

ELISA in Diagnosis of Respiratory Allergy

A Comparison with RAST and Skin Tests

A. C. NEGRINI, C. TROISE and S. VOLTOLINI

Servizio Autonomo di Allergologia, Ospedale S. Martino, Genova, Italy

An enzyme-linked immunosorbent assay (ELISA), employing beta-galactosidase conjugated anti-IgE, was used for the determination of specific IgE antibodies to common inhalant allergens (*Dermatophagoides pt.* and *f.*, *Parietaria* and four grass pollens) in serum samples from 82 adult patients with asthma and/or rhinitis. A total of 194 analysis were carried out and the results were compared with those of skin tests and RAST. The correlation coefficient (r) between ELISA and RAST results was high ($r=0.95$); the correlation between skin tests and ELISA ($r=0.93$) was greater than that between skin tests and RAST ($r=0.90$). No significant differences were found among the allergens used. We conclude that the version of ELISA used develops an overall good correlation with skin tests and RAST and seems to provide a satisfactory alternative to RAST for allergy diagnosis.

Key words: comparison; diagnosis; ELISA; prick-test; RAST; respiratory allergy.

Accepted for publication 20 October 1984

The enzyme-linked immunosorbent assay (ELISA) (7) is increasingly employed in a growing number of investigative and clinical studies (3, 13, 19, 23, 24, 25). In allergy and clinical immunology diagnosis it has been used to detect IgG antibodies to a variety of antigens (11, 14, 20, 21), total IgE (6, 12, 18, 21, 22) and specific IgE antibodies to both inhalant allergens (1, 2, 9, 15, 16, 17) and food allergens (4, 6, 10).

The aim of the present study is to evaluate the possibility of using ELISA in clinical practice for respiratory allergy diagnosis. For this purpose the ELISA results employing seven different allergens are compared with the outcome of prick-test as well as RAST results in adult allergic and non-allergic patients.

MATERIAL AND METHODS

The study comprises 82 adult patients (41 males and 41 females, 11-72 years of age, mean 34 ± 14) referred to our Service because of asthma and/or rhinitis. 66 had positive prick-test to one or several selected allergens; 16 had negative reaction to all allergens used and served as controls.

Allergen extracts of house dust mites (*Dermatophagoides pteronyssinus* and *farinae*) and pollens from *Parietaria* and four grasses (*Lolium perenne*, *Poa pratensis*, *Cynodon dactylon* and *Secale cereale*) have been included in the study, because these allergens are the most common allergic etiologic agents among the Italian people.

The skin prick-tests were performed on the volar side of the forearms with hydroglycerinated allergens purchased from Lofarma, Milan, using a conventional blood lancet. Titration of extracts was 1:50 w/v. Histamine chloride 1 mg/ml was used as a positive control and a solution of 4 mg/ml phenol and 50% glycerine in saline served as a negative control. The reactions were read after 15 min and graded on a 0-4+ scale, as described by Eriksson et al. (8). Thus, a wheal reaction of the same size as the positive control was recorded as three pluses (+++), and four pluses (++++) marked the reactions larger than the control. The reactions between the size elicited by the negative (scored as 0) and the positive reference were recorded as one or two pluses (+, ++). The scores of 2+ or more were judged as positive. The same physician, on the same day, performed all skin prick-tests to ensure reproducibility.

RAST and ELISA tests (Pharmacia, Uppsala, Sweden) were performed according to the manufacturer's directions. The enzyme used as marker was beta-galactosidase (26). The results were expressed in classes ranging from 0 to 4, 0 corresponding to an undetectable level of specific IgE and 4 to a very high level. Only the classes 2-4 were regarded as positive. The variation coefficient of the RAST technique used was 10.5% for the samples falling into the 3-4 classes and 21.3% for the sera belonging to the 1-2 classes. For the ELISA technique this coefficient was 10%.

RESULTS

The results of 194 assays for serum IgE antibodies against *Parietaria*, *Lolium p.*, *Poa p.*, *Cynodon d.*, *Secale c.*, *Dermatophagoides pt.* and *f.* with a beta-galactosidase technique have been compared with those obtained by the RAST procedure. Thus, of 55 analyses negative with the ELISA only seven were positive with the RAST. In sera with positive ELISA (159) three were negative with the RAST. The overall correlation was excellent ($r=0.95$) (Fig. 1).

A comparison between the results with prick-test, generally accepted as a major tool in the diagnosis of immediate-type hypersensitivity,

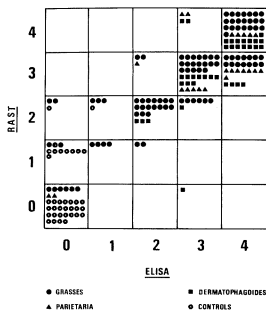


Fig. 1. Relationship between ELISA and RAST.

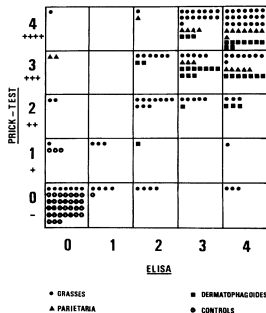


Fig. 2. Relationship between ELISA and prick-test.

and ELISA was also made. Excellent agreement was found ($r=0.93$) (Fig. 2), greater than that between skin-test and RAST results ($r=0.90$) (Fig. 3). No significant differences were found between the allergens used.

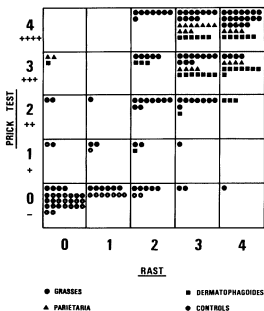


Fig. 3. Relationship between RAST and prick-test.

DISCUSSION

In the present study specific IgE antibodies against common inhalant allergens have been determined in serum by means of ELISA, and the results have been compared with those of the RAST and prick-test. Significant correlations have been found, suggesting that the enzyme immunoassay is able to determine allergen-specific IgE antibodies with the same precision as the RAST technique. This is in accordance with many authors (1, 2, 4, 5, 6, 15, 16, 18). A wide variety of allergens (grasses, ragweed, Parietaria, house dust mites, animal dander, food allergens, *Aspergillus fumigatus*) has been used, but the good agreement between the two tests does not seem to depend on the allergen employed. In contrast, other authors (9, 10) have shown marked discrepancies between the results obtained by the two techniques, with ELISA pointing out more cases of increased specific IgE than RAST.

In our study the two *in vitro* methods have shown discordance in a few tests. In six of these the ELISA results were in agreement with the patients' symptom history (2 negative and 4

positive), while four positive RAST results had clinical correspondence. When disagreement was found between ELISA and prick-test results, clinical symptoms agreed with the skin tests in eight cases (5 positive and 3 negative) and with the ELISA in six (all positive).

The disagreement among the various investigations might be due to different laboratory procedures, while there are no satisfactory explanations for the differences between RAST and ELISA results.

In enzyme immunoassays the use of purified allergens, the specificity of the antibody employed, and the quality of the enzyme-antibody conjugate are critically important for the sensitivity and specificity of the assay.

The enzyme-labelled anti-IgE is produced by Pharmacia Diagnostics from an antiserum raised in rabbits, and beta-galactosidase is conjugated to the antibodies with the SPDP method by forming covalent cross linkages between functional groups in the two proteins. Beta-galactosidase has a high molecular weight (598,000) but the size of the marker does not seem to be of significance. However, so far, no enzyme used as a label is without some disadvantage: in the alkaline phosphatase system the substrate seems to have low stability; in the horseradish peroxidase assay many acceptor substances are suspected of being highly carcinogenic. In conclusion, the ELISA seems to have the following advantages: high specificity, good accuracy and precision, high reproducibility, long shelf-life of reagents, ease of performance, elimination of the need for isotopic material and, last but not least, relatively low cost. Because of these significant advantages, and very good correlation between the ELISA and skin-test, we think that the enzyme immunoassay is able to provide a satisfactory alternative to radioimmunoassay for diagnosing reagin-mediated allergies in clinical practice.

REFERENCES

1. Ali, M., Nalebuff, D. J., Ramanarayanan, R., Fayemi, A. O. & Mesa-Teyada, R.: Assay of IgE antibodies against June and Timothy grasses by an immunoperoxidase technique. *Clin. Allergy* 10, 203-209, 1980.

2. Ali, M., Nalébuff, D. J., Fadal, R. & Fayemi, A. O.: Correlation of the diagnostic skin test with the immunoperoxidase assay in ragweed hypersensitivity. *Ann. Allergy* 45, 63-66, 1980.
3. Avrameas, S. & Guesdon, J. L.: ELISA and related techniques in allergy and clinical immunology diagnosis. In *Proceedings of the XII congress of the European Academy of Allergology and Clinical Immunology*, pp. 81-83. O. I. C. Medical Press, Florence, 1983.
4. Bamdad, S., Goodwin, 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. Bonini, S.: Revisione critica della metodologia e del significato clinico della ricerca di IgE specifiche mediante "immunoassays". In: *Atti del XV Congresso della Soc. Italiana di Allergologia e Immunologia Clinica*, pp. 187-202. Leonardo Ed., Rome, 1981.
6. de Montis, G., Deivigne, A., Dugué, M. A. & Georges, P.: Etude comparative des mesures d'IgE sériques totales et spécifiques par tests immunoenzymatiques et radioimmunologiques. *Rev. Fr. Allergol.* 22, 147-149, 1982.
7. Engvall, E. & Perlmann, P.: Enzyme-linked immunosorbent assay. ELISA. III Quantitation of specific antibodies by enzyme-labelled anti-immunoglobulin in antigen-coated tubes. *J. Immunol.* 109, 129, 1972.
8. Eriksson, N. E., 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.* 52, 335-346, 1976.
9. Eriksson, N. E. & Ahlstedt, S.: Diagnosis of reaginic allergy with house dust, animal dander and pollen allergens in adult patients. V. A comparison between ELISA, provocation tests, skin tests and RAST. *Int. Arch. Allergy Appl. Immunol.* 54, 88, 1977.
10. Fallstrom, S. P., Ahlstedt, S. & Honson, L. A.: Specific antibodies in infants with gastrointestinal intolerance to cow's milk proteins. *Int. Arch. Allergy Appl. Immunol.* 56, 97, 1978.
11. Grant, J. D., Goldblum, R. M., Rahr, R., Thuesen, D. O., Farman, J. & Gillaspay, J.: ELISA for IgG antibodies against insect venom. *J. Allergy Clin. Immunol.* 68, 112-118, 1981.
12. Hoffman, D. R.: Estimation of serum IgE by an enzyme-linked immunosorbent assay (ELISA). *J. Allergy Clin. Immunol.* 51, 103, 1973.
13. Longbottom, J. L.: ELISA. In: *Proceedings XI International Congress of Allergology and Clinical Immunology*, pp. 233-238. Macmillan Press, London, 1983.
14. Mantylari, R. A., Jousilahti, P. & Katila, M. L.: Antibodies to *Aspergillus fumigatus* in farmer's lung patients measured by ELISA. *Clin. Allergy* 10, 187-194, 1980.
15. Morandini, G. C., Finiguerra, M., Zanierato, G. & Cucco, S.: Studio comparativo tra metodo radioimmunologico (RAST) ed immunoenzimatico (RELIA) nella ricerca delle IgE specifiche. *Min. Pneumol.* 20, 2, 1981.
16. Negrini, A. C., D'Aste, N., Scarsi, P. G., Troise, C. & Voltolini, S.: La diagnostica immunoenzimatica nella sensibilizzazione a Parietaria: raffronto con tests cutanei e RAST. *Folia Allergol. Immunol. Clin.* 30, 211-215, 1983.
17. Norman, P. S., Lichtenstein, L. M. & Ishizaka, K.: Diagnostic tests in ragweed hay fever. *J. Allergy Clin. Immunol.* 52, 210, 1973.
18. Ormonroyd, P. & Robertshaw, D.: A comparison of the Phadebas and Phadezym IgE paper immunosorbent test kits. *Clin. Allergy* 13, 51-55, 1983.
19. Pal, S. B.: Enzyme-labelled immunoassays of hormones and drugs. Walter de Gruyter, Berlin, New York, 1978.
20. Sepulveda, R.: Measurement of IgG and IgE antibodies by ELISA technique. In: *Advances in allergology and applied immunology*, p. 555. Pergamon Press, Oxford, 1980.
21. Sepulveda, R., Longbottom, J. L. & Pepys, J.: Enzyme-linked immunosorbent assay, ELISA, for antibodies, IgG and IgE, to protein and polysaccharide antigens of *Aspergillus fumigatus*. *Clin. Allergy* 9, 359-371, 1979.
22. Stein, R., Evans, S., Milner, R., Rand, C. & Dolovich, J.: Isotopic and enzymatic assays in non-allergic subjects. *Allergy* 38, 389-398, 1983.
23. Voller, A., Bartlett, A. & Bidwell, D. E.: The use of ELISA in the serology of viral and parasitic diseases. *Scand. J. Immunol.* 8, 125, 1978.
24. Voller, A., Bidwell, D. E. & Bartlett, A.: The ELISA. A guide with abstract of microplate applications. Dynatech Europe, Gernsey (GB), 1979.
25. Voller, A. & Bonini, S.: ELISA. Metodologia e principali applicazioni. In: Serafini, U. (ed): *Immunologia ed Allergologia Clinica*, pp. 595-613. USES, Firenze, 1982.
26. Weltman, J. K., Franckelton, A. R. Jr, Szaro, R. B. & Rotman, M. B.: A galactosidase immunosorbent test for human IgE. *J. Allergy Clin. Immunol.* 57, 215, 1976.

Address:

Arsenio Corrado Negrini, M. D.

Servizio Autonomo di Allergologia

Ospedale S. Martino - XIII U.S.L.

Viale Benedetto XV - 16132 Genova

Italy

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.