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Comparison between RAST and Pharmacia CAP system: A new automated specific IgE assay

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RAST represents the standard technique to titrate serum-specific IgE and was found to have a higher efficiency in the diagnosis of IgE-mediated allergy than other in vitro tests. Pharmacia CAP system (CAP) is a new solid-phase immunoassay, fully automated, used for the titration of specific IgE antibodies. Results are listed in kilounits per liter, equilibrated against the World Health Organization standard for IgE. RAST and CAP were compared in 106 unselected patients (6 to 59 years) characterized by a detailed clinical history and skin prick tests with standardized allergen extracts. IgE to cat, Dermatophagoides pteronyssinus, Alternaria, orchard grass, olive, and Parietaria pollen were tested; 470 tests were run. The specificity, sensitivity, and efficiency of both in vitro tests ranged from 85.5% to 100% except for olive pollen, in which the sensitivity of both in vitro tests was low (68.2% for the new test and 63.6% for RAST). Except for orchard-grass pollen, the sensitivity and specificity of CAP were better than that of RAST. There was a highly significant correlation between both tests (r range, between 0.864 to 0.987). CAP competes favorably with RAST and has the advantage of being automated and eliciting results in kilounits per liter. (J ALLERGY CLIN IMMUNOL 1990;85:1039-43.)

Specific serum IgE is widely used in the diagnosis of IgE-mediated allergic diseases. Although several techniques have been proposed within the past 10 years, RAST represents the standard technique. In-

Abbreviations used

CAP: Pharmacia CAP system
PRIST: Paper radioimmunosorbent test
PRU: Pharmacia RAST unit
BU: Biological unit
RIA: Radioimmunoassay

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roduced in 1967 for the semiquantitative titration of allergen-specific IgE in serum,^{1,2} RAST was improved during the years,^{3,4} and its accuracy to diagnose specific IgE was widely documented.^{5,6} However, in

TABLE I. Sensitivity, specificity, and efficiency of RAST and CAP

	Sensitivity (%)			Specificity (%)			Efficiency (%)	
	N	CAP	RAST	N	CAP	RAST	CAP	RAST
<i>D. pteronyssinus</i>	44	93.2	88.6	46	97.8	93.4	93.2	91.1
Cat	22	90.9	90.9	63	100	95.2	97.6	94.1
<i>Alternaria</i>	14	100	85.5	60	100	100	100	97.2
Orchard-grass pollen	52	98.1	98.1	30	92.8	100	97.5	98.5
Olive pollen	22	68.2	64.0	47	100	97.9	89.8	86.9
<i>Parietaria</i> pollen	12	91.6	83.3	62	98.3	98.3	98.9	96.5

Only true positive and true negative patients were tested.

RAST, the exact concentration of IgE antibodies is not precisely determined.⁷ The results are expressed in PRUs per milliliter that are not related to total IgE levels, in rare cases, RAST results may be falsely positive in the presence of very high IgE levels,^{8,9} or levels of RAST may be reduced by interference with IgG,^{10,11} and it is not automated. Comparisons between RAST and other in vitro tests developed for the diagnosis of IgE-mediated allergy consistently demonstrated that RAST was more efficient.¹²⁻¹⁶

A new test system, CAP, was recently introduced.¹⁷ It consists of a new type of solid-phase RIA or enzyme immunoassay calibrated against the World Health Organization standard for IgE, making it possible to elicit results in international units. The instrumentation and information-management software was developed for measurements of total and specific IgE, and this assay can be fully automated.

CAP was compared with the Phadebas RAST (Pharmacia Diagnostics AB, Uppsala, Sweden) in 106 patients for the diagnosis of IgE-mediated allergy to the six most common allergens of the northern Mediterranean area to assess whether this new test system elicits a similar or better clinical relevance than RAST. Allergy diagnosis was based on a detailed clinical history and skin prick tests with standardized allergen extracts.

MATERIAL AND METHODS

Patients

One hundred six unselected patients (66 males and 60 female patients), ranging in age from 6 to 59 years (mean $\pm$ SD; 26.9 $\pm$ 12.8 years), who were first observed in the Allergy Unit of the Chest Clinic were enrolled in the study.

Diagnosis of allergy

The diagnosis of allergy and characterization of patients were based on case history and skin prick tests.

History of allergy. A detailed questionnaire was directed to patients on allergic symptoms, the time symptoms occurred in the year, and the onset of symptoms when the

patient was in contact with some known precipitating factors. All subjects had respiratory symptoms and were suffering from asthma (50%), rhinitis (81.0%), and/or conjunctivitis (30.2%). Seven patients had both asthma and atopic dermatitis. Three patients suffered from a food allergy associated with allergy to inhalants.

Skin prick tests. Skin prick tests were performed with glycerinated extracts provided by Stallergènes Laboratories (Fresnes, France), as previously described, with a 9% codeine phosphate-positive control solution.¹⁸ The extracts were prepared according to the proposals of the Allergen Standardization Subcommittee of the International Unions of Immunological Societies, and their preparation was previously described in detail.¹⁹ All the extracts used for the comparison of both IgE antibody tests were standardized by in vivo and in vitro tests¹⁹ and labeled in biologic units according to the Nordic Countries guidelines on allergen standardization.²⁰ Moreover, all the inhalant seasonal and perennial allergens prevalent in the Montpellier area and the six most common food extracts were tested according to a previous study.²¹ None of the patients had taken medications that might interfere with the performance of the test before the investigation.²²

Skin tests with the whole battery of extracts, as indicated in Table I, were done in all patients included in the study.

Skin tests were considered to be positive when the wheal size induced by the allergen was at least 75% of the wheal size induced by the codeine phosphate-positive control.

Only patients with either a definite clinical history to a given allergen and a concordant positive skin test (allergic group) or subjects with no history to a given allergen and a negative skin test (nonallergic group) were investigated.

Titration of serum-specific IgE

The titration of serum-specific IgE was done by both RAST and CAP.

RAST. The titration of serum-specific IgE was done by Phadebas RAST according to the package insert, and results were listed in PRUs per milliliter.

CAP. CAP is a new laboratory system representing a further development of Phadebas RAST. It consists of a solid-phase RIA, instrumentation, and information-management software for the automated measurement of total and specific IgE in undiluted serum or plasma. The

assay can be performed as an RIA or a fluorimetric assay. In this study, the RIA was used.

The system is built around a new type of solid-phase consisting of a flexible hydrophilic carrier polymer encased in a capsule (CAP). The carrier consists of a CNBr-activated cellulose derivative. This solid phase is in principle similar to that of the paper disk, but it has a three-dimensional structure increasing the surface and allowing a closer contact between the bound allergens and serum IgE antibodies. The new system can bind at least three times more protein than a corresponding paper disk and about 50 times more than can be adsorbed in the inner surface of a traditional coated tube. Equilibrium between bound allergen and serum IgE is reached within 20 minutes.

The system uses polyclonal and monoclonal antibodies ^{125}I -labeled or β -galactosidase-generating fluorescence by splitting the fluorogenic substrate 4-methylumbelliferyl- β -D-galactoside. The assay is calibrated against the World Health Organization standard for IgE, and results are expressed quantitatively in kilounits per liter for total IgE and kilounits per liter for specific IgE. There are two sets of calibrators: 0.35 to 100 kU/L for specific IgE and low range total IgE, and 2 to 2000 kU/L for wide range total IgE. The cutoff (positive/negative) remains the same as for the Phadebas RAST, and the interpretation is both in classes and kilounits of IgE.

CAP discriminates effectively low IgE antibody levels from background. The specificity of the system is very high. Twenty-eight normal blood donor samples tested with 20 common inhalant and food allergens were negative in 557 of 560 tests. Nonspecific binding of high total IgE, up to 3000 kU/L to 56 allergens, was found to be insignificantly different from blood-donor background. In another experiment, it was found that 20,000 kU/L of nonspecific IgE was not interfering with the six allergens tested. Cross-reactivity with other immunoglobulins is less than one per 2 million. Interference from specific IgG has not been observed.¹⁷

Allergen species tested. Six allergens were tested: *Dermatophagoides pteronyssinus*, cat dander, *Alternaria*, orchard-grass pollen, olive pollen, and *Parietaria officinalis* pollen.

Total serum IgE

Total serum IgE was measured by Phadebas PRIST according to the package insert.

Statistical analysis

All data were analyzed after log transformation. For each allergen species tested, we determined the specificity, sensitivity, and efficiency according to the method of Galen and Gambino.²³ Moreover, we calculated the linear correlation coefficient between the results of both in vitro tests in allergic patients.

RESULTS

The comparisons were performed on a total of 470 tests in which the classification of allergy was clear-

TABLE II. Mean values and correlation between RAST and CAP

	<i>r</i>
<i>D. pteronyssinus</i>	0.956
Cat	0.944
<i>Alternaria</i>	0.894
Orchard-grass pollen	0.987
Olive pollen	0.870
<i>Parietaria</i> pollen	0.864

r, Linear correlation coefficient between CAP and RAST for patients with positive skin tests and clinical history.

cut (true positive and true negative) with both in vitro tests measuring specific IgE. The results indicate that for all allergen species tested, CAP is at least as sensitive as the RAST, but both tests are not sensitive enough for olive pollen. Moreover, except for orchard-grass pollen, CAP is more specific than RAST (Table I), and the specificity of the new test is always >92.8%. The efficiency of CAP is greater than that of RAST for the whole study (CAP, 96.8%; RAST, 94.2%) and for all allergens except for orchard-grass pollen. Both tests have an efficiency of >85%, whichever allergen is examined.

There was no false-positive reaction in subjects with high total serum IgE levels. Ten patients with negative RAST and CAP had total serum IgE >1000 kU/L. The maximal values of total serum IgE in these patients ranged from 1452 kU/L for *D. pteronyssinus* to 1844 kU/L for olive pollen.

Mean values obtained in allergic patients ranged from 12.5 kU/L to 51.5 kU/L with CAP, and 3.4 PRU/mL to 27.0 PRU/mL with RAST (Table II). There was always an excellent correlation between results obtained with both tests (*r* ranging from 0.864 to 0.956).

DISCUSSION

CAP and RAST correlate very well the allergic history and skin prick tests. The new test has a slightly higher sensitivity, specificity, and efficiency than RAST, but because of the great quality of RAST, the differences are not very large in the present study.

Allergy was studied by means of the clinical history and skin prick tests with standardized allergen extracts. We wanted to assess the value of a solid-phase in vitro test, and we purposely used standardized allergens for skin tests coming from a different source than allergens used in the in vitro tests, since the quality of solid-phase immunoassays is largely dependent on the quality of allergens.^{24, 25} For skin tests we used high-quality extracts of Mediterranean pol-

lens and observed that neither of the two in vitro tests was sensitive enough in the diagnosis of olive-pollen allergy when these tests were evaluated against case history and skin prick tests. This discrepancy may be due to the difference in quality of allergens.

Many studies have already examined the correlations between skin test and RAST results. It has been observed that there is an overall excellent correlation for well-characterized and standardized allergens, as well as for well-characterized patients.²⁶⁻²⁹ However, RAST is usually less sensitive than skin tests.²⁷⁻³⁰ We observed a better correlation between results of skin tests and RAST than in most other studies, even though allergens for skin tests and in vitro assays were coming from different manufacturers. This may be due to the improvement in the accuracy of RAST and to the use of standardized allergen extracts for in vivo and in vitro tests. These results confirm therefore the importance of standardized extracts in the diagnosis of IgE-mediated allergy since it has already been observed for the comparison of skin test methods.³¹ The correlation between skin tests and CAP is even better and accords with other studies,¹⁷ indicating that the new solid-phase immunoassay competes favorably with RAST. It therefore appears that we, now for the first time, have a system for measuring IgE antibody superior to RAST.

In the present study, we did not observe any disturbance^{10, 11} in RAST or CAP by elevated total serum IgE because many sera containing between 1000 kU/L and 1844 kU/L of total IgE were tested, and there was no false-positive RAST or CAP result. This result agrees with data of Axén et al.¹⁷ who demonstrated no disturbance with sera containing at least 3000 kU/L of total serum IgE.

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Comparison of the intensity and duration of effects of inhaled bitolterol and albuterol on airway caliber and airway responsiveness to histamine

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*Inhaled β -agonists can produce bronchodilatation and reduce airway hyperreactivity in patients with asthma. Using these two measures, we compared inhaled bitolterol (three puffs, 1110 μ g), albuterol (two puffs, 180 μ g), and placebo administered by metered-dose inhaler in a blinded, crossover study of 40 subjects with chronic asthma. On each study day, subjects underwent histamine challenges at 1½ hours before, and ½, 2, 4, 6, and 8 hours after inhaling one of the three test-drug treatments. Both drugs produced significant bronchodilatation at 30 minutes through 4 hours and significant effects on airway reactivity at 30 minutes through 2 hours ($p < 0.05$). Bitolterol also produced small but significant bronchodilator effects at 6 hours and effects on airway reactivity at 4 hours ($p < 0.05$). Effects of bitolterol on airway reactivity diminished significantly more slowly than effects of albuterol in subjects with baseline provocative concentration causing a 20% fall in $FEV_1 \geq 1.0$ mg/ml of histamine (half-life of biologic effect 1.37 versus 0.92 hours; $p < 0.05$) but not in subjects with baseline provocative concentration causing a 20% fall in $FEV_1 \leq 1.0$ mg/ml (half-life of biologic effect of 1.01 versus 1.00 hours; $p > 0.05$). (*J ALLERGY CLIN IMMUNOL* 1990;85:1043-9.)*

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