

Purification and Characterization of Allergens

Summing-up

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This summary gives some general conclusions on the molecular structure, the interdependence of the structure and the activity, the antigenic and allergenic properties, and the different significance of allergens. On the basis of this knowledge a general recommendation of combinations of methods for the characterization and purification of allergens is given.

All important allergens isolated at present are common proteins. The general molecular weight range covered is 5,000–50,000 dalton, but smaller and larger molecules have been reported. The range of the isoelectric points does not differ from that of proteins in general. The solubility and the accessibility for chromatographic separations indicate that allergens are globular proteins. The carbohydrate content varies as in common proteins, and allergens with no carbohydrate content as well as with high carbohydrate content (20% w/w) have been described. The number of polypeptide chains in allergens differs from one to several, which may or may not be identical. The amino acid compositions do not show consistent features nor do the few allergens sequenced at present.

From the above-mentioned general molecular characterization of known allergens it cannot be argued that allergens exhibit specific features which facilitate the induction and production of IgE giving rise to clinical symptoms in the allergic patients.

The allergenic activity of isolated allergens is continuously influenced by any process which affects their stability in any irreversible way. Thus the allergenic activity is conformation-dependent. The failure to find any general structure of allergens or parts of allergens explains their variable stability towards physical, chemical and enzymatic agents. Thermal stability differs from allergen to allergen. Some allergens are irreversibly denatured by urea, guanidinium hydrochloride, and detergents—some are not. Chemical modifications, if causing degradation, charge-shift or polymerization, will influence the allergenic activity since the molecular structure and the conformation change. Enzymes which cause conformational change of allergenic molecules will change the allergenic activity too.

Molecular epitopes (determinants) may give rise to antibodies of any immunoglobulin class produced by any animal including man. At present a few examples can be drawn where only one class of immuno-

globulins directed against a unique epitope has been verified. This situation is due to the variable genetic capability of the individual's immunoglobulin producing system to recognize the antigens in low dosage and to their actual exposure (natural, or artificial by an immunization schedule). However, there is no scientific proof for distinguishing between allergenic (recognized by IgE) or antigenic (recognized mainly by IgG) determinants. This also implies that all allergens may be antigens and vice versa, and that it is, in theory, possible to raise antibodies against all potential allergens.

The term, major allergen, was originally introduced because some antigens more frequently than others give rise to a specific IgE response in patients due to reasons mentioned above. Allergens of major importance to the individual allergic patient always exist but a definition is needed of the major allergen of general importance to the group of patients reacting on the source material from which the allergen is extracted.

Major allergens were defined on the basis of the GRIE method as antigens to which 50% of the patients allergic to the allergen source in question react, and to which 25% bind strongly. Minor allergens are antigens to which less than 10% react, and the rest are defined as intermediate allergens. Although GRIE is not a quantitative method and, as discussed in this Workshop, is very dependent upon the reagents and the patient panel used, it is the only method today which distinguishes between individual allergens without previous purification. The significance of major allergens identified by this method has been verified by other methods. Purified allergens of major importance according to this definition are listed in Table 1.

The characterization and purification of allergen preparations based upon the above generalizations are obviously reduced to a protein chemical task. The preliminary characterization of the allergenic source material should include analyses which make it probable that: (1) all relevant material is represented; and which gives (2) an estimate of the total allergenic activity; (3) an identification of the major allergens; (4) determination of physico-chemical parameters (molecular weights and isoelectric points) for use in the separation procedure; and (5) content of protein, carbohydrate, and low molecular weight compounds. These analyses can be made by a combination of physico-

Table 1
The importance of some highly purified allergens

Source material		Major allergens	Other isolated allergens	Investigators
<i>Pollen:</i>				
(Grasses)	<i>Lolium perenne</i>	Group I	Group II, Group III	Marsh
	<i>Phleum pratense</i>	Ag 25 (Ag B)	Ag 3, Ag 30	Lowenstein, Malley
(Ragweed)	<i>Ambrosia elatior</i>	Ag E, Ag K	Ra 3, Ra 4 (BPA-R), Ra 5, Ra 6	King, Goodfriend, Griffith, Hussain
(Hazel)	<i>Corylus avellana</i>	H 1a		Borch
(Plane)	<i>Platanus acerifolia</i>	Allergen P		Anfosso
<i>Insect:</i>				
	Bee	PLA	Hyaluronidase, mellitin	Shipolini
	Yellowjacket	Ag 5	Hyaluronidase, PLA	King
<i>Fish:</i>				
	Cod	Allergen M		Elsayed/Aas
<i>Animal dander:</i>				
	Horse	Ag 6, Ag 9, Ag 11		Lowenstein/Weeke
	Cow	Ag 1, Ag 3	Ag 6	Prahl/Lowenstein
	Cat	Ag 1		Ohman
<i>Fungi:</i>	<i>Cladosporium herbarum</i>	Ag 32	Ag 54	Aukrust

chemical methods*, immunochemical methods**, and RAST technique†, without previous separation of the source material.

Any protein separation technique which does not change the native structure of the proteins may be convenient for the isolation of allergens. However, before deciding on the use of separation methods it is advisable to perform all relevant methods in microscale in order to predict a rational preparation of the desired allergen and to avoid superfluous use of material. The monitoring methods to be used during a purification procedure include combinations of physicochemical methods*, immunochemical methods**, and RAST technique†.

The purity of isolated allergens can be evaluated on the basis of the protein and carbohydrate contents, their concentration relative to starting material, the quality and quantity of impurities. These data can be obtained by a combination of physicochemical methods*, and immunochemical methods**. The allergenic activities of isolated allergens can be estimated on the basis of their potency relative to the source material on patient panels (serum pools) or on individual patients. This can be done as combinations of RAST technique† and provocation test††.

The final characterization of isolated allergens should

also include the stability of these compounds towards physical, chemical, and enzymatic degradation in order to evaluate the possible usefulness of modified allergens in future hyposensitization treatments.

The great number of well characterized major allergens isolated hitherto has provided us with detailed knowledge of the sort of material giving rise to allergic reactions. The diagnostic value of major allergens has also been verified in many cases by various methods. However, this information has still not influenced much of the current use of allergen extracts for hyposensitization. In the few cases studied, the use of major allergens *per se* for hyposensitization does not seem too promising and experience is needed to elucidate the efficacy of patient-tailored allergen extract compared with the conventional extract containing the whole spectrum of allergens.

Take the source material
do not put it on a grill
carefully analyse for "gen"
hope you know what that does mean.

Then select the patients right
and for research money fight
look upon impurities
don't forget activities.

Then at least in small amount
what suggested now was found
put it in a bottle nice
and just hope you get the price.

*Isoelectric focusing, SDS-gel electrophoresis protein analyses, carbohydrate analyses, light absorption (UV).

**Crossed immunoelectrophoresis (CIE), crossed radio-immunoelectrophoresis (CRIE), and other quantitative immunoelectrophoretic methods (QJE).

†Radio-allergosorbent test (direct RAST or RAST inhibition).

††Skin, nasal, and bronchial provocation test, histamine release.

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