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A simple method for the measurement of low levels of serum IgE

TETSUO GAMO *and* GIICHI MARUYAMA*

*Department of Pediatrics, Osaka Police Hospital, and *Department of Pediatrics, Hyogo College of Medicine, Japan*

Summary

A simple method for the estimation of low serum IgE levels is presented.

Radioimmunoassay often gives falsely high values because of the presence of non-specific factors in the serum when an excessive amount of serum is used in order to measure low serum IgE levels. Therefore the serum sample has to be diluted to minimize the influence of the non-specific factors and the lowest IgE level measurable in the serum is about 5 u/ml.

In this study, pretreatment of the serum with cellulose powder was found to remove the non-specific factors that yield falsely high values. These factors were adsorbed almost completely onto cellulose powder when the serum IgE level was less than 100 u/ml. Thus, low serum IgE levels could be determined by radioimmunoassay using a sufficient amount of serum pretreated by cellulose powder. The lowest serum IgE level measurable by this method was as low as 0·5 u/ml and the low IgE level in neonatal serum could be determined with sufficient accuracy. By this method the geometric level of IgE in cord serum was 2·45 u/ml with a ± 2 standard deviations range of 1·04 to 5·80 u/ml.

Introduction

Johansson (1967) reported the measurement of serum IgE by solid-phase radioimmunoassay (radioimmunosorbent assay) using IgND, which he had found. This method is very sensitive. However, the radioimmunoassay technique is often influenced by non-specific factors present in the serum, and gives falsely high values when an excessive amount of serum is used in order to estimate low serum IgE levels (Bazaral, Orgel & Hamburger, 1971; Gamo, 1972; Polmar, Waldmann & Terry, 1973; Johansson, Berglund & Kjellman, 1976). For this reason, conflicting figures have been reported for the serum IgE level in the neonatal period. For example, Johansson (1968) estimated the IgE level in cord serum as 36·3 ng/m by radioimmunoassay with Sephadex-coupled antibody, whereas Bazaral *et al.* (1971) found that it was 2·1 u/ml using bromoacetylcellulose-coupled antibody, and Stevenson, Orgel & Hamburger (1971) estimated it as 39·3 ng/ml by the double antibody technique. Furthermore, Kjellman, Johansson & Roth (1976) estimated the IgE level in cord serum as 0·22 u/ml

Correspondence: Dr T. Gamo, Department of Pediatrics, Osaka Police Hospital, 8 Komiya-cho, Tennouji-ku, Osaka, Japan 543.

by PRIST, and Johansson *et al.* (1976) estimated it as 3·79 u/ml and 0·39 u/ml by Phadebas IgE test and PRIST respectively*.

Through our studies on the adsorption properties of the bromoacetylcellulose–anti-IgE complex, cellulose particles were found to adsorb non-specific factors which give falsely high IgE values. This paper describes the application of this phenomenon to the measurement of low serum IgE levels in the neonatal period.

Materials and methods

Test serum

Cord and neonatal sera were obtained from newborn infants at the Department of Obstetrics and Gynecology in Suita City Hospital, whose parents had no past history of any allergic disease. Adult sera were obtained from healthy adults.

Bromoacetylcellulose–anti-IgE complex (BAC–anti-IgE)

Following the method of Mann, Granger & Fahey (1969), 2·7 g of BAC (Miles Laboratories Inc., Lot No. JC 512) were washed three times with 5 ml of 0·1 M phosphate–citrate buffer, pH 4·6. Then 2 ml of anti-IgE (sheep anti-human IgE serum, Pharmacia Laboratories Inc., Lot No. 7386) were added and phosphate–citrate buffer, pH 4·6, was added to give a final volume of 12 ml. A drop of toluene was added and the mixture was stirred vigorously for 20 hr at room temperature. Then the complex was washed three times with 0·1 M sodium bicarbonate buffer, pH 8·6, and stirred slowly for 20 hr at 4°C with 10 ml of the same buffer.

It was then centrifuged and the precipitate was suspended in 0·1 M sodium bicarbonate buffer containing 0·1 M monoethanolamine and adjusted to pH 8·6 with 1 N HCl. The suspension was stirred slowly for 20 hr at 4°C, washed three times with PBS and centrifuged. The precipitate was suspended at a concentration of 15% in PBS containing 0·1% NaN_3 and 0·25% human serum albumin (Wako Pure Chemical Industries Ltd), and stored at 4°C.

The protein content of the supernatants at each step, and in the washing fluids, were determined from the optical density at 280 nm. It was calculated that BAC was coupled with 73% of the sheep anti-IgE serum added.

BAC–human serum albumin complex (BAC–HSA)

BAC was coupled with 120 mg of human serum albumin (HSA) using the same procedure as for coupling of sheep anti-IgE serum.

Measurement of the optical density of the supernatant at 280 nm showed that BAC coupled with 50% of the HSA.

Radioimmunoassay of IgE

Determination of the IgE level was carried out mostly by the modified method of the Phadebas IgE test (radioimmunosorbent test using Sephadex-coupled antibody, Pharmacia Laboratories Inc.). In this modification, 0·75 ml of treated serum or treated standard serum, which contains 0·1 ml of original serum, and 0·25 ml of four-fold concentrated Sephadex–anti-IgE complex were used instead of 0·1 ml of serum (diluted

* The WHO Reference Standard 68-341 for IgE was evaluated as 2·42 ng of IgE per unit in terms of myeloma IgE by Bazaral & Hamburger (1972), and as $2{\cdot}35 \pm 0{\cdot}05$ ng per unit by Yunginger & Gleich (1973).

or undiluted) or standard serum and 1·0 ml of Sephadex–anti-IgE complex of the concentration advised in the original method.

Standard IgE serum

Human IgE control serum (Pharmacia Laboratories, Inc., Lot No. 0435) was used after calibration against the WHO reference standard 68–341.

Results

Adsorption of serum IgE by BAC–anti-IgE

Serial dilutions of 15% BAC–anti-IgE suspension were made with PBS containing 0·1% NaN_3 and 0·25% HSA. Volumes of 0·05 ml of each suspension were added to 0·5 ml of serum and stirred for 20 hr with a rotary mixer at 4°C and then centrifuged (2000 *g* for 3 min). The IgE levels of the supernatants with and without an eleven-fold dilution were determined by the original method of Phadebas IgE test (Gamo, 1972).

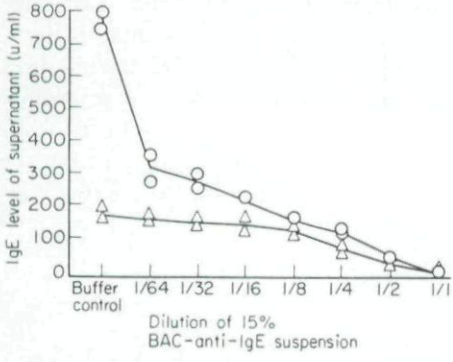

Fig. 1.

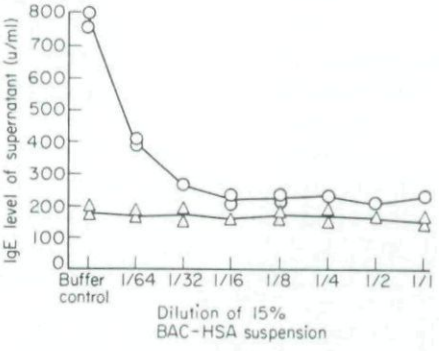

Fig. 2.

Fig. 1. Adsorption of serum IgE by BAC–anti-IgE. Various dilutions of BAC–anti-IgE suspensions and buffer as a control were added to samples of the serum. The mixtures were stirred for 20 hr with a rotary mixer at 4°C, and centrifuged. IgE levels of the supernatants were determined by use of undiluted (○) and eleven-fold diluted (△) supernatants by the original method of Phadebas IgE test.

Fig. 2. Adsorption by BAC–HSA of non-specific factors causing falsely high IgE values when the IgE level was measured in undiluted serum. Various dilutions of BAC–HSA and buffer (control) were added to the serum sample. The procedure was the same as for Fig. 1. IgE levels of the supernatants were determined by use of undiluted supernatant (○) and eleven-fold diluted supernatant (△).

As shown in Fig. 1, the IgE level determined by use of the undiluted supernatant was much higher compared with that determined by use of the diluted supernatant. However, as the increasing concentrations, up to 1/8 dilution, of BAC–anti-IgE were added, the IgE values determined by use of undiluted supernatants sharply decreased compared with a slight decrease determined by use of diluted supernatants, and then after this point both of the IgE levels, determined by the use of undiluted and diluted supernatants, decreased with the same slope (1/8 to 1/1). These results indicate that the non-specific factors causing falsely high IgE values can be adsorbed on a relatively small amount of BAC–anti-IgE, by which IgE in the serum was little adsorbed. A relatively large amount of BAC–anti-IgE seems to adsorb both non-specific factors and the serum IgE.

Adsorption of non-specific factors by BAC–HSA

The effect of treatment of serum with BAC–HSA was investigated to study whether non-specific factors are adsorbed by BAC particles or anti-IgE antibody. The same serum sample and the same procedure were used as for the results above except that BAC–HSA was used instead of BAC–anti-IgE.

The results are shown in Fig. 2. With eleven-fold diluted serum a constant IgE level was observed, within a range of experimental error, on varying concentrations of BAC–HSA. Undiluted serum gave falsely high IgE values at the lowest concentration of BAC–HSA. However, with an increase in the concentration of BAC–HSA, the IgE value approached to that estimated using the eleven-fold diluted serum. Thus it is concluded that BAC–HSA does not adsorb IgE in the serum but adsorbs only non-specific factors which cause falsely high IgE values.

Effect of treatment of serum with cellulose particles on determination of serum IgE level

The adsorption of non-specific factors which give falsely high IgE values was studied using BAC–HSA, Abicell SF (Funakoshi Pharmaceuticals Co., Lot No. 000932) and cellulose powder (Whatman CC 31). 15% suspensions of each powder in PBS containing 0·1% NaN_3 and 0·25% HSA were prepared, and 0·1 ml of each suspension was mixed with 1 ml of serum for 20 hr at 4°C using a rotary mixer. Then the mixtures were centrifuged and the IgE levels of the supernatants were determined by the original method of Phadebas IgE test.

Undiluted serum treated with buffer (control) gave a falsely high IgE value but the undiluted sera after treatment with cellulose particles gave nearly the same IgE values as the eleven-fold diluted sera (Fig. 3).

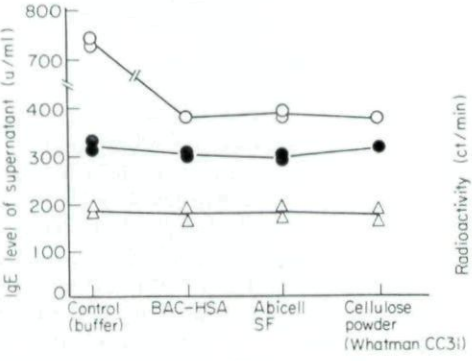

Fig. 3.

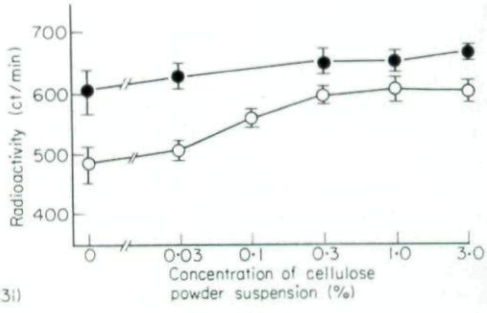

Fig. 4.

Fig. 3. Comparison of the adsorption of non-specific factors by cellulose particles (BAC–HSA, Abicell SF and cellulose powder). 15% suspensions of cellulose particles were prepared in PBS containing 0·1% NaN_3 and 0·25% HSA. Volumes of 0·1 ml of each suspension were mixed with 1 ml of serum sample or 1 ml of standard for 20 hr at 4°C. Then the mixtures were centrifuged (2000 *g* for 3 min) and the IgE levels of the supernatants were determined by the original method of Phadebas IgE test. (○) Sample determined by use of undiluted supernatant; (●) sample determined by use of eleven-fold diluted supernatant; (△) standard determined by use of undiluted supernatant.

Fig. 4. Effect of concentration of cellulose powder. Volumes of 1·3 ml of various concentrations of cellulose powder suspension were mixed with 0·2 ml of serum or standard IgE serum for 20 hr at 4°C. The mixtures were centrifuged (2000 *g* for 3 min) and the IgE levels of the supernatants were determined by the modified method of Phadebas IgE test. The ordinate shows the radioactivity of anti-IgE–Sephadex-bound radioiodinated IgE. Therefore the lower activity represents a higher IgE level. (●) 400 u/ml standard serum; (○) serum sample.

There were no significant differences in the IgE values among the different types of cellulose particles used for serum treatment. Therefore, further studies were made on cellulose powder only.

Effect of concentration of cellulose powder on adsorption of non-specific factors from serum

Volumes of 1·3 ml of various concentrations of cellulose powder suspension were mixed with 0·2 ml of serum or standard IgE serum for 20 hr at 4°C using a rotary mixer. The concentrations of cellulose powder used were 0·03, 0·1, 0·3, 1·0 and 3·0%. The mixtures were then centrifuged and the IgE concentration of the supernatant was determined by the modified method of Phadebas IgE test.

The results are shown in Fig. 4, where the ordinate shows the radioactivity of Sephadex–anti-IgE-bound radioiodinated IgE, so that lower activity represents a higher IgE level. Concentrations of less than 0·3% cellulose powder showed low adsorption. However, cellulose powder had a constant effect at concentrations of more than 0·3%. Therefore 0·5% cellulose powder suspension was used in subsequent experiments.

Effect of time of treatment with cellulose powder on adsorption of non-specific factors

Volumes of 1·3 ml of 0·5% cellulose powder suspension were mixed with 0·2 ml of serum for various periods of time at 4°C. Then the mixtures were centrifuged and the IgE concentration of the supernatant was determined by the modified method of Phadebas IgE test.

The results in Fig. 5 show that high radioactivity was found when serum was treated with cellulose powder for only a short time. This was more marked with the standard serum than with the serum sample.

The high radioactivity was observed for 30 min and then a constant level was obtained. This high radioactivity after a short treatment was not seen with the control of the serum sample and the control of the standard serum, which showed no significant variation in radioactivity throughout the 27 hr of the experiment. Thus it seems

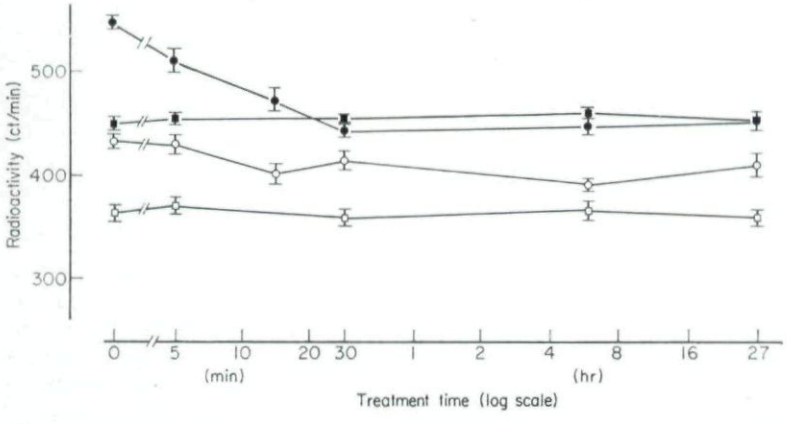

Fig. 5. Effect of time of treatment with cellulose. Volumes of 1·3 ml of 0·5% cellulose powder suspension are mixed with 0·2 ml of serum for the times indicated at 4°C. The ordinate shows the radioactivity of anti-IgE–Sephadex-bound radioiodinated IgE. (●) Standard serum, cellulose treatment; (■) standard serum, control; (○) serum sample, cellulose treatment; (□) serum sample, control.

likely that the high radioactivity observed soon after adding the cellulose powder was caused by adsorption of IgE onto the cellulose powder, and then the adsorbed IgE was gradually released again in the next 30 min, because the standard serum is the diluent of the high IgE serum and contains little of the non-specific factors. On the basis of these results, in subsequent experiments serum was treated with cellulose powder for 20 hr at 4°C.

Determination of IgE in serum samples

Serial dilutions of serum (undiluted and two-, four- and eight-fold diluted) were prepared. Volumes of 1·3 ml of 0·5% cellulose powder suspension were mixed with 0·2 ml of each serum dilution for 20 hr at 4°C. Then the mixtures were centrifuged and the IgE levels of the supernatants were determined by the modified method of Phadebas IgE test. The IgE levels were compared with those determined by the original method of Phadebas IgE test using eleven-fold diluted serum.

Fig. 6 shows that undiluted cellulose powder-treated sera generally gave higher IgE values than eleven-fold diluted sera which had not been treated with cellulose powder. However, the values of the four- and eight-fold diluted and treated sera were the same as that of the eleven-fold diluted and untreated sera. With sera containing less than 100 u/ml of IgE, the levels for undiluted and treated sera were similar to those for untreated and eleven-fold diluted sera.

IgE in cord and neonatal serum samples

A comparison was made of the IgE levels measured by the original method of the Phadebas IgE test, using undiluted untreated sera, and by the modified method of the

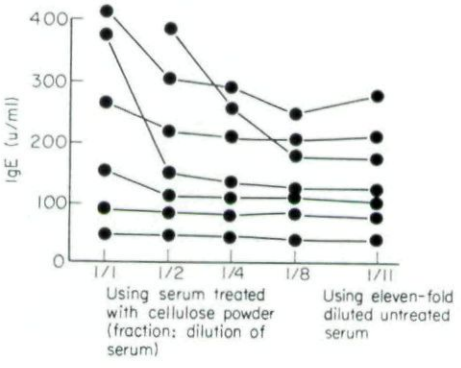

Fig. 6.

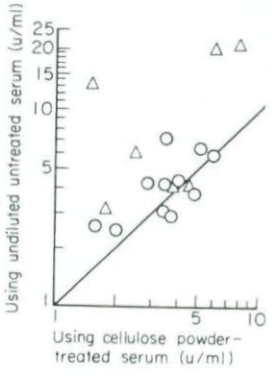

Fig. 7.

Fig. 6. Measurement of the IgE level in serum samples. Four dilutions of serum (undiluted and two-, four- and eight-fold diluted) were prepared. 1·3 ml of 0·5% cellulose powder suspension were mixed with 0·2 ml of each dilution for 20 hr at 4°C. The mixtures were centrifuged and the IgE levels of the supernatants were determined by the modified method of Phadebas IgE test. These IgE levels were compared with the levels determined by the original method of Phadebas IgE test using eleven-fold diluted serum.

Fig. 7. Measurement of the IgE levels in cord and neonatal serum samples. IgE levels of samples were measured by the original method of Phadebas IgE test, using undiluted untreated samples, and by the modified method of Phadebas IgE test, using same samples treated with cellulose powder, and the levels were compared. (○) Cord serum; (△) neonatal serum.

Phadebas IgE test, using sera treated with cellulose powder. As shown in Fig. 7, the untreated sera generally gave higher values, and in four samples of untreated neonatal serum the levels were more than 200% of those of the corresponding treated sera.

The IgE levels in thirty-three samples of cord serum were determined by the modified method of the Phadebas IgE test using cellulose-treated sera. The geometric mean level (± 2 standard deviations range) was 2·45 (1·04–5·80) u/ml.

Discussion

The method of Mancini, Carbonara & Hermans (1965) for determining serum IgE is of low sensitivity, and only high IgE levels (more than about 1000 u/ml) can be determined. Various modifications of this method have been devised, but the maximum sensitivity is about 20 u/ml, in the method of Arbesman, Wypych & Wicher (1972) or of Rowe (1969). Thus the low IgE levels of newborn infants cannot be determined by these methods.

Radioimmunoassay is the most sensitive method and can measure as little as 0·5 to 1 u/ml of IgE, but in the double antibody technique, an excessive amount of serum is known to interfere with the precipitating step (second reaction) (Morgan, Sorensen & Lazarow; 1964). Polgar *et al.* (1972) reported that the double antibody technique is less influenced by non-specific factors than the BAC or Sepharose technique (solid-phase techniques). However, under certain conditions the double antibody technique also seems to give falsely high levels (Johansson *et al.*, 1976). For the original method of the Phadebas IgE test the sample has to be diluted more than eight-fold because an excessive amount of serum gives falsely high values (Gamo, 1972). Consequently, the minimum IgE level measurable in serum is about 5 u/ml in radioimmunoassay techniques, and determination of the IgE level in the newborn infant is not accurate (Johansson *et al.*, 1976).

Bazaral *et al.* (1971) determined the IgE level of undiluted serum, and that of the same serum after adsorbing IgE with Sepharose–anti-IgE, and calculated the actual serum IgE level from the difference. In the present study on the adsorption of serum IgE by BAC–anti-IgE, it was found that BAC–anti-IgE adsorbs not only IgE but also non-specific factors which give falsely high values. Moreover, it was found that cellulose powder alone could adsorb these non-specific factors. Cellulose powder is used for the radioimmunoassay of insulin. It adsorbs free hormone but not antibody-bound hormone (Wright & Malaise, 1966; Zaharko & Beck, 1968), and so it is used to separate free and bound insulin. Macromolecular IgE did not seem to be adsorbed by cellulose powder, only non-specific factors being adsorbed. However, they could not be removed by dialysis.

In PRIST, it is thought theoretically that non-specific factors may interfere with the binding of IgE to paper disc-coupled anti-IgE and lead to a falsely low IgE value. However, Johansson *et al.* (1976) reported that the influence on the IgE values was not so pronounced. They speculated that the washing step performed before the addition of the labelled reagent will probably eliminate some serum factors, and an excess of anti-IgE bound to each disc will make it less likely for antibodies to bovine gamma globulin to interfere sterically with the binding of IgE to anti-IgE. In addition, it is also thought that cellulose in the paper disc may adsorb non-specific factors and eliminate the influence of these factors. Anyhow, PRIST tends to give a falsely low IgE value, but not a high one.

Although non-specific factors were adsorbed by cellulose powder, their adsorption

was not complete. However, these non-specific factors were adsorbed completely when sufficient BAC–anti-IgE was added. Moreover, they were adsorbed almost completely on cellulose powder when the serum IgE level was less than 100 u/ml. Thus the adsorption of non-specific factors seemed to be interfered with by the presence of IgE.

The IgE levels in serum samples of newborn infants were determined by this method, and the geometric mean level (± 2 standard deviations range) for thirty-three samples of cord serum was 2·45 (1·04–5·80) u/ml. This value is much lower than those of 36·3 ng/ml estimated by radioimmunosorbent assay by Johansson (1968) and 39·3 ng/ml estimated by Stevenson *et al.* (1971) using the double antibody method. Moreover, this value is much higher than those of 0·22 u/ml (Kjellman *et al.*, 1976) and 0·39 u/ml (Johansson *et al.*, 1976) estimated by PRIST. However, this value is more consistent with 2·1 u/ml obtained by Bazaral *et al.* (1971) from the consideration of non-specific factors.

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