

IgE antibody levels to ingested soya protein determined in a normal adult population

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Summary

Levels of soya protein-specific IgE were measured in a normal adult population (seventy-four males, fifteen females) who ingested soya-containing and control diets during two 4-week periods. Increases in soya-specific IgE were observed for some individuals following ingestion of the soya-containing diet, and for the female group the increase in soya-specific IgE was statistically significant ($P=0.02$). The increase of soya-specific IgE was small and led to lower levels than that associated with adverse effects. The increase in soya-specific IgE in the female group was accompanied by a significant increase ($P=0.02$) in total immunoglobulin A. Changes in the level of soya-specific haemagglutinating antibody, soya-specific IgG, IgA and IgM as measured by ELISA and the immunoconglutinin titre could not be related to ingestion of the soya-containing diet.

Introduction

Allergic reactions to ingested foods mediated by IgE antibody are a frequent occurrence in childhood, but this anaphylactic sensitization usually wanes or disappears by adult life (Parish, 1971). RAST has been widely used in the diagnosis of allergic reactivity to ingested foods (Hoffman & Haddad, 1974; Chua *et al.*, 1976; Dannaeus *et al.*, 1977; Aas, 1978; Wraith *et al.*, 1979), but the interpretation of such tests has been made difficult by the frequent finding of IgE antibodies in the sera of asymptomatic children, and occasionally adults tolerant to the food in question. The biological significance of these antibodies in normal persons has remained unestablished.

Soya proteins are widely used in small amounts in prepared foods and may be regularly eaten by many people in Western countries. Although allergic reactions to ingested as well as inhaled soya proteins have been reported, soya proteins have been regarded as being less allergenic than proteins of mammalian origin (e.g. cow's milk) (Halpern *et al.*, 1973). IgE antibodies to soya proteins have, however, been detected in the sera of asymptomatic children and adults by RAST (Dannaeus *et al.*, 1977; Aas, 1978).

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This paper describes the measurement of soya-specific IgE and total immunoglobulins in approximately 100 normal adults who took part in feeding trials designed to evaluate the physiological effect of high dietary levels of soya protein (Van Stratum 1978; Van Stratum & Rudrum, 1979). The results of the trial show that sera from some normal adults may contain IgE antibodies to food proteins including soya, and that the level of soya-specific IgE may increase in some subjects, without adverse effect, as a result of incorporation of high dietary levels of soya protein.

Materials and methods

Subjects examined and feeding trial design

Main trial. Full details of the feeding trial, and a preliminary report of the immunological findings have been published (Van Stratum, 1978; Van Stratum & Rudrum, 1979). The feeding trial design is shown in Fig. 1. Briefly, a group of eighty-nine volunteer Dutch laboratory personnel (seventy-four males, fifteen females)

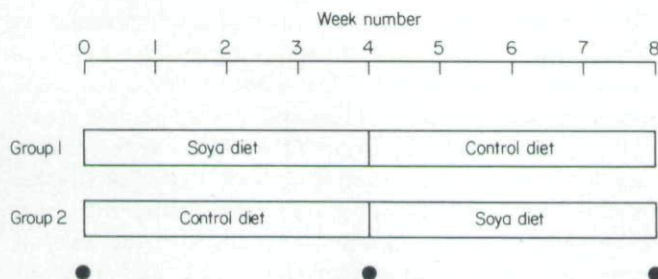


Fig. 1. Main feeding trial design. ●, Blood sample taken.

were fed a test soya diet and control diet using a cross-over design. The test diet, containing a large variety of heated soya protein products, was given in a 4-week menu cycle in the form of varied fully acceptable meals. The control diet, having the same protein content, was given in a similar menu cycle with the same meals as the test diet but using conventional proteins.

Serum samples for antibody analyses were obtained before the test (week 0) and at weeks 4 and 8. The experimental diets eaten by the volunteers during the trial provided an average adult a protein intake of 100 g/day, and test diet contained on average 23 g soya protein.

Second trial. In a second trial designed to evaluate the effect of flatulence factors in unrefined soya protein (Van Stratum & Rudrum, 1979), soya-specific IgE was measured in a further nine subjects. The subjects were fed less-refined soya protein in a test meal at day 0, and also high soya protein meals over a 4-day period 4 weeks later. Blood samples were taken before the trial, and immediately before and 1 week after the second period of soya ingestion.

Serological procedures

Total immunoglobulin G, A and M levels were estimated by the single radial diffusion procedure (Mancini *et al.*, 1965) using commercial test plates and standard sera (Behringwerke, Hoechst Pharmaceuticals).

Total IgE levels were estimated by the RIST procedure using a commercial kit system (Phadebas RIST, Pharmacia).

RAST for the detection of soya-specific IgE was carried out as originally described (Wide *et al.*, 1967), using soya flour allergosorbents prepared to the criteria described by Goodwin & How (1976). Soya flour extracts for the preparation of allergosorbents were prepared by extracting commercial, unheated soya flour (1 g) in 0.1 M sodium bicarbonate pH 8.0 (10 ml) at room temperature for 15 min, followed by centrifugation. Allergosorbents prepared in this way were capable of detecting soya-specific IgE in the sera of soya-intolerant patients. Immunosorbent purified anti-IgE was obtained from Pharmacia (Phadebas RAST). All RAST analyses were carried out at the same time using the same batches of reagents, and the results are expressed as the uptake of anti-IgE (ct/min).

Passive haemagglutination tests were carried out using sheep erythrocytes sensitized to unheated soya flour extract using glutaraldehyde (Avrameas *et al.*, 1969). Soya flour extracts were absorbed before coupling with sheep erythrocytes (1.5 ml washed packed erythrocytes/25 ml soya extract diluted 1:32 in phosphate buffered saline pH 7.2) to remove the natural haemagglutinin.

Enzyme-linked immunosorbent assay (ELISA) was carried out as described by Voller *et al.*, (1974) using Removawells (Dynatech). Extracts (1 g/10 ml) of USF in 0.05 M carbonate buffer pH 9.8 were diluted (1:200) and used to coat the wells overnight at room temperature. Excess antigen was removed and the wells washed ($\times 5$) with phosphate buffered saline (PBS) containing 0.15% Tween 20. The washing procedure was repeated before the addition of each new reagent. Aliquots (200 μ l) of test sera, diluted 1:1000 in PBS/Tween 20 were added to the wells and incubated at room temperature for 3 hr. IgG, IgA and IgM antibodies were detected using IgG (7s) fractions of specific sheep antisera (Nordic) labelled with alkaline phosphatase (type VII, Sigma) as described by Engvall & Perlmann (1972). The optimum dilution (1:200) of these reagents in PBS/Tween 20 was determined in preliminary experiments. Following incubation at room temperature for 2 hr, bound enzyme activity was detected by addition of 200 μ l of p-nitrophenyl phosphate (Sigma 104, 1 mg/ml in 0.05 M carbonate buffer pH 9.8 containing 0.005 M magnesium chloride) and an overnight incubation at room temperature. The optical density of the contents of each well was determined at 400 nm, and corrected for the background substrate hydrolysis observed in wells where test sera were omitted.

Immunconglutinins were detected in sera using EAC43 prepared according to Lachman, Hobart & Aston (1973). Suramin BP was obtained from Bayer U.K. Limited, Haywards Heath, West Sussex.

Results

Total immunoglobulin levels

There was no significant difference between the IgG, IgM and IgE levels for male or female subjects following their 4-week period on soya-containing or control diet (Table 1). Female subjects showed a significant increase in total serum IgA following ingestion of the soya-containing diet, but a similar increase was not observed for male subjects who as a group showed significantly higher IgA levels than the females.

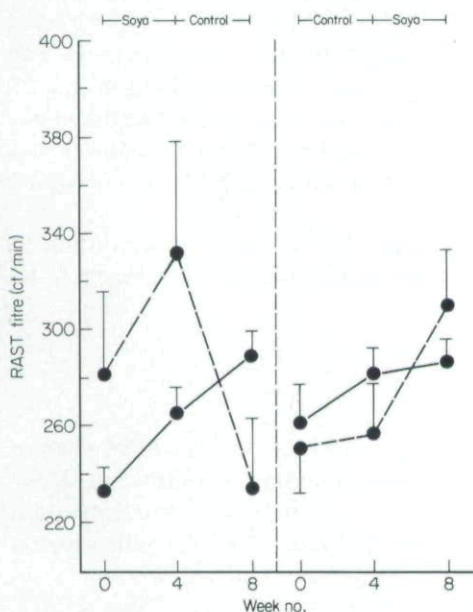
Soya-specific IgE levels

The observed changes in soya-specific IgE following ingestion of soya-containing and

Table 1. Statistical difference between total immunoglobulin levels and soya-specific IgE levels (RAST titres) following ingestion of soya-containing and control diets

Parameter	Units	Number of subjects	Soya containing diet (A)	Control diet (B)	Difference (A) - (B) $\pm$ s.e. mean	Significance
IgG	iu/ml	89	140	139	0.6 ± 1.7	Not significant
IgA(male)	iu/ml	74	117	116	1 ± 1.4	Not significant
IgA(female)	iu/ml	15	94	87	7 ± 2.5	$P = 0.02$
IgM	iu/ml	89	161	160	1 ± 1.6	Not significant
IgE	iu/ml	89	96	93	2 ± 12	Not significant
Soya-specific IgE (male)	ct/min	74	276	285	-9 ± 9.5	Not significant
Soya-specific IgE (female)	ct/min	15	327	245	82 ± 30	$P = 0.02$

control diets in the main trial are shown in Fig. 2. Only the female subjects showed a significant increase in soya-specific IgE (Table 1). Subjects with an elevated level of soya-specific IgE (> 1.5 group mean) at the beginning of the trial showed a decrease in RAST titre between 0 and 4 weeks and an increase between 4 and 8 weeks irrespective of whether soya or control diet was eaten. None of the subjects experienced symptoms of allergic reactivity during ingestion or after ingestion of the soya-protein diets.

**Fig. 2.** Soya-specific IgE response in male (—) and female (---) subjects participating in main feeding trial. (●) Mean $\pm$ s.e. mean in male.

There was no correlation between changes in the soya-specific IgE level (or total immunoglobulin A) for the female subjects and their estimated soya consumption. Females tended to eat less soya than males, but had a similar soya intake per kg body weight.

Changes in soya-specific IgE for subjects taking part in the second trial are shown in Fig. 3. Subjects were divided into two groups according to their level of soya-specific IgE in the pre-test sample. Subjects with low pre-trial soya-specific IgE (Fig. 3a) showed an increase at week 4 and a sharp decrease in soya-specific IgE after the second period of soya ingestion. Three subjects with slightly higher soya-specific IgE levels at the beginning of the trial (Fig. 3b) showed a decrease in soya-specific IgE at week 4, and in two subjects a sharp increase after the second period of soya ingestion.

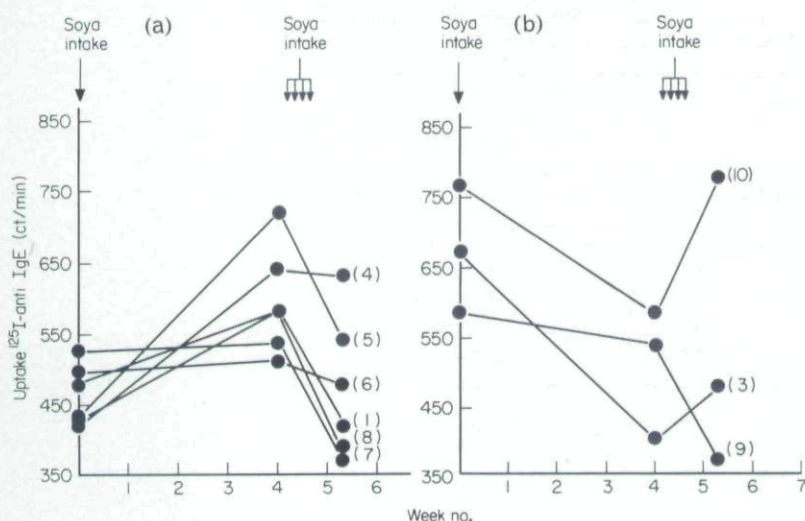


Fig. 3. Soya-specific IgE responses in subjects participating in second trial. (a) Subjects with low pre-trial RAST. (b) Subjects with slightly elevated pre-trial RAST.

Soya-specific antibodies measured by ELISA and passive haemagglutination

Soya-specific IgG antibody was detected by ELISA in the sera of some subjects at the beginning of the main trial, but did not alter significantly as a result of ingestion of the soya diet. Soya-specific IgA and IgM were not detected in any of the sera, or in the sera of female subjects who showed a significant increase in total IgA as a result of the soya diet.

Thirty-two of the eighty-nine sera tested contained detectable ($\geq$ titre of 1:5) soya-specific haemagglutinating antibody at the beginning of the trial (range 1:5–1:160). There was considerable variation in the titres during the trial, which could not be related to either soya or control diet.

Immunoconglutinin determinations

Immunoconglutinins were detected in five sera at the beginning of the trial, in fourteen sera after 4 weeks and in twenty-three after 8 weeks. The immunoconglutinins were in most cases only detectable in neat serum and could not be related to ingestion of either

soya-containing or control diet. An increased incidence of colds and throat infections in the area was noted during the trial.

Incidence of soya-protein, rice, wheat grain, and grass pollen-specific IgE in pre-trial sera
Sera from subjects participating in the main trial, and from additional volunteers from the same laboratory population, were examined for rice, wheat grain and grass pollen-specific IgE in addition to soya protein-specific IgE (Table 2). Soya-specific IgE was found less frequently than the cereal-specific IgE and elevated levels of rice-specific IgE were the most frequently observed. No correlation was observed between the levels of wheat grain, rice or grass pollen-specific IgE, although two subjects with elevated wheat-specific IgE also had elevated grass pollen-specific IgE levels.

Table 2. Incidence of elevated soya protein, rice wheat grain and grass pollen-specific IgE in a normal adult population

Allergen	Number of sera tested	Number of sera with elevated level of specific IgE antibody	
		1.5 - 2 × mean uptake*	≥ 2 × mean uptake
Soya protein	106	4	0
Rice	99	7	4
Wheat grain	96	1	1
Grass pollen	99	0	9

* Mean uptake calculated for all sera tested for a particular allergen.

Discussion

In the normal healthy adult populations examined in this study, ingestion of soya bean products over a long period resulted in a small but significant increase in the level of soya-specific IgE, as measured by RAST, in some subjects. This increase in soya-specific IgE was not accompanied by any adverse reaction to the ingested protein, and the level of soya-specific IgE antibody detected in these individuals was smaller than that normally associated with allergic reactivity to ingested or inhaled allergens. Females tended to show a greater response than males, and on the basis of a limited second trial, secondary responses were not observed.

Soya protein-specific IgE was less frequently observed than IgE antibodies to the two cereal proteins wheat grain and rice, although levels of IgE antibody to these ingested cereal antigens may have been influenced by cross-reactions between the cereals and inhaled grass pollen (Hoffman, 1975) or inhaled cereal allergens. However, as described by Hoffman (1975) and confirmed by Wraith *et al.*, (1979), some sera showed raised levels of IgE to either cereals or pollen, and the possibility that the allergenic exposure responsible for the cereal IgE formation was by ingestion rather than inhalation cannot be excluded. Also, the cereal RAST levels were measured at a time (pre-pollen season) when the airborne level of pollen or cereal-derived allergens would be low. Although non-specific binding of IgE to soya-allergosorbents in RAST has been suggested by Aas (1978), the strong correlation between the level of

soya-specific IgE and soya intake observed in this study suggest that the increased levels of IgE are due to antibody synthesis rather than assay artefact. If this is true, then levels of specific IgE to other food antigens in normal or asymptomatic subjects may reflect the exposure of the individual to the ingested antigen. Such levels of antibody measured by RAST would be below the level regarded as clinically significant (RAST class I), but below this point the level of antibody may reflect exposure to the antigen in a manner analogous to some inhalant allergens (Pepys *et al.*, 1973).

The biological consequence of IgE antibody at this level is a matter of conjecture, and it is unlikely that such low levels of antibody detected by radioimmunoassay would be detected by passive sensitization procedures (Parish, 1971) or mediate an adverse reaction. However, if the measurements of IgE antibody by RAST in normal adults represent IgE antibody synthesis, then it is possible that this antibody acts locally in the gut, possibly causing increased gut permeability and facilitating the elimination of antigenic material by formation of antigen antibody complexes (Paganelli *et al.*, 1979). The IgE response would at the same time be controlled by the various mechanisms proposed (Jarrett, 1977) for the control of IgE antibody synthesis in non-allergic subjects, and an adverse reaction would not result.

The observed increase in soya-specific IgE in the female subjects was accompanied by an increase in the total serum level of IgA. There was not, however, a corresponding increase in the soya-specific haemagglutinating antibody titre or the levels of soya-specific IgA or IgG and IgM antibodies detected by ELISA. The increased total IgA in these subjects may reflect an increased antibody synthesis to soya proteins or be the result of changes in exposure to other antigens derived from the diet or gut flora. If the increase in total serum IgA is the result of soya protein ingestion, then the inability of passive haemagglutination or ELISA to detect such antibody may be analogous to that described for *E. coli* (Jodal *et al.*, 1977). Alternatively, such antibody may be in the form of IgA-antigen complexes (Paganelli *et al.*, 1979), which would probably not be detected by the techniques used in this study to detect free antibody or by immunoconglutinin titration.

The results of these feeding trials suggest that IgE antibody levels to some ingested foods may fluctuate in normal adults as a result of antigenic stimulation. Further studies of the IgE response of normal adults as well as patients with food allergy may lead to a better understanding of the normal role of this immunoglobulin.

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References

- AAS, K. (1978) The diagnosis of hypersensitivity to ingested foods. Reliability of skin prick testing and the radioallergosorbent test with different materials. *Clinical Allergy*, **8**, 39.
- AVRAMEAS, S., TANDOU, B. & CHUILON, S. (1969) Glutaraldehyde, cyanuric chloride and tetraazotized o-dianisidine as coupling reagents in the passive haemagglutination test. *Immunochemistry*, **6**, 67.
- CHUA, Y.Y., BREMNER, K., LLOBET, J.L., KOKUBU, H.L. & COLLINS-WILLIAMS, C. (1976) Diagnosis of food allergy by the radioallergosorbent test. *Journal of Allergy and Clinical Immunology*, **58**, 477.

- DANNAEUS, A., JOHANSSON, S.G.O., FOUCARD, T. & OHMAN, S. (1977) Clinical and immunological aspects of food allergy in childhood. I. Estimation of IgG, IgA, and IgE antibodies to food antigens in children with food allergy and atopic dermatitis. *Acta paediatrica Scandinavica*, **66**, 31.
- ENGVALL, E. & PERLMANN, P. (1972) Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labelled anti-immunoglobulin in antigen-coated tubes. *J. Immunol.*, **109**, 129.
- GOODWIN, B.F.J. & HOW, M.J. (1976) RAST using crude and purified anti-IgE. *Clinical Allergy*, **6**, 441.
- HALPERN, S.R., SELLARS, W.A., JOHNSON, R.B., ANDERSON, D.W., SAPERSTEIN, S. & REISCH, J.S. (1973) Development of childhood allergy in infants fed breast, soy or cow's milk. *Journal of Allergy and Clinical Immunology*, **51**, 139.
- HOFFMAN, D.R. (1975) The specificities of human IgE antibodies combining with cereal grains. *Immunochemistry*, **12**, 535.
- HOFFMAN, D.R. & HADDAD, Z.H. (1974) Diagnosis of IgE-mediated reactions to food antigens by radioimmunoassay. *Journal of Allergy and Clinical Immunology*, **54**, 165.
- JARRETT, E.E.E. (1977) Activation of IgE regulatory mechanisms by transmucosal absorption of antigen. *Lancet*, **ii**, 223.
- JODAL, U., AHLSTEDT, S., HANSON, L.A., LIDIN-JANSON, G. & SOHL AKERLUND, A. (1977) Intestinal stimulation of the serum antibody response against E. Coli 083 antigen in healthy adults. *International Archives of Allergy and Applied Immunology*, **53**, 481.
- LACHMAN, P.J., HOBART, M.J., & ASTON, W.P. (1973) Complement technology. In: *Handbook of Experimental Immunology*, Vol. 1 (ed D. M. Weir), 2nd ed, p. 51 Blackwell Scientific Publications, Oxford.
- MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.
- PAGANELLI, R., LEVINSKY, R.J., BROSTOFF, J. & WRAITH, D.G. (1979) Immune complexes containing food proteins in normal and atopic subjects after oral challenge and effect of sodium cromoglycate on antigen absorption. *Lancet*, **i**, 1270.
- PARISH, W.E. (1971) Detection of reaginic and short-term sensitizing anaphylactic or anaphylactoid antibodies to milk in sera of allergic and normal persons. *Clinical Allergy*, **1**, 369.
- PEPYS, J., WELLS, I.D., D'SOUZA, M.F. & GREENBERG, M. (1973) Clinical and immunological responses to enzymes of *Bacillus subtilis* in factory workers and consumers. *Clinical Allergy*, **3**, 143.
- VAN STRATUM, P. (1978) Physiological effects of a high soya-bean protein diet in man. *Cereal Foods World*, **23**, 235.
- VAN STRATUM, P. & RUDRUM, M. (1979) Effects of consumption of processed soy proteins on minerals and digestion in man. *J. Am. Oil Chemists' Soc.*, **56**, 130.
- VOLLER, A., BIDWELL, D.E., HULDT, G. & ENGVALL, E. (1974) A microplate method of enzyme linked immunosorbent assay and its application to malaria. *Bulletin of the World Health Organisation*, **51/2**, 209.
- WIDE, L., BENNICH, H. & JOHANSSON, S.G.O. (1967) Diagnosis of allergy by an *in vitro* test for allergen antibodies. *Lancet*, **ii**, 1105.
- WRAITH, D.G., MERRETT, J., ROTH, A., YMAN, L. & MERRETT, T.G. (1979) Recognition of food-allergic patients and their allergens by the RAST technique and clinical investigation. *Clinical Allergy*, **9**, 25.

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