Estimation of serum IgE by an enzyme-linked immunosorbent assay (ELISA)

Donald R. Hoffman, Ph.D. *Los Angeles, Calif.*

A reliable and relatively simple method for the estimation of serum IgE is presented. It is a modification of radioimmune inhibition methods in which the marker is an enzyme, alkaline phosphatase, not radioactivity. The method does not require radioactivity or expensive equipment, and the reagents are stable for long periods of time. As presented it has a minimum sensitivity of about 10 ng. per milliliter. The reproducibility of the method is ± 3 per cent and for correlation with radioimmunoassay $\rho = 0.97$.

Several methods are available for determination of serum IgE. The classical radial immunodiffusion method can be used only for sera with high IgE content.[1] Radial immunodiffusion procedures can be amplified directly by the use of radiolabeled anti-IgE[2] or indirectly by the use of a radiolabeled second antibody.[3] The most sensitive techniques for determining IgE all use inhibition of binding of radiolabeled IgE[4-7] and can be made sensitive to as little as 0.1 ng. per milliliter. However, all of these methods that are useful in the normal clinical range of serum IgE concentrations require the use of radioactive materials, which are unstable. In addition, the inhibition procedures require expensive counting equipment.

In the procedure described in this paper we use an enzyme, alkaline phosphatase, as a marker instead of radioactive iodine. The conjugate is stable for long periods of time, at least 4 months, with no detectable decrease in activity.[8] This method, the enzyme-linked immunosorbent assay, ELISA, was introduced by Engvall and Perlmann[9] for determination of rabbit IgG. In this study it is shown to be useful for determining serum IgE from a lower limit of 10 ng. per milliliter.

MATERIALS AND METHODS

IgE protein

A sample of purified IgE myeloma protein ND was kindly supplied by Dr. S. G. O. Johansson. W. H. O. reference serum 68/341 was obtained from the N. C. I. Immunoglobulin

From the Division of Pediatric Allergy and the Department of Pediatrics, Los Angeles County-University of Southern California Medical Center.

Supported in part by a grant from the General Research Support Fund of the Los Angeles County-University of Southern California Medical Center.

Received for publication Oct. 16, 1972.

Reprint requests to: Dr. Donald R. Hoffman, General Lab. Bldg., Los Angeles County-University of Southern California Medical Center, Los Angeles, Calif. 90033.

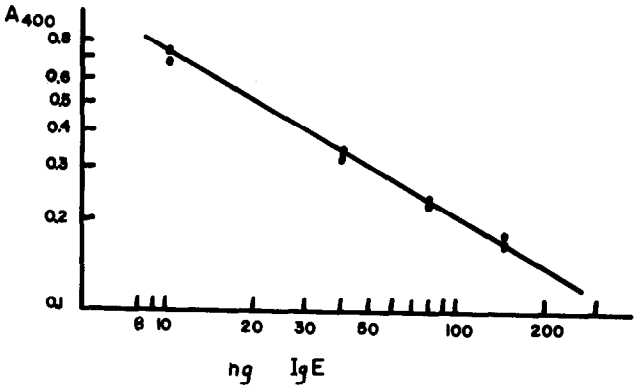

FIG. 1. Standard plot for ELISA method of determining serum IgE. The ordinate shows the absorbance of 400 nm. after incubation for 30 minutes at 22° C., and the abscissa the concentration of IgE determined by radioimmunoassay. The line is the best straight line for determinations using 35 sera. The points indicated are dilutions of 2 standard preparations.

Reference Center and found to have a concentration of 22 μg per milliliter of IgE in agreement with other investigators.[2, 10]

Buffers

Incubation buffer: 0.15M sodium chloride containing 0.05M phosphate pH 7.4, 0.3 per cent human serum albumin, 0.05 per cent sodium azide, and 0.05 per cent Tween 20.

Coating buffer: 0.07M sodium barbital in deionized triple distilled water containing 0.1 per cent sodium azide, pH 9.6 to 9.8.

Washing solution: 1 per cent Tween 20 in 0.15M sodium chloride.

Enzyme buffer: 0.05M sodium bicarbonate containing 0.001M magnesium chloride pH 9.8.

Materials

Alkaline phosphatase, Sigma type VII, with a specific activity of 860 U. per milligram, and p-nitrophenyl phosphate were obtained from the Sigma Chemical Company, St. Louis, Missouri.

Antisera

Anti-IgE was prepared by immunizing a horse with PS myeloma protein. The serum was made monospecific by absorption with insolubilized IgG, IgA, and normal human serum,[11] and the globulin fraction prepared by ammonium sulfate fractionation.[12] In some experiments the anti-IgE was specifically purified by use of an adsorbent prepared by coupling ND myeloma protein to agarose[13]; the antibody was eluted with 3M NaSCN.[14] The specifically purified antibody was more than 30 per cent bound to insoluble IgE after radiolabeling.

Other anti-IgE preparations tested were produced by immunizing a rabbit, a guinea pig, and a goat with PS IgE myeloma protein. All were monospecific by immunodiffusion testing with ND myeloma protein and normal human serum.

Enzyme-IgE conjugate

The conjugate was prepared by the method of Engvall and Perlmann[8] with minor modifications. One milligram of alkaline phosphatase suspension was centrifuged and the supernatant discarded; 50 μl of 0.2M phosphate buffer pH 8.3 and 50 μl of 10 mg. per milliliter ND myeloma protein were added. The mixture was dialyzed overnight against phosphate-buffered saline pH 7.3 containing 0.001M magnesium chloride. The volume was about 0.5 ml. after

dialysis. After warming to room temperature, 20 μl of 5 per cent glutaraldehyde was added. After 2 hours at room temperature, the mixture was diluted to 1 ml. and dialyzed against 3 changes of 1 L. of phosphate-buffered saline pH 7.4 containing 0.001M $MgCl_2$. The conjugate was then diluted to 10 ml. with 0.05M Tris buffer pH 7.8 containing 5 per cent human serum albumin and 0.001M $MgCl_2$. The conjugate was stored at 4° C.

Coated tubes

Falcon Plastics No. 2038 10 × 75 mm. plastic tubes were coated with 1 ml. of 1 μg per milliliter antibody either purified or globulin fraction in coating buffer. The tubes were allowed to stand 4 hours at room temperature and then stored at 4° C. with the antibody solution. Immediately before use the antibody solution was aspirated and the tubes washed 3 times.

Determination of IgE by ELISA

A suitable dilution of serum or standard in 0.9 ml. incubation buffer was added to each washed antibody-coated tube. Then 0.1 ml. of a 1:100 dilution of conjugate in incubation buffer was added. The tubes were capped and rotated overnight at a 30° angle. The tubes were then aspirated, washed 3 times, and 1.0 ml. of 1 mg. per milliliter p-nitrophenyl phosphate in enzyme buffer was added to each tube. The tubes were then rotated for 30 or 60 minutes and the enzyme reaction stopped by the addition of 0.5 ml. of 0.3N sodium hydroxide. The absorbance of each solution was then determined at 400 nm. (Fig. 1).

Determination of IgE by RIST

The Phadebas procedure (Pharmacia Laboratories, Inc.) was used for determination of IgE levels of all sera.

RESULTS

The IgE levels of a series of 35 patients' sera and 3 preparations of standard were determined by both a radioactive inhibition method and the enzyme-linked inhibition method. The patients' sera were found to have IgE levels of from 24 to 2,400 ng. per milliliter. The rank order correlation coefficient for agreement between the two methods was 0.97 ($p < 0.001$). Fig. 1 shows the calibration line obtained from the best fit of the 35 samples. It is a plot of absorbance at 400 nm. after 30 minutes incubation with substrate against nanograms of IgE by radioimmunoassay. The points superimposed on the line are IgE standards from two sources.

Both methods give similar values for international reference standard 68/341, 22 μg per milliliter by radioimmunoassay, 20 μg per milliliter by ELISA. Other investigators report 22 μg per milliliter[2, 7] and 24 μg per milliliter.[10]

Of the 5 preparations of anti-IgE tried, only one was suitable for this test, although all appeared monospecific by immunodiffusion analysis. Four preparations were unsuitable because of low sensitivity and anomalous results with many sera, probably caused by low-grade cross-reactivity with other serum components. Similar results were obtained with these 4 preparations in a RAST system. The horse antibody was suitable for both tests.

The ELISA method was quite reproducible from day to day, and it was necessary to run only 2 or 3 standards for daily calibration. Duplicate samples in the same run are reproducible to ± 3 per cent.

The conditions for IgE determination by ELISA given in *Materials and Methods* are those that give the best range consistent with the desired sensitivity.

Both the concentration of antibody used for coating the tubes and the amount of conjugate may need to be adjusted for different preparations.

DISCUSSION

The ELISA method for serum IgE determinations has several advantages over other methods. It does not require radioactivity or expensive equipment, uses stable materials, does not require elaborate calibration of each run, and is usable in the clinically important range for serum IgE of greater than 10 ng. per milliliter. Values of serum IgE determined by the ELISA method correlate well with values obtained by radioimmunoassay. In addition, the ELISA method is relatively simple to carry out and determinations require less than one day.

The major problem encountered in developing this method was finding a suitable antiserum. Although apparently monospecific by immunodiffusion analysis with a second IgE myeloma protein and human serum, 4 out of 5 antisera were unsuitable for this test. The probable reasons for this are a low-grade cross-reactivity with other immunoglobulins and low affinity for IgE. The horse antiserum used also gave excellent results in a radioallergosorbent system. If the antiserum is of high enough titer, it is possible to use the globulin fraction to coat the tubes, but in other cases it is necessary to specifically purify the anti-IgE. At the present time, due to the limited availability of IgE, this is a drawback to the method.

The coated tube procedure introduced by Catt and Tregear[15] was used for convenience. Since this method relies upon physical adsorption, which is reversible, for insolubilization, it would probably improve the accuracy of the method if the antibody were bound to insoluble carrier by covalent bonds as in the RAST method.[16]

Since the property measured in the ELISA test is related to the concentration of enzyme marker in a time- and temperature-dependent manner, it is important to adequately consider and control these variables. We have found incubation time of 30 minutes with substrate at a temperature of 20 to 22° C. adequate for most experiments. Less-potent antisera require higher temperatures and longer incubation times for equivalent accuracy.

REFERENCES

1 Mancini, G., Carbonara, A. O., and Heremans, J. F.: Immunochemical quantitation of of antigens by single radial immunodiffusion, Immunochemistry 2: 255, 1965.
2 Arbesman, C. E., Ito, K., Wypych, J. I., and Wicher, K.: Measurement of serum IgE by a one-step single radial radiodiffusion method, J. ALLERGY CLIN. IMMUNOL. 49: 72, 1972.
3 Rowe, D. S.: Radioactive single radial diffusion: A method for increasing sensitivity of immunochemical quantitation of proteins in agar gel, Bull. W. H. O. 40: 613, 1969.
4 Johansson, S. G. O., Bennich, H., and Wide, L.: A new class of immunoglobulin in human serum, Immunology 14: 265, 1968.
5 Salmon, S. E.: Serum and secreted IgE in man, Clin Res. 18: 432, 1970.
6 Gleich, G. L., Averbeck, A. K., and Swedlund, H. A.: Measurement of IgE in normal and allergic sera by radioimmunoassay, J. Lab. Clin. Med. 77: 690, 1971.
7 Bazaral, M., Orgel, H. A., and Hamburger, R. N.: IgE levels in normal infants and children and an inheritance hypothesis, J. Immunol. 107: 794, 1971.
8 Engvall, E., and Perlmann, P.: Enzyme-linked immunosorbent assay, ELISA, J. Immunol. 109: 129, 1972.

9 Engvall, E., and Perlmann, P.: Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G, Immunochemistry **8**: 871, 1971.
10 Bazaral, M., and Hamburger, R. N.: Standardization and stability of immunoglobulin E (IgE), J. ALLERGY CLIN. IMMUNOL. **49**: 189, 1972.
11 Axen, R., Porath, J., and Ernback, S.: Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides, Nature **214**: 1302, 1967.
12 Campbell, D. H., Garvey, J. S., Cremer, N. E., and Sussdorf, D. H.: Methods in immunology, New York, 1970, W. A. Benjamin, Inc., p. 189.
13 Cuatrecasas, P.: Protein purification by affinity chromatography, J. Biol. Chem. **245**: 3059, 1970.
14 Carrel, S., Theilkas, L., Morell, A., Skvaril, F., and Barandun, S.: Isolation of normal immunoglobulin E by means of an immunoadsorbent, Biochem. J. **122**: 405, 1971.
15 Catt, K., and Tregear, G. W.: Solid-phase radioimmunoassay in antibody-coated tubes, Science **158**: 1570, 1967.
16 Ceska, M., Eriksson, R., and Varga, J. M.: Radioimmunosorbent assay of allergens, J. ALLERGY CLIN. IMMUNOL. **49**: 1, 1972.