

EDITORIAL

Airways Disease/Editorial

“Molecular extracts” for allergy diagnostics and therapy

1 | EXTRACT STANDARDIZATION: THE ETERNAL PROBLEM OF ALLERGOLOGY

Allergen-specific immunotherapy (AIT) is the only attempt to cure allergic diseases by attacking their antigen-specific immunologic mechanisms. As the first pioneer treatment was performed over 100 years ago, this approach has a long tradition.¹ Since then, a broad set of research has been performed and AIT has evolved in all its aspects: indications, allergen content, vehicle, adjuvant, route and schedule of administration, production, distribution, regulation and documentation.²

For over 90 years, allergens used for diagnosis and therapy have been produced by extraction from primary sources. Manufacturing stages are distinguished in native, intermediate and bulk allergenic preparations. Intermediate extracts often undergo different modifications, including chemical (such as cross-linking with formaldehyde or glutaraldehyde) and physical processes (such as adsorption onto aluminium hydroxide, calcium phosphate/tyrosine or inclusion into liposomes/microspheres) as well as addition of biologically active agents (such as LPS derivatives).³ With these methods, the manufacturer first produces the final reagent and then tries to define its composition.

Standardization of allergens means both consistent composition and potency of the allergenic preparations. The latter should be of course related to clinical efficacy. The Monograph on Allergen Products (MAP), a European regulation, permitted a wide range of variation (from 50% to 200%, tested by IgE inhibition tests) of the content of individual allergenic molecules in an extract.⁴ This means that for a simple allergen extract containing only three major allergenic proteins, there can be batches with very low and very high allergenic contents including all the intermediate possibilities.

Therefore, allergen extracts from similar allergen sources by different manufacturers greatly differ in their molecular composition and potency, and allergen standardization remains a difficult, if not impossible, mission.⁵⁻⁸ It is not surprising that the wheal reaction elicited by allergen extracts of the same pollen or mite species in the individual patient is highly influenced by the manufacturer.⁶⁻⁸ Similarly, part of the variability in the outcome of *in vitro* IgE tests to the extracts of the same allergen source is probably dependent on the extract composition.⁹ In conclusion, the standardization of allergen extracts for diagnosis and therapy is still an open issue in Allergology.⁵

Recently, new European regulations established that allergen extracts for *in vivo* diagnostics (eg, SPT) must undergo the same registration procedures as those used for the equivalent therapeutic products.¹⁰ As the registration costs for each of the allergen extracts used in the clinical routine are too high, the battery of SPT reagents available on the market will soon be reduced to those few products corresponding to a registered immunotherapy preparation.¹¹

2 | MOLECULAR ALLERGOLOGY: A MISSED OPPORTUNITY?

The advent of molecular allergology had raised new hope for a better standardization of allergen products for diagnosis and therapy. A preparation containing one single recombinant allergen molecule (eg, Der p 1, Phl p 1) is as precise and standardized as any therapeutic preparation containing a recombinant biologic (eg, recombinant α -IFN or a MoAb). Recombinant allergen molecules were proposed for a patient-tailored allergen immunotherapy (component-resolved immunotherapy, CRIT), individually designed after *in vitro* molecular assays (component-resolved diagnostics, CRD) defining the patient's molecular IgE sensitization profile. Two factors undermined the implementation of this concept: (a) European regulations only allow the registration of pre-defined mixtures^{4,11}; and (b) the molecular sensitization profiles of individual patients are extremely heterogeneous (eg, many dozens for grass pollen or *Dermatophagoides pteronyssinus*)¹²⁻¹⁴ and vary by age or geographic region (eg, peanut).^{15,16} Given the current regulations, manufacturers should register dozens of mixes of grass pollen or *D. pteronyssinus* molecules to provide a patient-tailored CRIT for grass pollen or mite allergic patients. Also the use of individual native or recombinant molecules for SPT, proposed over 15 years ago,¹⁷ is still limited to clinical research. The production and registration costs of allergen products for *in vivo* diagnostics are so high that no recombinant allergen has been so far registered just as a prick test solution, if there is no chance to use it also as a therapeutic preparation.^{4,11}

3 | BEYOND THE MOLECULES-EXTRACT DUALISM: “MOLECULAR EXTRACTS” (ME)

So-called “molecular extracts”(ME) are pre-defined mixes of all known relevant allergen molecules of a given allergen source (eg, *Phleum pratense*) so far identified, cloned and produced with

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recombinant DNA technology (Figure 1). These “molecular extracts” would have the following characteristics:

- **Composition**—The panel and isoforms of the molecule included in the ME should be fixed “a priori” considering the average allergenic relevance of each molecule in the geographically targeted patient population (epidemiologic criterion).
- **Cross-reactivity**—The panel of molecules to be mixed should be selected in order to identify genuine sensitization to the allergen source or strictly related ones.
- **Absence of interfering substances**—Being produced by assembling a molecular preparation, instead of by extraction from a complex primary source, the “molecular extract” would be deprived of any contaminants interfering with IgE reactivity.
- **Titration**—The exact concentration of each molecule in the ME, based on international consensus, has to be declared by the manufacturer and is therefore known to the doctor and regulatory agency.
- **Standardization**—The batch-to-batch variability of such a product would be kept to a minimum.
- **Comparability**—Different manufacturers would produce similar extracts (in terms of molecular allergen composition).
- **SPT**—Molecular extracts would be used for *in vivo* diagnosis. Skin test with recombinant extracts with the same declared molecular composition would be highly comparable in terms of results in the same patient in a batch- and manufacturer-independent fashion.
- **IgE assays**—In a diagnostic workup, the “molecular extract” could be used as an initial screening step leading, if positive, to test its individual molecules.
- **Therapy**—A balanced mix of the relevant allergen molecules could be used for AIT.

Until a few years ago, the idea of producing “molecular extracts” would have been unrealistic. However, the number of relevant allergen molecules identified, cloned and produced as recombinant molecules has been rapidly growing and reached 1583 in September 2018.¹⁸ For the most relevant allergen sources (grass, birch, olive, cypress, pellitory, ragweed, mugwort pollens; *D. pteronyssinus/farinae*; cat and dog; cow's milk, hen's egg, peanut), the list of cloned allergen molecules is almost complete. For example, the grass-specific IgE reactivity of most grass pollen allergic patients is totally or almost totally directed against eight allergen molecules (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11 and Phl p 12).¹⁹ A strong correlation has also been found when measuring the cumulative IgE reactivity assessed with panels of individual molecules and the one measured with the corresponding allergen extract of birch and hazel pollens, *D. pteronyssinus* and cat.²⁰

4 | HYBRID MOLECULES (DOULADIRIS ET AL IN THIS EDITION OF PAI)

Hybrid technology offers the possibility of producing recombinant molecules consisting of several unrelated antigens or epitopes. In

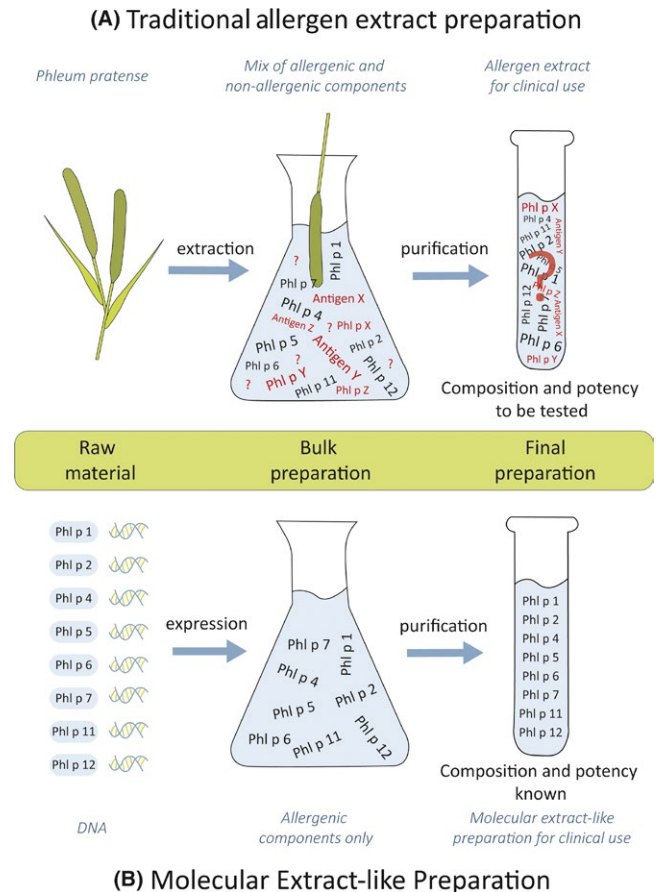


FIGURE 1 “Traditional extracts” vs “molecular extracts” for allergy diagnosis and therapy. A, Traditional Extracts—Traditional allergen extracts are manufactured through native, intermediate and bulk allergenic preparations (reviewed in³). The final product is a mix of allergen components and a myriad of other components deprived of any diagnostic value. With this approach, the manufacturer first produces the final reagent and then tries to define its composition. However, the exact qualitative and quantitative composition of this type of extract is poorly determined. Products with a similar label from different manufacturers produce different results in the same patient.^{5–9} This implies that the clinical decision taken by the doctor is strongly dependent on the composition of the allergen extract used for diagnosis. B, Molecular Extract-like Preparation—So-called “molecular extracts” are pre-defined mixes of all the relevant allergen molecules of a given allergen source (eg, *Phleum pratense*) so far identified and cloned. The isoform and concentration of each molecule should be fixed according to the average allergenic relevance of that molecule in the patient population. The exact qualitative and quantitative composition is therefore established “a priori” by the manufacturer and known to the doctor and to the regulatory agency. The batch-to-batch variability of such a product can be kept to a minimum. Once scientific organizations have established which components and isoforms should be present in a “molecular extract” and in which amount, different manufacturers will be able to produce similar, well-defined extracts

addition, parts of a given antigen can be rearranged in the form of a new “mosaic antigen” or antigens/epitopes can be combined with other molecules.²¹ For various allergenic sources, these advantages

have been utilized to investigate the potential merits of hybrid molecules in diagnosis and therapy.²²⁻²⁶

4.1 | Hybrid molecules for allergy diagnosis

Rudolf Valenta et al. developed a recombinant hybrid molecule for in vivo diagnostic purposes consisting of the four major allergenic proteins of Timothy grass (*Phleum pratense*; Phl p 1, Phl p 2, Phl p 5 and Phl p 6).²² Containing the most important IgE epitopes, the hybrid molecule was successfully tested in a cohort of 32 grass pollen allergic adults, showing a good correlation between IgE levels obtained with the hybrid molecule and natural grass pollen extract measured by ELISA ($r = 0.98$, $P < 0.0001$) and by ImmunoCAP ($r = 0.98$, $P < 0.0001$).²⁷ Douladiris et al.²⁸ (this issue of PAI, page X) examined this hybrid construct now as an SPT reagent in 64 grass pollen allergic children (6-17 years) with positive SPT or IgE test to grass pollen extract. All patients were tested for skin reactivity with the single recombinant *Phleum pratense* molecules as well as the hybrid molecule. The latter elicited a positive SPT result in 85% of the patients with grass pollen allergy. Notably, nine out of 61 patients, previously tested positive for SPT with mixed grasses, showed negative results when tested with the hybrid molecule. For Timothy grass extract, this was the case in five out of 57 patients. Measurement of specific IgE then revealed a pronounced sensitization to LTPs, Phl p 11, and Phl p 4, which could explain the positive results to extracts. These results underline the improvement in the precision of SPT when performed with hybrid molecules. No side effects were reported. Hence, the authors conclude that the recombinant hybrid molecule is a sensitive and specific tool for in vivo diagnosis of genuine grass pollen sensitization, although creating higher costs than the traditional, extract-based approach.²⁸

4.2 | Fusion proteins for therapy

A mixture of fusion proteins, designated BM32, combines non-allergenic peptides derived from the same four major allergens of *Phleum pratense*, fused to the hepatitis B-derived PreS domain, for immunotherapy.²⁹ The PreS domain functions as a non-allergenic carrier protein, providing T-cell help for the production of allergen-specific blocking IgG antibodies after immunization.²⁹ BM32 has been also recently tested in a large multicentre trial for grass pollen allergen immunotherapy.³⁰ In contrast to the hybrid molecule tested by Douladiris et al, these constructs are hypoallergenic and therefore not suitable for diagnostic purposes.

These examples of two hybrid approaches, which are successfully being tested for in vivo diagnostics and immunotherapy, represent prototypes of a new, promising category of allergen preparations, which may be expanded to other allergen sources. By combining the most important antigenic and allergenic components in a composition of recombinant molecules, the problem of standardization is being minimized and the variation of test results might decline dramatically. Yet, it has to be mentioned that this advantage is related to higher costs than the production of native extracts.

5 | THE COMING ERA OF "PRECISION ALLERGOLOGY"

The use of molecules for allergy diagnostics and therapy was theorized by Rudolf Valenta et al.³¹ Hence Molecular Allergology has outgrown infancy and adolescence and reached adulthood. Most clinicians, initially sceptic, are now accepting the evidence that molecules add value to allergen extracts by providing additional information on potential risk indicators, genuine sensitizations and cross-reactivity, especially valuable for poly-sensitized patients.^{32,33} Molecular extracts, as proposed here, and hybrid allergens may solve the eternal problem of diagnostic and therapeutic product standardization for IgE-mediated allergies. Whether this approach is economically feasible remains to be established now, given the proof of similar performance. If the advantages of increased simplicity and improved standardization outweigh the production costs, this opens, at last, the Era of Precision Allergology.

CONFLICT OF INTEREST

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