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Summary An in-vitro method, called the radio-allergosorbent test, has been developed for the detection of allergen-specific antibodies of a new immunoglobulin class, provisionally called IgND. Antibodies to 14 different allergens were detected in sera of allergic patients. A 96% agreement was obtained between results from provocation tests and the in-vitro test for allergy. The results strongly support the hypothesis that reagins belong to the immunoglobulin class IgND. The method described might become of great importance for the clinical diagnosis of hypersensitivity to various allergens.

Introduction

THE existence of a new class of immunoglobulins, provisionally called IgND, was revealed by the finding of an atypical myeloma protein (Johansson and Bennich 1967a) and the corresponding protein in normal serum (Johansson, Bennich, and Wide 1968). The concentration of IgND in normal serum, although much lower than that of the other four immunoglobulins, could still be assayed by the sensitive radioimmunosorbent technique of Wide and Porath (1966). Elevated levels of IgND were found in sera of patients with asthma and hay-fever of proven allergy (Johansson 1967, Johansson and Bennich 1967b). This observation, together with the following points, supports the hypothesis that IgND and reagins are similar: the physicochemical properties of IgND are similar to those of skin-fixing antibodies (Sehon and Gyenes 1965,

Bennich and Johansson 1967); IgND does not pass the placental barrier freely (Johansson and Bennich 1967b); myeloma protein ND specifically inhibits the Prausnitz-Küstner reaction (Stanworth, Humphrey, Bennich, and Johansson 1967); IgND seems to be antigenically related to γE (Johansson, Bennich, Ishizaka, and Ishizaka 1967).

If reagins belong to the IgND class it should be possible to detect allergen-antibodies of this class in sera of allergic patients. On this assumption a method for the detection of allergen antibodies of the IgND class was developed. The principle of the method, the radioallergosorbent test (R.A.S.T.), is as follows: an allergen coupled to an insoluble polymer is added to the serum to be investigated, if antibodies to the allergen are present they should react with the conjugate; after the removal of all unbound serum components ^{125}I-labelled anti-IgND antibodies are then added, they will bind to the antibodies of the IgND class which have reacted with the polymer-coupled allergen; the uptake of labelled antibodies, measured in terms of radioactivity, on the particles is essentially proportional to the amount of IgND allergen antibodies.

Materials and Methods

Sera

Sera from thirty-one patients (sixteen males and fifteen females, mean age 20 years and age range 8–42 years) with asthma or hay-fever of allergic genesis were analysed. The samples were collected during the summer and autumn 1967 at the Allergy Outpatient Clinic and at the Children's Allergy Outpatient Clinic, University Hospital, Uppsala. The sera were kept at −20°C until analysed. The diagnoses were established by clinical history and by skin and provocation tests using commercial allergen preparations (Vitrum AB, Stockholm and Dome Chemicals Incorporated, New York). The provocation tests were made in cases of asthma by inhalation and measured by a peak-flow meter, and in cases of hay-fever by nose or eye tests. Four of the patients had been treated with hyposensitisation. Results from tests for antibodies to allergens with which these patients had been hyposensitised have been excluded. None of the patients received any other kind of treatment (e.g., steroids). Besides the sera from allergic patients, ten sera from individuals with no history of allergic diseases were tested.

Insoluble Polymer-allergen Conjugates

Each of the following 14 allergens was coupled to cyanogen-bromide-activated insoluble dextran ('Sephadex', Pharmacia AB, Uppsala): animal dandruff from horse, dog, cat, cow, and rabbit; pollen from birch, reeds, daisy, and three types of grass (*Phleum pratense*, *Festuca pratensis*, *Artemisia vulgaris*); a fungus mixture; and extracts from shellfish and house dust. The allergen in 1 ml. standardised allergen solution, 1/10 (Vitrum AB, Stockholm) or 10,000 protein-nitrogen units (Dome Chemicals Incorporated, New York), was coupled to 100 mg. CNBr-acti-

vated (Axén et al. 1967) sephadex G 25, ultrafine as described for the coupling of antibodies (Wide et al. 1967). The particles were then suspended in a concentration of 1 mg. per ml. of 0·1 *M* tris (hydromethylamino) methane (" tris ") buffered saline solution of pH 7·4 with 1% ' Tween 20 ' and 0·2% bovine serum-albumin. Sephadex-allergen conjugates were stable for at least 3 months at +4°C and −20°C.

^{125}I-labelled Anti-IgND Antibodies

Specific antibodies against the Fc-fragment of IgND (Bennich and Johansson 1967) were isolated by an immunosorbent technique (Robbins et al. 1967) using a bromacetylcellulose/myeloma-ND conjugate. The purified antibodies were labelled with ^{125}I using the chloramine T method of Hunter and Greenwood (1962). 20 μg. of antibodies were mixed with 4 mC ^{125}I and 20 μg. chloramine T in 0·1 *M* phosphate buffer of pH 7·4 and the reaction was stopped after 1 minute by the addition of 100 μg. sodium metabisulphite in 0·1 *M* borate-buffered saline solution of pH 8·6. The labelled antibodies were separated from denatured products and free iodine by gel filtration on sephadex G 150. The specific activity was estimated to be 50–75 mC per mg. Up to 33% of the labelled antibodies could be bound to a sephadex-coupled preparation of crude IgND isolated from serum of an allergic patient. Labelled antibodies diluted in 0·1 *M* tris-buffered saline solution with 5% bovine albumin could be stored for 2–3 months at 4°C without loss of antibody activity.

Performance of R.A.S.T.

Step 1.—5–50 μl. serum and 0·5 ml. of a suspension of a polymer-allergen conjugate were mixed in a test tube (50 × 10 mm.) and incubated for 6–24 hours at room temperature with slow vertical rotation of the tubes. The suspension was centrifuged at 3000 r.p.m. and washed three times with 0·1 *M* tris-buffered saline solution of pH 7·4 containing 1% tween 20 leaving about 0·2 ml. washing solution together with the particles in the tube. *Step 2.*—100 μl. of labelled anti-IgND antibodies in a concentration corresponding to about 40,000 counts per minute was added to the tube containing the washed particles. The mixture was incubated, centrifuged, and washed as described in *step 1.* The radioactivity bound to the particles was measured in a scintillation detector. The results obtained with unknown sera were compared with known non-allergic sera and with diluent.

The results were regarded as positive (+) when the radioactivity uptake of the particles was 2–5 times that of the control and strongly positive (++) when the radioactivity was higher. The test capacity is 50–100 tests per day and the results can be obtained within 24 hours. A total number of 374 tests were made by the R.A.S.T. All these tests were made in duplicate.

Quantitative Assay of IgND in Serum

The concentration of IgND in serum was determined by a radioimmunosorbent assay (R.I.S.A.) (Wide and Porath 1966) using carefully absorbed antisera to the Fc fragment of IgND as described by Johansson, Bennich, and Wide (1968). The

antibodies were coupled to CNBr-activated sephadex G 25, ultrafine, as previously described (Wide et al. 1967).

Results and Discussion

The R.A.S.T. is based upon the following observations: (1) allergens of different types can be chemically coupled to an insoluble polymer-like cross-linked dextran (sephadex); (2) these polymer-coupled allergens retain their capacity to bind allergen antibodies of the IgND class; (3) allergen antibodies bound to a sephadex-allergen conjugate can further bind anti-IgND antibodies; and (4) the anti-IgND antibodies can be purified and labelled with a radioactive isotope.

Specificity Studies

When patients allergic to known allergens were tested for antibodies against the same allergens high amounts of labelled antibodies (radioactivity corresponding to 10–100 times that of the controls) were bound to the particles. On no occasion did sera from non-allergic individuals give a significantly higher uptake than the diluent control. These results indicated the presence of allergen antibodies of the IgND class in sera from allergic patients. Allergen antibodies of the IgND class have been detected for all 14 different allergens which were polymer coupled.

Addition of up to 100 μg. of purified IgA, IgD, IgG, or IgM before *step 2* did not decrease the amount of anti-IgND antibodies to be bound to the conjugates, while addition of 1 μg. IgND or Fc fragment from IgND completely inhibited the binding of the anti-IgND antibodies. These results indicate that the labelled antibodies did not react with any immunoglobulin other than IgND.

When an allergen, dissolved in water, was added to serum from a patient sensitive to this allergen an inhibition of the reaction was found in the R.A.S.T. system when tested for antibodies to this particular allergen. No decrease was observed in the binding of antibodies to other allergens to which the patient also was sensitive. Results from one of these studies are shown in table I. These results indicate that the R.A.S.T. reaction is allergen specific.

In the R.I.S.A. system addition of allergens in solution had no influence on the level of IgND. This is a further indication that the specific antigenic determinants on the antibodies of the IgND class are still available for reaction, although the antibody-combining sites are blocked. In addition, it seems likely that the allergen-antibody complex is soluble.

Incubation of allergic serum with the corresponding sephadex-coupled allergen and subsequent separation of

TABLE I—SPECIFICITY OF R.A.S.T. FOR THE DETECTION OF ALLERGEN ANTIBODIES*

—	Results from R.A.S.T. with polymer-allergen conjugate:		
	Extract from shellfish	Dandruff from horse	Pollen from timothy grass
Allergen added to serum before test:			
Extract from shellfish ..	−	−	++
Dandruff from horse ..	++	−	++
Pollen from timothy grass (*P. pratense*)	++	−	−
Controls:			
Untreated serum	++	−	++
Test on non-allergic serum	−	−	−

*The tests were done after the addition of allergens, dissolved in water, to a serum from a patient hypersensitive to extract from shellfish and pollen from timothy grass, but not to dandruff from horse.

the serum from the particles showed, by the R.A.S.T., a decrease in serum of the amount of antibodies to that particular allergen. No influence on antibodies to other allergens was observed. In the R.I.S.A. system a significant decrease in the concentration of IgND was found. However, no decrease in the IgND level was obtained by absorption with sephadex-coupled allergen to which the patient was not allergic.

Clinical Application

The detection of high amounts of allergen antibodies of the IgND class in sera from allergic patients suggested the use of the R.A.S.T. as a simple in-vitro test for the diagnosis of allergy. A comparison was therefore made between the occurrence of allergen-specific antibodies of the IgND class as measured with the R.A.S.T. and the results from skin and provocation tests on patients with proven allergy. The results are shown in table II. An agreement in the results between skin tests and the R.A.S.T. was obtained in 94 out of the 140 tests (68%). In those cases

TABLE II—COMPARISON BETWEEN THE OCCURRENCE OF ALLERGEN-SPECIFIC ANTIBODIES OF THE IgND CLASS AS MEASURED BY R.A.S.T. AND THE RESULTS FROM 140 SKIN TESTS AND 51 PROVOCATION TESTS ON PATIENTS WITH PROVEN ALLERGY

Test	Result of test	Result of R.A.S.T.		Agreement (%)
		+	−	
Skin	+	47	41	68
	−	5	47	
Provocation	+	28	1	96
	−	1	21	

where a disagreement between the two methods was seen, there was a predominance (41 out of 46) of positive skin tests and negative R.A.S.T. reactions. It is well known that the skin test is not an ideal test for allergy and that false-positive reactions are common. In this respect the provocation tests are considered to be much more accurate. The comparison between the R.A.S.T. and provocation tests showed a 96% agreement. Of the 51 tests only 2 failed to give concordant results.

The mean IgND level in the non-hyposensitised allergic patients was 1126 ng. per ml. (range, 120–5850 ng. per ml.). The mean IgND level in the group of patients with known allergy to a single allergen was 633 ng. per ml. compared to a mean level of 1307 ng. per ml. in patients allergic to two or more allergens. Fourteen out of twenty-six non-hyposensitised patients had an IgND concentration in serum which was significantly higher ($P < 0.05$) than that for non-allergic individuals. This accords with the results found in allergic asthma by Johansson (1967).

This seems to be the first time that a good correlation has been obtained between provocation tests and an in-vitro test for allergy which is based upon the detection of allergen-specific antibodies of a certain class of immunoglobulins. The clinical importance of this in-vitro technique for the diagnosis of allergy is being investigated. Our results strongly support the hypothesis that reagins belong to the IgND class. However, this does not exclude the existence of reagins belonging to other immunoglobulin classes.

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