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IgE-Based Radioimmunoassays for the Quantitation of Allergens

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For many allergens there is now good evidence that the corresponding allergy is IgE antibody mediated. Since the IgE allergen interaction can be quantitated conveniently by radioimmunoassay such as the radioallergosorbent test (RAST) these procedures can in principle be used for allergen quantitation. I will briefly review advantages and disadvantages of such procedures, of which the principles are given in Table 1. Emphasis will be mainly on two problems specific for

allergen estimation by radioimmunoassay:

1. The IgE antibodies used are in general not monospecific.
2. Allergens with only one determinant ("monovalent allergens") cannot induce mast cell triggering, and might even inhibit such a process.

It is therefore of relevance to discriminate between monovalent and polyvalent allergens.

Table 1
IgE-based solid phase procedures for allergen RIA

	Step 1 unknown allergen plus	Step 2	Step 3
<i>Inhibition tests</i>			
1 labelled reagent allergen	S-aE-E+A _R *°		
2 insolubilized reagent allergen	S-A _R +E	S-A _R -E	+aE-°
<i>Binding tests</i>			
3 labelled unknown allergen	°	A-°	+S-aE-E
4 insolubilized unknown allergen	S (reactive)	S-A	+E +aE-°
5 insolubilized anti-allergen	S-aA	S-aA-A	+E +aE-°
6 insolubilized anti-IgG	S-aG +aA+E	S-aG-aA-A-E	+aE-°

S, solid phase. aE, anti-IgE. E, IgE anti-allergen. A_R, reagent allergen. A, unknown allergen. *¹²⁵I. aA, IgG anti-allergen. aG, anti-IgG.

INHIBITION SYSTEMS 1 AND 2

For all inhibition assays it is essential that the reagent allergen can be distinguished from the unknown allergen. In general this means that some allergen derivative has to be prepared; this can be either a labelled derivative or a derivative with different physicochemical or immunochemical properties. Insolubilization is a most convenient modification, but hapten-modified allergens in combination with anti-hapten antibodies may be used instead.

When the relative potency of an unknown allergen extract is estimated relative to some standard preparation, more often than not the (log dose)-response curves are not strictly parallel. This obviously indicates qualitative and/or quantitative differences. If for practical reasons only one single potency value has to be given, it is customary to use the amount of allergen required for 50% inhibition (3). This quantity, however, depends to a marked extent on the experimental conditions.

My suggestion is to use instead the initial part of the dose-response curve: on a linear scale a straight line is in general found up to over 20% inhibition. Estimation of the initial slope (or the amount of allergen required for 20% inhibition) will give a less arbitrary value for the potency of the unknown allergen extract.

Inhibition systems have some disadvantages:

1. One additional biological reagent, a modified allergen, is required, apart from the IgE anti-allergen antibodies, the reference allergen preparation and the labelled anti-IgE. This is a complication as far as standardization is concerned; it is, however, an advantage with respect to the specificity of the assay.
2. The modification of the allergen reagent may inactivate an allergenic site.
3. Non-specific inhibition, e.g. due to enzymatic activity or to lectin-like activity in the allergen extract, has to be excluded.
4. Monovalent allergens are detected also.

DIRECT BINDING SYSTEMS

In direct binding assays (i.e. non-competitive assays) reagents can in principle be used in excess. Large reagent excess will result in linear dose-response curves, which makes comparison of potencies straightforward.

Systems which require physical or chemical modification of the unknown allergen: system 3 and 4

For these tests one has to prepare a derivative of the allergen in a poorly characterized allergen preparation. Such a derivation reaction, be it iodination, covalent coupling to a solid phase, or adsorption to a solid phase, may be influenced by substances in the unknown allergen preparation. Other disadvantages of these systems are shared with inhibition assays:

% bound ^{125}I -anti-IgE

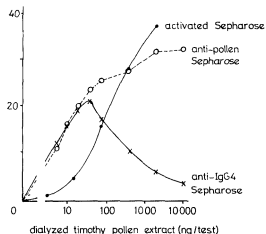


Fig. 1. Comparison of systems 4, 5 and 6 for the estimation of timothy pollen allergen. The allergen was incubated with 0.5 ml of a 1:300 (v/v) suspension of Sepharose. In system 4 (activated Sepharose, ●—●), commercially available CNBr-activated Sepharose was used in a buffer which consisted of 0.1M NaHCO_3 , 0.5M NaCl and 0.1% Tween-20. The tubes were incubated overnight on a rotator. The Sepharose was washed with a buffer of pH 8.5 which contained 0.5M glycine. Then, 10 μl serum PB with a high IgE antibody titre to grass pollen was added and after further incubation and washing, IgE-binding was detected with ^{125}I -labelled anti-IgE antibodies.

In system 5 (anti-pollen Sepharose, ○—○), 0.6 μg anti-grass pollen antibody coupled to CNBr-activated Sepharose was used per test. The rest of the procedure used was as for system 4.

In system 6 (anti-IgG4 Sepharose, x—x), 5 μg anti-IgG4 antibody coupled to CNBr-activated Sepharose was used per test. The allergen, serum PB (10 μl) and the anti-IgG4 Sepharose were mixed; serum PB also contains, in addition to the IgE antibodies, a moderate amount of IgG4 antibodies to grass pollen allergen. IgG4-allergen-IgE complexes formed were bound by the Sepharose. After washing, IgE-binding was detected with ^{125}I -labelled anti-IgE antibodies.

1. The above-mentioned risk inherent in modification reactions.
2. Monovalent allergens are detected also.

Systems which require the binding of at least two antibodies to the allergen: systems 5 and 6

For detection in these tests, the allergen has to be combined both with IgE antibody and with some other anti-allergen antibody. Theoretically, the second anti-

allergen antibody should preferably be an IgE antibody also, since then the biological situation (one allergen molecule cross-linking two IgE anti-allergen antibodies on the surface of the mast cell) would be closely mimicked. For practical reasons the second anti-allergen antibody will be an IgG antibody, either human or heterologous.

In system 5 (2), the IgG anti-allergen antibody, coupled to a solid phase, is incubated with the unknown allergen; IgE anti-allergen antibody is then added, and IgE binding to the solid phase is detected with labelled anti-IgE.

In system 6 (1), a mixture of the IgG antibody, the IgE antibody, and the allergen is incubated with some insoluble anti-IgG reagent; IgE-binding to this anti-IgG reagent is again detected with labelled anti-IgE. The latter test is suitable when the IgE and the IgG antibodies are present in the same human serum.

As anti-IgG reagent we have used rheumatoid factor, anti-IgG antibodies, or anti-IgG4 antibodies. In the grass pollen system a steeper dose-response curve was obtained with anti-IgG4 antibodies than with anti-IgG antibodies. This presumably indicates that the ratio: grass pollen-specific Ig to total Ig, is higher for IgG4 than for the other Ig subclasses. Comparison of system 5 with system 6 indicates a similar sensitivity; however, due to the extra washing step included in system 5, no prozone effect is found (Fig. 1).

Disadvantages of these systems are:

1. IgG anti-allergen antibodies are required.
2. For system 6, an anti-Ig reagent is required.
3. Aggregated allergens are possibly overestimated.
4. Non-specific effects, either increasing or decreasing IgE-binding, are theoretically possible.

Advantages are:

1. No allergen modification is required.

2. The requirement of a second anti-allergen antibody increases the specificity, especially if heterologous antibody is used.
3. Monovalent allergens will not be detected.

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In vitro Standardization of Basophil Histamine Release

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For many years it has been known that allergic mediators as histamine and SRS-A are responsible for the symptoms in allergic diseases such as asthma, rhinitis and urticaria. Investigations during the last 10 years have further elucidated the cellular mechanisms of the IgE-mediated release of these mediators from mast cells and basophils (1), and it is likely that an estimation of basophil histamine release would be of more diagnostic value than a RAST estimation, since it is the

cell-attached IgE that reacts with allergen, resulting in the release of allergic mediators.

The determination of histamine released from basophils after *in vitro* provocations with the suspected allergens is being increasingly used for diagnostics of type I allergic reactions, since the response is correlated to *in vivo* provocation (3). Investigations with patients with exogenous bronchial asthma have thus shown that *in vitro* provocation of the patient's basophil leukocytes

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