

Comparative evaluation between two immunoenzymatic techniques (FAST and Phadezym) and the Phadebas RAST in food allergy

D. A. MONERET-VAUTRIN,¹ J. L. GUEANT,² A. ABDEL-GHANI,² Y. MARIA¹
& J. P. NICOLAS²

¹Service de Médecine D, Immuno-Allergologie, CHU de Nancy-Brabois, and ²Groupe de Biochimie-Immunologie, INSERM U 308 et Laboratoire de Biochimie Médicale et Pédiatrique, Faculté de Médecine, Université de Nancy I, France

The diagnosis of food IgE-dependent hypersensitivity is based on the demonstration of specific IgE, completed by provocation tests. Two immunoenzymatic techniques, the Phadezym and the FAST, are compared with the Phadebas RAST, in 86 sera (23 controls, 28 from patients with a reported food allergy and 35 with a positive RAST to a food allergen). The within-run variation coefficient of class 0–2 sera was 9% for the Phadebas RAST, and higher than 20% for the Phadezym and the FAST. It was in order of 8.7%, 9.4% and of 17.6% respectively for Phadebas RAST, Phadezym and FAST when estimated with class 3–4 sera. The specificity was higher than 95% for the three techniques. The sensitivity was 75% for Phadebas and 43% for Phadezym and FAST. The FAST test is much less sensitive with allergens of vegetal origin than those of animal origin ($P < 0.01$). This work indicates the high percentage of false negative results of immunoenzymatic techniques when food extracts are tested. This could be explained either by an enzyme-substrate reaction or by a non-specific inhibition of the enzyme linked to the anti-IgE IgG.

Key words: food allergy; RAST Phadebas; Phadezym; FAST; specific IgE

Accepted for publication 7 August 1989

The diagnosis of food allergy is based on the demonstration of specific IgE and is confirmed by performing provocation tests with foods (1, 2, 3, 6, 8, 9, 12, 16, 23). Specific IgE are usually detected by the RAST, a radio-immunologic reference technique. The sensitivity of RAST for food allergens varies from 50% to more than 90% (8, 12). The discrepancies observed for estimation of RAST sensitivity depend mainly on the criteria for patient selection. Nowadays, the radio-immunologic techniques are concurred by immunoenzymatic tests (enzyme linked immunosorbent assays). Until now, the studies which compared the sensitivity of these techniques with that of the RAST were contradic-

tory (4, 17). In addition, an interference with specific IgG has been observed with ELISA techniques (15–18). A recent study of our group showed that the sensitivity of the RAST was superior to that of the FAST, especially concerning food allergens (10).

In the present study we compare two immunoenzymatic techniques, the Phadezym and FAST (Fluoro-allergo-sorbent-test), with the RAST for the diagnosis of food allergy. The tests were practiced in 86 sera: 23 controls, 28 sera of patients with a reported food allergy and 35 sera with a RAST positive to a food allergen. The results confirmed the better performance of the radioimmunologic method. The difference in sensitivity between ELISA

Table 1
Characteristics of RAST, Phadezym and FAST

	RAST	Phadezym	FAST
Allergen-solid phase	paper	paper	polystyrene cupule
IgE reference serum	anti-birch	anti-birch	anti-cokle
Enzyme	-	galactosidase	alkaline phosphatase
Incubation delay with serum	3 h	3 h	2 h
Incubation delay with the anti-serum	16-20 h	16-20 h	2 h
Substrate	-	0-nitrophenyl- -galactosidase	4 methyl-umbelliferyl-l-phosphate
Incubation conditions	-	2 h at 37°C	1/2 h at 20°C
Detection	γ-counting	colorimetry	fluorometry

techniques and RIA varied depending on whether the allergen tested was of vegetal or animal origin, suggesting an interaction between the linked enzyme and the vegetal allergens.

MATERIAL AND METHODS

Selection of sera. The 23 control sera belonged to healthy volunteers, non-atopic, in whom the skin tests to food extracts were negative. The 63 sera of patients were collected in two ways. 28 sera were from individuals with a food allergy of immediate type (5 children aged from 1 to 8 years, 13 females and 10 males aged from 16 to 31 years). The

symptoms were: anaphylactic shock (*n* = 2), urticaria (*n* = 10), asthma and eczema (*n* = 6), urticaria and eczema (*n* = 2), asthma (*n* = 5), rhinitis (*n* = 3). These clinical symptoms were confirmed by means of prick and provocation tests to the foods. The foods tested were: milk: 17; egg white: 13; fish: 6; shrimps: 2; wheat flour: 14; soya bean: 5; peanuts: 2; carrots: 2; orange juice: 2.

35 additional sera with a positive RAST to a food were included in the study.

Techniques. The main characteristics of Phadebas, Phadezym and FAST tests are presented in Table 1. The three tests were performed according to the manufacturer's instructions

Table 2
Within-run assay of standard sera

	Determination		Theoretical values	Correlation (<i>n</i> = 8)
	I	II		
Phadebas RAST (PRU/ml)	17.70	19.90	17.50	<i>r</i> = 0.996 <i>P</i> < 0.001
	3.01	4.41	3.50	
	0.91	0.70	0.70	
	0.38	0.36	0.35	
Phadezym RAST (PRU/ml)	17.5	15.91	17.50	<i>r</i> = 0.996 <i>P</i> < 0.001
	4.99	4.34	3.50	
	0.725	0.725	0.70	
	0.29	0.28	0.35	
FAST (IU/ml)	20.56	14.19	20.00	<i>r</i> = 0.973 <i>P</i> < 0.001
	4.12	4.12	4.00	
	1.03	1.07	0.80	
	0.10	0.10	0.10	

and interpretation was made in 4 positive classes, as described recently (10).

Biochemical performance. Standard undiluted sera were tested as samples. Each standard sera was introduced in duplicate and the obtained values were compared with the theoretical values given by the manufacturer.

The within-run reproducibility was studied with 12 RAST-positive sera. These sera were assayed eight times by the three tests in the same session by the same technician. The food allergens tested were egg white, milk, fish, flour, peanuts, soya bean and carrot. The variation coefficient of each allergen was calculated.

Statistical analysis. The results obtained by the three techniques were compared using χ^2 test with Yates' correction.

RESULTS

With the three methods a good correlation was observed between the theoretical values and the measured values of standard sera (Table 2). The within-run variation coefficient for sera of classes 0-2 was estimated to 9%,

24.3% and 21% with RAST, Phadezym and FAST, respectively. With sera of classes 3-4 it was 8.7%, 9.4% and 17.6% for RAST, Phadezym and FAST, respectively.

The specificity of the three methods for eight foods (23 control sera 184 tests) was very satisfactory: It was estimated to 95.4% for RAST and FAST and to 96.6% for Phadezym.

RAST showed 75% sensitivity and Phadebas and FAST 43% when estimated with the 28 sera from 28 patients with a reported food allergy (Table 3).

When taking into account the 63 patient sera (28 reported food allergies and 35 sera positive to RAST), the sensitivity of Phadezym and FAST was respectively estimated to 39.7% and 44%. The difference in sensitivity between Phadezym and FAST was not statistically significant. When only the allergens of animal origin were considered, the FAST was more sensitive than the Phadezym (55.3% and 31.6%, respectively, $P < 0.05$). The sensitivity of Phadezym and of RAST for detecting IgE against vegetal allergens was 44.0% and 28.0%, respectively (difference not significant).

Table 3
Comparison between Phadebas-RAST, Phadezym-RAST and FAST among 28 individuals with food allergy

Allergen		Positive tests vs total tests		
		RAST	Phadezym	FAST
Allergens from animal origin	egg-white	4/5	3/5	2/5
	milk	3/5	1/5	3/5
	fish	1/3	0/3	1/3
	shrimp	2/2	2/2	2/2
	sensitivity	10/15 (66.6)	6/15 (40.0)	8/15 (53.3)
Allergens from vegetal origin	wheat flour	3/4	2/4	1/4
	soya bean	3/4	1/4	2/4
	peanuts	2/2	1/2	0/2
	orange	2/2	2/2	1/2
	carrot	1/1	0/1	0/1
	sensitivity	11/33 (84.6)	6/13 (46.2)	4/13 (30.8)
sensitivity of the 28 cases		21/28 (75)	12/28 (42.9)	12/28 (42.9)

Values in parenthesis correspond to sensitivity expressed in percentage

The FAST was more sensitive for detecting animal allergen specific IgE than vegetal allergen specific IgE ($P < 0.01$).

DISCUSSION

The classic reference method for measuring specific IgE is the Phadebas RAST (11). The Phadezym uses the same allergens coupled to cellulose disks. In both tests, the results are expressed in PRU/ml. The results of FAST are expressed in IU/ml (13). Schroder has demonstrated that there is, at least for high values, an equivalence between the two systems of units (20). There are works that compare these techniques for cases of allergy to inhalant allergens, obtaining a variation coefficient close to 10 % for the three methods. The Phadebas and Phadezym give very similar results regarding graminaceous, herbaceous or tree pollens, and animal danders. False negatives for the Phadezym are under 1 %.

The present study refers only to food allergy. The specificity of the three methods for detecting anti-food allergen IgE is excellent, over 95 % (established from 184 determinations). This value is similar to that estimated by Carron on 70 non-allergic children for the RAST to milk, egg, fish, wheat flour and orange (7). According to Schroder et al. (21) the false positive results of Phadebas and Phadezym are mostly obtained for sera with total IgE levels higher than 6000 kIU/l. None of the sera included in our study had an IgE level in that range. The FAST specificity is under discussion (17, 19, 22). It seems to be poor according to Pecoud's study (17).

The within-run reproducibility (9 %) of the radioimmunologic method was satisfactory whatever the level of specific IgE. This was not the case with the immunoenzymatic methods, which both presented a variation coefficient higher than 20 % for classes 1-2. This means that sera with immunoenzymatic tests of class 1 for food allergens might correspond to false positive results (19, 20). For this reason, they are not recommended for the assay of specific IgE in umbilical cord blood (17). The frequency of false positives with the FAST has been

recently noted in a comparative work between the PRICK and the FAST in 253 individuals: 23 % of negative PRICK corresponded to a positive FAST (22).

The sensitivity of the Phadebas RAST (75 %) is significantly better than that of immunoenzymatic methods (Table 3). The sensitivity of immunoenzymatic tests depended on the animal or vegetal origin of the food extracts. For animal allergens, the FAST was more sensitive than the Phadezym but for vegetal allergens, the performance of the FAST was rather poor. The discordance of sensitivity between the Phadebas RAST and the immunoenzymatic methods may depend on the type of enzyme linked to the anti-IgE. It can be explained by a specific reaction of the enzyme with a substrate present in the allergenic extract. This is clearly the case for milk, an allergen extract which reacts with the betagalactosidase coupled to the anti-IgE of the Phadezym. The reactive site of the enzyme does not remain free and cannot react with the substrate of the developer solution (Table 1).

In the FAST, the linked enzyme (alkaline phosphate) can react with both animal and vegetal allergen extracts. Another possible interaction of the linked enzyme with the allergen extract is a non-specific inhibition of the enzyme by alcohol of phosphatidic esters or by amino acids as L-phenylalanine, L-tryptophane or L-cysteine (5). This could explain in part the poor sensitivity of the FAST in detecting specific IgE against vegetal allergens.

The biologic diagnosis of IgE-dependent food allergy can be efficiently established by the Phadebas RAST. However, the sensitivity of RAST is much better for pollens and acarians than for allergens from food (10). The sensitivity of RAST for this type of allergens could be improved by technical advances in the purification of food antigens (14). Such improvement could also avoid some false positive results which are due to a cross-reactivity between graminaceous pollen and foods (7). The immunoenzymatic methods do not seem suitable for food allergy diagnosis, because of their too low sensitivity. However, the risk of false positives is increased because the within-run reproducibility for class 0-1 sera is too high.

REFERENCES

1. Aas, K. & Lundkvist, U.: The radioallergo sorbent test with a purified allergen from codfish. *Clin. Allergy* 3, 255-261, 1973.
2. Amlot, P. L., Urbanek, R., Youlten, L. J. F., Kemeny, M. & Lessof, M. H.: Type I allergy to egg and milk proteins: comparison of skin tests with nasal, buccal and gastric provocation tests. *Int. Arch. Allergy Appl. Immunol.* 77, 171-173, 1985.
4. Bamdad, S., Goddwin, B. F. J. & Hill, J. E.: IgE antibodies to food allergens detected by ELISA, RAST and monkey PCA. *Clin. Allergy* 13, 89-97, 1983.
5. Barman, T. E.: Enzyme handbook, Vol. II, Springer-Verlag, Berlin, Heidelberg, New York, 1969.
7. Carron, R., Guerrier, G., Guilloux, L. & Ville, G.: Fréquence des réactions positives aux trophallergènes chez l'enfant. *Allergie et Immunologie* 17, 158-163, 1985.
8. Chua, Y., Bremner, K. et al.: Diagnosis of food allergy by the radio allergo sorbent test. *J. Allergy. Clin. Immunol.* 66, 477-482, 1976.
9. Gavani, O., Hyde, J., et al.: Hypersensitivity to milk and egg white. Skin tests, RAST result, and clinical intolerance. *Ann. Allergy* 40, 314-318, 1978.
10. Guéant, J. L., Moneret-Vautrin, D. A., Déjardin, G., Algalarondo, C., Nicolas, J. P., Grilliat, J. P.: Comparative evaluation of RAST and FAST for 11 allergens in 288 patients. *Allergy* 44, 204-208, 1989.
11. Guérin, B., Didierlaurent, A., Derer, M. & Stadler, B.: Seuil de positivité: interprétation des résultats du RAST. *Rev. Fr. Allergol.* 25, 189-195, 1985.
12. Haddad, Z. H. & Hoffman, D. R.: Specific IgE antibodies and serum IgE levels in children with immediate hypersensitivity-type diseases. *Excerpta Med. Int. Conf. Ser.* 16, 300, 1973.
13. Halpern, G. & Tsay, Y. G.: Le test fluoro-allergo-sorbent pour l'IgE (IgE FAST): concepts et applications cliniques. *Med. Hyg.* 43, 1094-1098, 1985.
14. Heiner, D. C.: The use of RAST in the study of allergy to foods. *Advances in diagnosis of allergy. RASY: Medical Book Publishers*, pp. 153-164, 1975.
15. Kilshaw, P. J., McEwan, F. J., Baker, K. E. & Cant, J.: Studies on the specificity of antibodies to albumin in normal human serum: chemical consideration in the use of ELISA methods. *Clin. Exp. Immunol.* 66, 481-489, 1986.
16. Moneret-Vautrin, D. A., Gérard, H., et al.: Allergie alimentaire de type immédiate: évaluation critique du radio-allergo sorbent test et du test de dégranulation de basophiles. *Nouv. Presse Med.* 8, 3176, 1979.
17. Pecoud, A., Peitrequin, R., Fasel, J. & Frei, P. C.: 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* 41, 243-249, 1986.
18. Reid, M. J., Kwasnicki, M. J., Moss, R. B. & Nak-Kong, V. C.: Underestimation of specific immunoglobulin E by microtiterplate enzyme-linked immunosorbent assay. *J. Allergy Clin. Immunol.* 76, 172-176, 1985.
19. Sarmini, H., Robinet, D., Lesure, J. & Funès, A.: Fluoro-allergo-sorbent test (IgE FAST): concepts et performances. *Med. Hyg.* 43, 1101-1106, 1985.
20. Schroder, H., Edman, P. & Johanson, S. G. O.: Evaluation of allergenic fluorometric assays for IgE and IgE antibody. *Allergy* 41, 163-165, 1986.
21. Schroder, H., Kober, A., Yman, L.: New developments in specific IgE antibody measurement - Phadezym RAST. Correlation between EIA and RIA (Phadebas RAST). *Allergol. Immunopathol.* 46, Suppl. 4, 46-50, 1981.
22. Selzer, J., Halpern, G. M. & Tsay, Y. G.: Correlation of allergy test results obtained by IgE FAST, RAST and Prick-Puncture methods. *Ann. Allergy* 54, 25-30, 1985.
23. Szocska, M., Szenickzey, K., et al.: Diagnosis of food allergy through bronchial challenge. *Allergol. Immunopathol.* 7, 1-6, 1979.

Address:

D. A. Moneret-Vautrin

Service de Médecine D, Immunoallergologie

CHU de Nancy-Brabois

54511 Vandoeuvre les Nancy Cedex

France

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.