

In Vitro Diagnosis of Reagin-Mediated Allergy

One of the first problems that meets the allergologist who plans examination of a patient with possible allergic disease is to decide whether he is confronted with a case of reagin-mediated allergy or if other mechanisms are causing the symptoms, e.g. intolerance, IgG/complement-mediated non-atopic type III disease, C₁-esterase inhibitor deficiency, alterations of prostaglandin synthesis, etc. Several of the diagnostic procedures used by the allergologist aim at differentiating between these possibilities, and the decisions he makes in his diagnostic and therapeutic work are arranged accordingly.

In this respect the case history is important: reagin-mediated allergic disease is frequently associated with atopy in the family, the patient may have suffered from atopic symptoms from other organs earlier in life, and there may be clinical symptoms of atopy from several organ systems at the time of examination. Investigations will often reveal an increased level of IgE in serum and excretes, positive RAST and skin reactions, and positive effect of allergen elimination from the milieu and/or specific hyposensitization. In contrast to the reagin-mediated allergy, the endogen "allergies" more frequently occur in families without atopic disease. They occur later in life, not infrequently associated with symptoms of intolerance to acetylsalicylic acid, perennial rhinitis, recurrent nasal polyps and other severe "allergic" symptoms. In these cases we usually find pronounced eosinophilia in blood and excretes, but the IgE level is normal, the skin tests are negative, and the patients do not improve when specific anti-allergic treatment is attempted. Patients with "exogen" and "endogen" allergy in the airways present a hyper-reactivity of the mucous membranes, manifest by non-specific reactions to irritants in inhaled air. This may cause misinterpretation as to existence of specific allergy. Therefore it is important that the allergologist is clear himself which methods of investigation he wishes to use in each case to disclose the mechanism behind the patient's allergy or "allergic" condition. The demand for a correct allergen-specific diagnosis is especially high if the measures and treatment involve complete elimination of a specific allergen from the milieu, change of work/housing, or initiation of specific immunotherapy.

In this issue of *Allergy* Johansson gives a presentation of the *in vitro* methods which by now are generally available to the clinician. It is difficult to imagine an allergologic examination without some kind of case history, and this must always be the initial step. Whether the allergologist thereafter chooses to perform skin tests or RAST will depend on available possibilities, but generally the skin test is preferable. The practising general physician may possibly wish a confirmation of supposed reagin-mediated disease by RAST with a few allergens selected on the basis of the case history. The allergologist cannot perform a satisfactory investigation without skin testing. This leads to the many problems involved in producing biologically standardized allergen extracts and their use, which has previously been described in this journal by Aas.

The allergologist can be recommended to use a grading of the results of the various investigations. On the basis of such gradings it is possible to build up a diagnostic probability of a suspected allergy. A system of this type has been practised in Scandinavia. The case history is given marks from 0 to 3; similarly the outcome of the skin test with the specific allergen is given marks from 0 to 3. Clinical investigation has shown that a total of 6 marks or more is tantamount to manifest allergy, and further investigation is unnecessary. If the score is below 6 marks, the investigation must be supplemented by further examinations, possibly even provocation tests. At this stage the RAST is excellent and handy. With the RAST similarly graded from 0 to 3, it is possible to supplement the two above-mentioned sources of information and thereby make the specific aetiological diagnosis in 80–90% of the patients. The best concordance with provocation tests is found in those patients that react to very small doses of allergen. The limitations of the RAST are due to the fact that not all allergens are equally fit for the analytical system. This becomes particularly apparent when mixed extracts are employed, such as house dust, fungi, feathers, and several food stuffs; the analysis and extracts are often good with pollen and animal hair. Blocking factors, perhaps IgG, may inhibit or abolish a positive RAST, especially with sepharose as immunosorbent (as in the original RAST) or aluminiumhydroxide (as described by Weeke), whereas RAST with paper discs is less influenced by blocking factors. Addition of small amounts of allergen to the serum sample before the RAST (RAST inhibition) can remove the blocking factors and thus demask an otherwise undemonstrable IgE binding. RAST inhibition is very time-consuming and therefore not suited to routine analysis.

As mentioned by Johansson, the antigen-induced basophil histamine release assay is sensitive and specific, and in several studies has shown a better concordance with the allergic state than RAST and skin test if the allergic state is diagnosed clinically and by provocation tests. The capacity of the histamine release assay is limited since analysis of blood from one patient for reactivity against 10–20 allergens means a whole day's work for one laboratory technician. In its original design, the analysis requires 10–20 ml blood. However, present developmental work attempts to alter this method by using the principle behind histamine release from basophil granulocytes in assays that require smaller amounts of blood and permit more allergens to be investigated. It seems also possible, as indicated by Norn (personal communication), to preserve the blood viable for the analysis at least for 1 day by adding tissue culture medium to the blood sample. This enables samples for this assay to be transported over long distances. It is feasible that this development will soon make the principle behind histamine release from basophil granulocytes just as useful for the clinical investigation as the RAST is today. Histamine determination in whole blood with an auto-analyser, as suggested by Siraganian, has not found extensive clinical use, probably due to technical difficulties associated with the apparatus and principle of analysis.

During the last few years the use of the crossed radioimmuno-electrophoresis (CRIE) technique has provided us with new possibilities to obtain *in vitro* identification of those allergen extract components that can react specifically with the IgE molecules of the patient. The skin test, the RAST, and

the histamine release assay give an overall expression of the presence of IgE with specific reactivity against a certain allergen extract. But the CRIE makes it possible to obtain a precise indication of those specified extract components that can react specifically with the IgE of the patient. It is namely possible, by means of gel electrophoresis, to separate the individual antigenic components of the allergen extract and thereafter to bind the specific IgE of a certain patient serum to the electrophoretic pattern in the gel. When this procedure is followed by a marking of the IgE-containing precipitates with radiolabelled anti-IgE, it becomes possible to visualize and identify the fractions that represent the allergens. The CRIE is already of major importance in the characterization and fractionation of allergen extracts, but the technique can also be used for selection and identification of the few components of an allergen extract that induce the clinically relevant allergy, e.g. in an extract of the patient's own house dust. It is well known that house dust contains components from animal hair, pollen, fungi, and mites, etc.

CRIE on the house dust extract makes it possible to identify the individual allergens and use this for a more individual aetiological and immunologically specific diagnosis as a basis for milieu, sanitation and perhaps specific immunotherapy. Since extract of purified house dust is one of the preparations most commonly used for "specific" immunotherapy of allergic patients, it seems highly desirable to develop a more exact aetiological diagnosis by identification of the relevant allergen in the house dust of each individual patient.

In his review in this issue of *Allergy* Johansson also emphasizes that mechanisms independent of IgE may be behind diseases which clinically manifest themselves as immediate reactions. It is therefore important for the clinician to have assays for determination of specifically reactive precipitating IgG antibodies, complement activation in plasma samples, and the possibility to identify complement split products. It is also possible that future investigations on lymphocytes will reveal a pathogenetic role of specifically reactive T-lymphocytes.

Presently, several new methods make it possible to answer many of the problems which challenge the clinician in his daily work. Improvements of the diagnostic methods by means of RAST, histamine release assay, protein determination on the serum and excretes, and improvement of the purity, specificity and standardization of allergen extracts all tend to increase our knowledge of allergic disease and will eventually improve our possibilities to plan and carry out an effective treatment directed against the very mechanism behind the allergic condition. It must still be remembered that about one third of those patients with allergic symptoms, who are referred to a specialist, suffer from "allergic" disease which is probably not mediated by IgE. Therefore it is necessary to have a wide range of methods for clinical and laboratory investigation and from this sortiment to select the proper analytical programme for a good diagnosis and characterization of the patient. On this basis adequate treatment can be planned and carried out.

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