Recombinant allergens

Better than the natural product

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Traditionally, allergens for diagnostics and therapy are isolated from natural sources. But nowadays the natural substances are increasingly being replaced by genetically produced proteins. Products obtained this way enable more sensitive and specific in vitro and in vivo tests and present a lower risk for patients undergoing hyposensitisation.

Key words: *allergy, allergy tests, recombinant proteins*

Allergens are protein substances from different biological sources, such as pollen, insect venoms or food, which have manifold biological effects. Alongside structural proteins are lipocalins, enzymes, enzyme inhibitors, etc. Despite their heterogenicity, they have one common feature: in predisposed persons they trigger an excessive immune reaction which can range from harmless itching to fatal shock.

However, the association between allergen and allergic reaction is not always obvious. For example, the clinical picture of WDEIA (wheat dependent exercise induced anaphylaxis) is such that the consumption of wheat products with subsequent physical exertion – and only this – can lead to a severe allergic reaction, which is triggered by IgE antibodies against omega-5 gliadin.

For many years, allergens for diagnostics and therapeutics were obtained from natural sources. But nowadays the natural products are increasingly replaced by genetically produced recombinant proteins, since these are much easier to standardise.

Test	Detection	Principle, comments
Prick test (epicutaneous test)	Allergen-specific IgE antibodies (in vivo)	Well-established skin test; considered the gold standard in adults; risk of a strong allergic reaction.
RAST/CAP (Multiarray procedure)	Allergen-specific IgE antibodies (in vitro)	Most important and most standardised assay; does not necessarily correlate with clinical symptoms.
Basophile activation test, CAST	Indirect, allergen-specific IgE detection (in vitro)	Determination of CD63, CD203c etc. on activated basophiles (e.g. if no defined allergens are available).
Lymphozyte transformation test (LTT)	In vitro supplementary tests to the prick test (e.g. when IgE is not responsible for the allergic reaction).	Detection of increased DNA synthesis in lymphocyte cultures after allergen stimulation (radioactive thymine).
ELISPOT assay		Enzyme-linked immunospot assay; detection of secreted cytokines or antibodies from immune cells after allergen stimulation

Important in vivo and in vitro procedures for detection of allergies.

Disadvantages of native extracts

Even extracts that are carefully extracted and purified always contain a mixture of major and minor allergens as well as (primarily non-allergenic) accompanying substances. This heterogenicity can, for example, impair the success of hyposensitisation. New allergies against one of the accompanying substances may occur, and it is often impossible to differentiate between an allergic reaction and a cross reaction.

Many influencing factors have to be taken into consideration already when the source is chosen. For example, in grasses, different growth conditions can lead to variable concentrations of components. Extraction methods also differ from manufacturer to manufacturer, so that important allergens such as the previously mentioned omega-5 gliadin may be underrepresented, leading to false negative test results.

A particular risk arises from sugar side

chains, especially CCD (*cross reactive carbohydrate determinants*), which according to today's knowledge do not actually trigger an allergic reaction. However, in in vitro tests they can lead to false positive reactions.

A clinically important example is CCD-induced cross reactivity to natural wasp and bee venom extracts. Particularly with potentially life-threatening insect venom allergies, the doctor needs a clear-cut result as to whether the patient has a reaction to bee or wasp venom or is one of the rare cases with a combined sensitivity to wasp and bee. Biotechnologically produced wasp venom and bee venom allergens such as rVes v5 and rApi m1 in combination with further specific components enable a clear differentiation, allowing targeted hyposensitisation and effective protection of the patient.

Molecular allergy diagnostics

Over the last 20 years, nearly all important allergens have been cloned, sequenced and expressed in bacterial, yeast or insect cells. The products demonstrate similar IgE binding characteristics to their natural counterparts and yield largely comparable reactions in in vivo and in vitro tests as well as in hyposensitisation.

For these innovative assays the term "molecular" (sometimes also "component-based") allergy diagnostics has become established, expressing that the allergens are biotechnologically synthesised based on their gene sequences, rather than isolated from natural protein mixtures. Since these preparations contain precisely defined allergens and are free of non-allergenic components, the sensitivity and specificity of the antigen-antibody reactions is significantly increased.

Leading manufacturers of component-based allergy diagnostics such as Phadia and Euroimmun (see below) nowadays combine multiple allergens in a single assay. This multiplex approach is in principle also possible with native allergen extracts, but is not useful here due to the low specificity. The major advantage of multiple-component kits is that with the smallest sample volume numerous allergens can be analysed in one test run. This is particularly advantageous in paediatrics with its small blood volumes.

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