

IgE antibodies to food allergens detected by ELISA, RAST and monkey PCA

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Summary

IgE antibody to twelve common food and inhalant allergens was measured by enzyme-linked immunosorbent assay (ELISA) in the sera of thirteen atopic patients with one or more allergic disorders (asthma occurring in eleven; rhinitis in ten; eczema in six; urticaria in four; mouth and gastro-intestinal symptoms in six), of twelve non-atopic patients with various clinical symptoms (asthma in four; rhinitis in four; eczema in one; urticaria in two; mouth and gastro-intestinal symptoms in four) and sixteen cord blood sera. The atopic patients had significantly higher IgE ELISA values to the twelve allergens tested than non-atopic persons ($P < 0.01$) and cord blood sera ($P < 0.001$).

Further investigation of IgE antibody to egg white in a group of twenty-two maternal-infant-paired sera using RAST and ELISA techniques showed two cord blood sera that had reproducible RAST values significantly greater than the mean of the maternal group. This was confirmed by RAST inhibition studies but not by monkey PCA tests. High IgE ELISA values to egg white extract did not correlate with RAST results.

Introduction

Food allergy in general and egg allergy in particular is thought to be an important aetiological factor in certain anaphylactic disorders; angio-edema, eczema, urticaria, asthma, allergic rhinitis and gastro-intestinal disturbances, especially in children (Golbert, 1972; Hoffman & Haddad, 1974; Dannaeus *et al.*, 1977; Aas, 1978).

Several biological tests have been used to detect the IgE antibodies believed to mediate these disorders. *In vivo* tests such as the classic passive transfer of serum to humans (Prausnitz & Kustner, 1921) or monkeys (Parish, 1971) may be used to detect both IgE and IgG-STS antibodies to ingested antigens. Among the *in vitro* tests are

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those designed to detect sensitivity of the donor by the addition of antigen to the cells, and those designed to detect anaphylactic antibody (mainly IgE) by treating tissues of normal persons with sera from sensitized donors and challenging the tissues with antigen. Some techniques, e.g. determination of histamine release from passively sensitized human lung challenged with antigen (Parish, 1967) or from monkey lung are impracticable as diagnostic tests because of the requirement for fresh tissue.

Non-biological tests to detect specific antibodies by antigen-binding include the radio-allergosorbent test (RAST). This test (Wide, Bennich & Johansson, 1967) is a sensitive, reliable and reproducible method for detecting IgE antibodies against food allergens (Hoffman & Haddad, 1974; Chua *et al.*, 1976; Wraith *et al.*, 1979) and egg white RAST has shown a significant correlation with clinical egg sensitivity (Schur, Hyde & Wypych, 1974; Dannaeus *et al.*, 1977).

The enzyme-linked immunosorbent assay (ELISA) (Engvall & Perlmann, 1972) has been shown to be sensitive, specific and reliable assay for IgG antibodies to a variety of antigens, including viral and parasitic antigens (Voller, Bidwell & Bartlett, 1976). *Micropolyspora faeni* antigens in former lung disease (Bamdad, 1980) and to protein and polysaccharide antigens of *Aspergillus fumigatus* (Sepulveda, Longbottom & Pepys, 1979). It has also been used to detect total IgE (Hoffman, 1973) and specific IgE antibodies to some inhalant allergens (Eriksson & Ahlstedt, 1977).

In this study, ELISA has been adapted to assay IgE antibodies to common food and some inhalant allergens in adult atopic and non-atopic patients with various allergies. In addition, the results of egg white ELISA, RAST and monkey PCA tests for specific IgE in paired maternal-infant sera are presented.

Materials and methods

Subjects

Group A. The sera of twenty-five adult patients were studied; twelve were skin-prick test negative (non-atopics) to thirty-two common allergens, of whom six had clinical symptoms of allergy (Table 1). One had a history of house dust and another of grass pollen sensitivity and the remaining four had neither clinical sensitivity nor positive skin tests. The other thirteen patients has positive skin-prick test(s) to common allergens ranging from 1+ to 4+ and had various clinical allergies (Table 1) often associated with and aggravated by ingestion of a particular food and improving on elimination diet. Sixteen cord blood samples were included as controls in the analysis of these samples.

Group B. Sera were obtained from twenty-two random paired mothers and infants.

Table 1. Number of patients grouped according to symptoms and atopic status

Diagnosis	Asthma	Rhinitis	Eczema	Urticaria	Mouth & G.I.
Atopics <i>n</i> = 13	11	10	6	4	6
Non-atopics <i>n</i> = 12	4	4	1	2	4?

Allergens

The following allergen extracts were used for both *in vivo* and *in vitro* tests. Bencard's skin test solutions of egg white, egg yolk, cow's milk, mixed nuts, fish, wheat flour, coffee, maize, orange, grass pollen-B₂, house dust and *Dermatophagoides pteronyssinus* were used to test the Group A sera. Test material for Group B, both *in vivo* (PCA) and *in vitro* (ELISA and RAST), was prepared from a crude extract of egg white.

The whites from twelve fresh eggs were separated and defatted in ether for 48 hr before being extracted in Coca's solution for 7 days at +4°C. The extract was filtered through Whatman paper No. 1 prior to being Seitz-filtered, dialysed against running water overnight at +4°C and freeze-dried.

Enzyme-linked immunosorbent assay (ELISA) for specific IgE

The method was a modification of that of Sepulveda *et al.* (1979). The optimal concentration of the allergens, serum and anti-IgE were determined by chequer-board titration using known positive and negative sera. After coating the plates (Dynatech, microelisa M129A) with antigen extract (diluted 1:100) overnight, they were incubated with test serum (1:5) for 6 hr followed by incubation with rabbit anti-human IgE (Behringwerke) overnight. The plates were then incubated with an alkaline phosphatase-conjugated goat anti-rabbit IgG (Miles, Lot No. S415) (1:1500) prepared according to the method of Voller *et al.* (1976) using Sigma alkaline phosphatase (Lot No. 107C-0139). After the final wash, *p*-nitrophenyl phosphate (Sigma 104 phosphatase substrate) 1 mg/ml in 10% diethanolamine buffer (pH=9.8) was added and the reaction was stopped after 30 min by addition of 3M NaOH. All tests were carried out in duplicate and the absorbance was determined at 405_{nm}.

In another experiment, Fc-specific goat anti-human IgE, purified by affinity chromatography (kindly supplied by Dr C.A. Arbesman, Buffalo, U.S.A.) was used in place of the commercially available anti-human IgE and used in conjugation with alkaline phosphatase conjugated rabbit anti-goat IgG (Miles) (1:3000).

Results were expressed as the ELISA value or optical density at 405_{nm}, values greater than 0.25 being considered as significant.

ELISA for egg-white-specific IgG

Plates were coated with egg white extract (50 µg/ml). Sera from Group B were examined in duplicate using test conditions described previously for detecting IgG antibodies in farmers' lung disease (Bamdad, 1980).

ELISA inhibition

Inhibition tests were done in duplicate, incubating increasing concentrations of egg white extract (0.01–100 µg/ml) with known positive and negative sera prior to being used in the experiment.

IgE assay

Sepharose-4B (Pharmacia, London) was activated essentially as described by March, Parikh and Cuatrecasas (1974). Anti-human IgE serum (Behringwerke) was coupled to activated Sepharose-4B according to the method of Ceska and Lundkvist (1972). The goat anti-human IgE (Dr Arbesman) was labelled with ¹²⁵I Na using the chloramine T method of Hunter and Greenwood (1962).

The total IgE assay was done in duplicate using 100 µl volumes of 1:4 suspension of

the solid phase anti-IgE in phosphate-buffered saline (PBS) containing 0.3% human serum albumin (HSA) with 50 μ l of test or standard serum. After overnight incubation at room temperature on a rotary shaker and three washes with PBS, an excess of ^{125} I-labelled anti-human IgE was added. After further overnight incubation and six washes with PBS containing 0.5% Tween-20, residual radioactivity was measured using a Wallac gamma counter. Values of IgE were determined from the standard curve determined from the British Working Standard 75/502 for IgE and an internal laboratory standard.

Radio-allergosorbent test (RAST)

Cyanogen bromide activated cellulose discs (Whatman No. 1, 6 mm diameter) (by the method of Ceska, Eriksson & Varga, 1972) were coupled to the egg white freeze-dried extract (200 μ g/ml).

RAST tests were made in duplicate, using 50 μ l volumes of test serum. Bovine serum albumin (Armour Pharmaceutical Co. Ltd.) (20 μ l of 0.2%) was added to each tube in order to achieve comparable conditions to those used in the RAST inhibition. After overnight incubation, one coupled cellulose disc was incubated with the serum in each tube as before. The discs were then washed four times with PBS before being incubated with the ^{125} I-labelled anti-human IgE (50 μ l containing 80 000 cpm, previously absorbed with a few allergen coupled discs to reduce the non-specific binding). After six washes with PBS containing 0.5% Tween-20, residual activity was counted. Known positive and negative sera were included.

RAST inhibition

In a pilot inhibition assay study it was found that the prior addition of 40 μ g of freeze-dried egg white extract or 10 μ l of anti-human IgE (Behringwerke), provided sufficient antigen or anti-IgE to give approximately 90% inhibition of 50 μ l of known strong RAST positive control sera.

RAST inhibition was performed in duplicate by incubating the test sera (50 μ l) with 20- μ l volumes of 0.2% egg white extract in PBS overnight and then following the RAST procedure above.

Passive transfer tests in monkeys (PCA)

The paired maternal-infant sera together with known positive and negative control sera were used in PCA tests for IgE and IgG-STS antibodies in four cynomolgous monkeys (Parish, 1971). Sites on the thorax and abdomen were injected with unheated sera 24 hr and heated sera (56°C for 2 hr) 3 to 4 hr before testing. After intravenous injection of 5 ml of 5% Evans' blue dye, antigen challenge was carried out by intracutaneous injection of egg white extract (500 μ g/ml). Experiments were carried out blind, and in duplicate, using undiluted sera.

Results

ELISA for IgE antibodies to food and inhalant allergens (Group A)

Sera from thirteen atopic, twelve non-atopic and sixteen cord blood sera were examined for IgE antibodies against twelve allergens (Table 2). The atopic sera had significantly higher ELISA values than the non-atopic sera ($P < 0.01$) and the cord blood sera ($P < 0.001$).

Table 2. ELISA results for IgE to twelve various foods and inhalant allergens in sera from Group A

	Thirteen atopics		Twelve non-atopics		Sixteen cord blood sera	
	0*	≥0.25*	0*	≥0.25*	0*	≥0.25*
Egg white	3	10	4	8	10	6
Egg yolk	5	8	6	6	13	3
Cow's milk	4	9	6	6	12	4
Mixed nuts	5	8	7	5	16	0
Fish	5	8	6	6	15	1
Wheat flour	7	6	6	6	16	0
Coffee	8	5	10	2	16	0
Maize	9	4	11	1	16	0
Orange	4	9	9	3	15	1
Grass pollen B ₂	2	11	5	7	15	1
House dust	4	9	8	4	16	0
<i>D. pteronyssinus</i>	3	10	5	7	14	2

* Number of sera with ELISA value, which is the optical density at 405 nm. Values ≥0.25 OD units were considered as significant in the test.

ELISA values greater than 0.25 optical density units were obtained with cord blood sera in eighteen instances, egg-white-specific IgE being detected most frequently (Table 2).

ELISA and ELISA-inhibition studies for egg-white-specific IgE and IgG in paired maternal-infant sera (Group B)

In the assay of specific IgG antibodies to egg white extract in the twenty-two maternal-infant paired sera, three cord sera had ELISA values greater than their mothers whilst the remainder had similar amounts of antibody as their mothers (Table 3).

The tests for specific IgE to egg white extract in the same sera are shown in Table 4. High ELISA values (≥1.00) were obtained in three maternal and three infant sera, and in only one instance from a paired sample. Similar results were obtained using Fc-specific anti-human IgE, (Dr Arbesman) and rabbit anti-human IgE (Behringwerke).

Table 3. ELISA results for IgG antibodies to egg white extract in twenty-two maternal-infant pair sera

Sera	ELISA value							
	<0.25	0.25-0.49	0.50-0.74	0.75-0.99	1.00-1.24	1.25-1.49	1.50-1.99	2.00
Newborn <i>n</i> = 22	10	4	2	2	1	1	1	1
Maternal <i>n</i> = 22	10	5	2	2	1	—	1	1

Table 4. ELISA results for IgE antibody to egg white extract in twenty-two maternal-infant pair sera

Sera	ELISA value							
	<0.25	0.25-0.49	0.50-0.74	0.75-0.99	1.00-1.24	1.25-1.49	1.50-1.99	2.00
Newborn <i>n</i> = 22	10	4	3	2	1	1	—	1
Maternal <i>n</i> = 22	7	5	6	1	1	—	2	—

The specificity of the results obtained by ELISA was confirmed by inhibition of the antibody binding to the antigen-coated surface by prior incubation of the sera with increasing concentrations of egg white extract.

RAST and RAST-inhibition studies for egg-white-specific IgE in paired maternal-infant sera (Group B)

Three cord blood sera and higher egg white RAST values than the mean value for the twenty-two maternal sera. The positive RAST to egg white was inhibited by prior incubation with egg white, although in the case of one sera, the positive RAST and RAST inhibition could not be repeated on re-test a few months later. Patients with eczema had the highest values of egg white RAST as compared with atopics without eczema and non-atopic subjects.

The positive egg white RAST in the two cord sera, and a positive control could also be inhibited by prior incubation with anti-IgE.

Total IgE levels in paired maternal-infant sera (Group B)

The range of total IgE values for the cord sera was <0.5 to 3.4 i.u./ml as compared with the range of values for maternal sera of 4.5 to 220 i.u./ml. No correlation was found between the cord and maternal serum total IgE levels. Two cord sera with high RAST values had total IgE values of 2.2 and 3.4 i.u./ml, which were among the three cord sera with highest values. The highest total IgE values were obtained in atopic patients with eczema.

Monkey PCA tests on paired maternal-infant sera (Group B)

None of the twenty-two maternal-infant-paired sera sensitized monkey skin for PCA when tested for IgE for IgG-STs activity by intracutaneous injections of egg white antigen. Negative control sera (no. antibody to egg white) all gave negative responses and positive control sera, including sera from three patients with eczema and high values for RAST anti-egg white gave positive 24-hr, but not 3-hr PCA reactions.

Discussion

There are several reports indicating that the foetus may form IgE reagenic antibody *in utero* and be born with atopic sensitivity. Positive skin tests (Balyeat, 1928; Bell & Ericksson, 1931), PK reactions (Allansmith & Buell, 1964) and both phenomena in the same newborn infant (Kaufman, 1971) have been reported to be inducible at birth. Antibodies against a penicillin-hapten group have also been found in the sera of

newborn infants (Levin, Altman & Sela, 1970). Though it has been shown by the use of ^{14}C -labelled amino acids that at 11 weeks the human conceptus is capable of synthesizing IgE (Miller, Hiroven & Gitlin, 1973), very few babies give positive responses to anti-IgE until about 3 weeks old (Stevenson *et al.*, 1971), and PK reactivity of cord sera is usually very difficult to detect (Bell & Ericksson, 1931; Kuhns, 1965). This is consistent with our results in which IgE antibody was detected by the very sensitive RAST and ELISA, but none was detected by monkey PCA. The PCA test is about ten-fold less sensitive than the PK procedure in man (Parish, 1969).

Food allergy in infants particularly to egg and milk is a major problem contributing to development and/or aggravation of atopic eczema, allergic rhinitis, and asthma. Buffum and Settupane (1966) in a follow-up study of 518 asthmatic children, reported that continued or intractable asthma was usually associated with (1) a positive scratch test to egg, (2) the onset of asthma before the age of 2 years, and (3) a history of or the presence of eczema. It is agreed that early identification of children at risk is desirable to start appropriate management, and it is well known that a very large percentage of adverse reactions to foods are IgE-mediated. However, although good correlations have been obtained between the results of RAST tests for egg white and clinical symptoms, a high incidence of egg-white-specific IgE may be detected in egg tolerant patients (Foucard, 1973; Hoffman & Haddad, 1974; Schur *et al.*, 1974; Dannaeus *et al.*, 1977; Wraith *et al.*, 1979).

In the present study, the disparity between total and egg-white-specific IgE measured in maternal-infant sera suggests that the high egg-white-specific IgE antibody levels in some infant sera may be due to active sensitization of the foetus by antigen crossing the placenta. This may well be one of the explanations for the report of Buckley (1973) that in infants with atopic eczema, skin tests with egg white were observed to be often positive before the infant had eaten egg. However, other sources of antigen exposure such as the presence of food antigens in human breast milk must also be considered.

Comparisons between ELISA and RAST for specific IgE measurements to some inhalant and ingested allergens have shown marked discrepancies between the results obtained in the two tests (Ericksson & Ahlstedt, 1977; Fallstrom, Ahlstedt & Hanson, 1978), with ELISA showing more cases of increased specific IgE than RAST. By contrast, Sepulveda *et al.* (1979) using a double antibody ELISA for *A. fumigatus*-specific IgE found ELISA to be a very sensitive and useful alternative to other tests.

In the present study, an ELISA double antibody technique has been used to detect IgE antibodies to twelve different foodstuffs and inhalants. Although it was found that atopics had significantly higher ELISA values than non-atopics ($P < 0.01$) and cord blood ($P < 0.001$) there were high ELISA values particularly to egg white extract in some non-atopics and even in cord blood. Comparing the results of egg white RAST and ELISA there was some agreement, though the ELISA tended to show more increased values than did the RAST. There are many possible explanations for the differences obtained between RAST and ELISA. First, ELISA may measure antibodies to different component(s) of the allergen extract, which may bind preferentially to the plastic surface of the ELISA well. Secondly, non-specific and/or blocking factors or antibody may compete for allergen in RAST but not ELISA, and thus ELISA may detect a population of high affinity IgE antibodies. Whatever the reason, ELISA has proved to be a sensitive, safe and useful technique for the measurement of antibodies of classes other than IgE to a wide variety of allergens.

Further development of ELISA for the detection of IgE antibodies would therefore be valuable, in view of the inherent drawbacks which are involved in the handling of radio-isotopes in RAST.

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