FB, Bousquet J, Greillier P, Robinet-Levy M, Coulomb Y. Comparison of cord blood immunoglobulin E concentrations and maternal allergy for the prediction of atopic diseases in infancy. *J Allergy Clin Immunol* 1980; 65:422-30.

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.