

The radioallergosorbent test with a purified allergen from codfish

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Summary

The radioallergosorbent test (RAST) was performed with a purified allergen (DS 22) from cod, coupled to CNBr-activated cellulose discs. A 100% correlation was found between a positive RAST and the presence of clinical allergy to cod in fifty-six children as compared to a negative RAST in all sera from fifty-six allergic children tolerating fish. Positive results were obtained with discs treated with DS 22 in a concentration as low as 0.4 ng/ml.

Introduction

The radioallergosorbent test (RAST) is an antiglobulin reaction based upon the detection of specific antibodies of the IgE type, by ^{125}I -labelled anti-IgE on sorbent material to which allergens have been coupled (Wide, Bennich & Johansson, 1967). It has been reported that RAST may be most valuable for experimental work in allergology (Ceska, 1972; Ceska, Eriksson & Varga, 1972a, b; Ceska & Hultén, 1972; Varga & Ceska, 1972a, b; Foucard, Aas & Johansson, 1973; Ponterius *et al.*, 1973; Yman, Brandt & Ponterius, 1973), and that it is a valuable *in vitro* supplement in the diagnosis of clinical allergy (Aas & Johansson, 1971; Berg, Bennich & Johansson, 1971; Berg & Johansson, 1971a, b; Fagerberg & Wide, 1970; Johansson, Bennich & Berg, 1971; Johansson, Bennich & Berg, 1972; Stenius & Wide, 1969; Wide *et al.*, 1967).

In two previous studies (Aas & Johansson, 1971; Berg *et al.*, 1971), an overall correlation between the clinical diagnosis and RAST was established in 73-74% of the cases investigated. In one of the studies (Aas & Johansson, 1971), the correlation depended on the type of allergen used. There may be different explanations for this. Different allergens may result in different degrees of immunological sensitization with consequences for the specificities and titres of reagin occurring in serum of some of the patients. Qualitative differences between allergen extracts used in the clinical diagnosis and those used in RAST may also play a part. The lack of satisfactorily standardized extracts and hence availability only of qualitatively and quantitatively heterogeneous

protein mixtures for both *in vitro* and clinical tests represent probably the most important limitations in correlation studies (Aas, 1972; Aas & Belin, 1973).

The present investigation was undertaken to study the reliability and sensitivity of a new modification of the RAST-technique with use of a partially purified and well characterized allergenic material.

Material and methods

Allergen

A purified allergen (DS 22) from cod was used as the model allergen. It was produced as described before (Aas & Jebsen, 1967), and stored in the freeze-dried state until used. For clinical tests it was resolved in sterile saline (0.9% NaCl) containing 0.5% phenol and used in tenfold dilutions from a 1 mg/ml starting concentration. For the *in vitro* tests, the material was resolved in sterile, distilled water.

Patients

Fifty-six children allergic to cod were included in this study. The clinical data have been described before (Aas, 1966a, b). All of them suffered from immediate type allergy to cod with bronchial asthma and/or urticaria or angioedema. All patients had an unequivocal history of allergy to fish. Each child showed strong positive (+ + + or more) wealing reactions to scratch tests with the crude cod extract in a 0.2 mg/ml total protein concentration and to DS 22 in a 0.002 mg/ml concentration (Aas, 1966a, b; Aas & Jebsen, 1967; Aas, 1969). In every instance, at least two exposure tests or control provocative feedings with cod had been done, in each instance resulting in immediate hypersensitivity reactions (Aas, 1966a, b). Passive transfer tests with *in vivo* cross-neutralization with the sera from six randomly selected children allergic to cod and with the reference serum (see below), showed that DS 22 represented the only major allergen in cod. This was confirmed also with experiments in which sensitized and neutralized PK-sites were challenged by ingestion of DS 22 and cod, respectively (Aas & Jebsen, 1967).

As control subjects were selected an equal number of children with asthma and/or urticaria shown to be allergic but without any past or present history of fish allergy, frequently eating cod without untoward reactions, and with negative skin test reactions to fish extracts including cod and DS 22. The controls were matched with the cod allergic patients with respect to sex and age.

Serum was collected by venepuncture under sterile conditions and stored without any additives at -20°C until use. They were collected at the time when the clinical allergy was manifest and were stored for 3–10 years before used in the radioallergo-sorbent test. A few of the sera have been thawed and refrozen one to three times during this period of storage.

Serum was collected from an adult male patient allergic to cod (Aas, 1966a, b), and was used for passive transfer tests and for adjustment of the RAST-technique modification to be used. This serum has been used as the reference serum in a number of studies previously reported (Aas, 1966a, b, 1969; Aas, Foucard & Johansson, 1971; Elsayed & Aas, 1971). It has been shown to be able in high dilutions to sensitize human skin *in vivo* and chopped human lung *in vitro* for challenges with cod extracts and DS 22, and to react in RAST coupled to Sephadex particles (Foucard *et al.*,

1973). As a negative control serum, the serum from one of the control patients tolerating fish was used.

Passive transfer (PK) tests were performed as described before (Aas, 1966b) on a non-allergic male volunteer. Serial tenfold dilutions of serum were made with saline containing 0.5% phenol and used to sensitize skin on the back of the recipient and on the volar surface of the forearms. In one series of experiments serum DE was diluted in the similar way with the use of the control serum as diluent.

Intradermal challenges were made with DS 22 0.0001 mg/ml to establish the lower end point of serum dilutions capable of sensitizing the skin for a positive reaction (a distinct weal and flare reaction not occurring in control sites). The PK titre was expressed as the reciprocal of this serum dilution.

To establish the lower end point for DS 22 able to elicit a PK-reaction, serial tenfold dilutions of the allergen were used to challenge sites sensitized with undiluted serum. The methods used have been described in detail in previous work (Aas, 1966b).

CNBr-activation of cellulose discs

Source of paper discs as well as the method for CNBr-activation is described elsewhere (Ceska & Lundkvist, 1972). The allergen preparation DS 22 was prepared at concentrations of 0.1–5 µg/ml in 0.9% saline. Aliquots of 0.4 ml of the DS 22 solutions were added to 3.6 ml of 0.1 M NaHCO₃ containing 100 discs. The mixture was shaken for 3 hr, and the discs were extensively washed as described before (Ceska & Lundkvist, 1972). The exact amount of DS 22 on the discs has so far not been determined.

The radioallergosorbent test (RAST)

The assay was performed in disposable polystyrene test tubes of Ellerman type (round bottom, 10 × 57 mm). One paper disc on to which DS 22 was coupled, was used per test tube. To each tube was added 50 µl serum (sample, reference or negative control). All assays were performed in duplicates. The tubes were covered with a sheet of parafilm and left on the laboratory bench to incubate for 3 hr. The liquid phase was aspirated, and the discs were then washed three times with 2.5 ml 0.9% saline. Between each washing the discs were incubated for 10 min in the washing liquid. Then 50 µl (approximately 7 ng) ¹²⁵I-a-IgE (ND)* were added to the paper discs. The tubes were again covered with parafilm and incubated for approximately 18 hr. After termination of this latter incubation the tubes were washed three times with 0.9% saline. The radioactivity on the paper discs was counted in a γ-counter, and the results were given as counts per 2 or 5 min.

Results

Passive transfer tests with reference serum DE and control serum, and challenges with serially diluted DS 22

Challenges with DS 22 in control serum sites were negative in all instances. Challenges with serial dilutions of DS 22 of sites sensitized with the cod allergic reference serum DE were positive with a lower end point at 10⁻¹⁴ g/ml. Challenges of sites sensitized with serially diluted cod allergic serum DE elicited distinctly positive reactions with a

* Immunosorbent purified material of rabbit origin obtained from Dr H. Bennich, Wallenberg Laboratory, Uppsala, and labelled by the ICL technique at Pharmacia AB,

lower end point activity at 1/160 to 1/200 of the serum with saline as the diluent. The same endpoint was found with the negative control serum as a diluent.

CNBr-activated cellulose discs incubated with the DS 22 allergen solution in serial dilutions were used in RAST with the positive reference serum and with a negative control serum. It was found that distinct *in vitro* reactions were obtained when DS 22 was used at a concentration of 40 ng/100 discs during coupling. The maximal response was obtained when 800 ng DS 22 in the coupling mixture were used per 100 discs. The dose-response curve obtained when coupling different concentrations of DS 22 to the activated discs is shown in Fig. 1, using the positive reference serum DE for the detection of the bound DS 22.

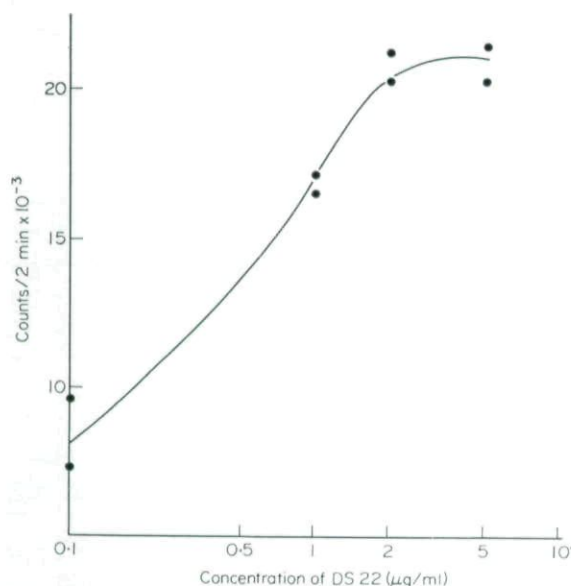


Fig. 1. The dose-response curve obtained when different concentrations of DS 22 were used for the coupling to the discs. The response was assayed by means of RAST using the positive reference serum from a cod sensitive individual (D.E.).

None of the sera from control patients tolerating cod showed a RAST result above 4500 counts/5 min (range 1395–4477 counts/5 min). The highest count obtained with the reference system was 70041 counts/5 min (background subtracted). The highest count similarly obtained with the DS 22 in the cod allergic patients was 94660 counts/5 min given as the mean of duplicate tests with background subtracted.

RAST was positive with serum samples from all of the cod allergic individuals (Fig. 2) with the weakest reaction at 6700 counts/5 min.

In two patients allergic to cod, RAST was, however, found to be negative with some serum samples although being positive when performed with serum collected at other times. The serum samples collected at different times during 6 years from one of these patients (T.N.) were studied in a separate series of experiments in which both RAST negative and RAST positive ones were used in PK tests (Table 1). All samples were positive in PK tests with lower end point titres varying from 10 to 400. With these serum samples (from T.N.) no direct correlation was found between PK titres and RAST results.

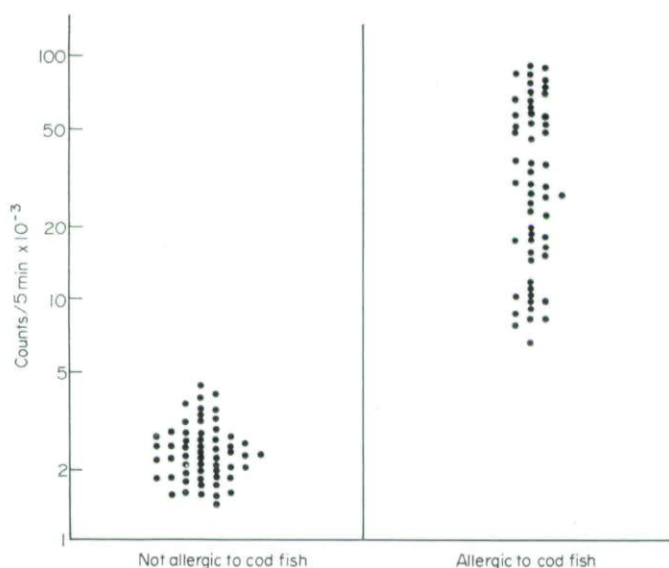


Fig. 2. The RAST response obtained with serum from fifty-six cod sensitive and fifty-six allergic patients tolerating cod.

Discussion

The use of potent and rather pure allergenic material appears to provide higher precision both of clinical tests and *in vitro* techniques (Aas, 1972). The material used in the present study is not, however, a completely pure single component. It is composed of two main protein fractions distinguishable by disc electrophoresis. The two main components are antigenically and allergenically identical, however, and are considered to be so-called iso-allergens. DS 22 has been further purified (Elsayed & Aas, 1971; Elsayed, Aas & Christensen, 1971). Further purification is, however, cumbersome and gives too low a yield for practical purposes. The semipurified material was therefore

Table 1. PK titres and RAST counts/5 min with ten different serum samples from one cod-allergic individual (T.N.)

Serum sample*	PK-titre	RAST† (counts/5 min)
TN 4326 ·011965	160	33932
TN 145 ·031965	40, 80	3502
TN 45233·071965	10, 10	10655
TN 145 ·101965	10, 40	2708
TN 125 ·111968	80, 200, 200	3417
TN 70 ·031969	80, 160	18712
TN 34 ·031971	40, 40	3313
TN 26 ·051971	10, 40	1870
TN 30 ·061971	400	12807
TN 74 ·071971	20, 20	5631

* The last six figures indicate the month and year when the serum sample was collected.

† Background 1712–2450 counts/5 min.

selected as a model, representing a degree of purification which may be achieved rather easily. It seems that this degree of purification is sufficient to reach optimal precision in the *in vitro* test system studied. This potent and rather pure allergen also gives similarly high precision and reliability to skin tests when these are adequately performed.

The radioallergosorbent technique used was very sensitive as regards ability to detect small amounts of the purified allergen coupled to the solid phase. The CNBr-activated cellulose could easily bind allergen from a 4×10^{-9} g/ml solution in amounts sufficient to give distinct reactions with cod allergic sera. The passive transfer test (PK) is still more sensitive, however. In previous experiments it has been possible to detect even 10^{-17} g with this system (Aas & Jebsen, 1967).

Other investigations have shown that the PK-system is also the most sensitive one to detect specific reagenic antibodies, although the RAST-technique comes very close to it in this respect (Foucard *et al.*, 1973). The lack of correlation between PK titre and the finding of a positive or negative RAST with one serum (T.N.) could be explained by the presence of varying amounts of blocking antibodies in these samples. Another explanation would be that in this serum reagenic properties may be present connected both to IgE antibodies and to other immunoglobulin classes not detectable by the anti-IgE serum used (Aas *et al.*, 1971). Whatever the explanation, this problem seems to be rather rarely encountered.

The demonstration of varying RAST results for the same allergen in two cod allergic skin-positive subjects, seems to confirm the previous suggestions that the biologically active tissue-bound reagin may not always have its counterpart as free circulating antibody (Aas, 1972). For the fish-allergen this was important only for two of the fifty-six patients, however. For other allergens, the proportion of serum-negative, tissue-positive patients may be higher. With a low degree of sensitization the very sensitive reagin/mast cell system may perhaps give reactions in particular tissues without detectable amounts of reagins being present in serum.

It seems that the reliability of RAST depends very much on availability of sufficiently potent, purified and characterized allergens, and it is to be hoped that more allergens may be purified for this and similar purposes.

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