

EDITORIAL

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How important is it to include all epitopes in grass pollen extracts for specific immunotherapy?

This editorial discusses the findings in the paper by Chabre et al. [11]

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Up to 20% of the population in developed countries are affected by atopic diseases caused by airborne allergens such as grass and tree pollens, house dust mites, and animal dander. These conditions are increasing in prevalence [1] and have significant economic impact and adverse effects on the quality of life [2]. Treatment consists of (a) identifying allergic triggers and avoiding them where possible; (b) pharmacotherapy, which is essentially a non-specific approach, independent of the triggering allergens; and (c) in selected and more severe cases, allergen-specific immunotherapy (SIT).

SIT involves the administration of allergen extracts to achieve clinical tolerance of the allergens, which cause symptoms in patients with allergic conditions. Immunotherapy has been shown to be effective in patients with mild forms of allergic disease, and also in those who do not respond well to standard drug therapy. Recent studies suggest that SIT may also modify the course of allergic disease, by reducing the risk of developing new allergic sensitizations and inhibiting the development of clinical asthma in children treated for allergic rhinitis. An effect on regulatory T cells seems most probable, associated with switching of allergen-specific B cells towards IgG₄ production [3]. While some progress has been made in developing allergen-specific therapies, there is clearly scope for further improvement. For example, even in those who do currently respond to immunotherapy, we need to shorten the duration of treatment, improve the safety of therapy, and increase the overall level of efficacy. For those who appear suitable but do not currently respond, we need to understand the reasons why, in order to modify our vaccines and treatment regimes appropriately.

One area that may prove fruitful in this quest is to explore allergen structures, and in particular the epitopes that are recognized by IgE antibodies [4]. The Poaceae family of temperate pasture grasses contains > 2000 species, about 20 of which are implicated in grass pollen allergy (Fig. 1). These 20 species can, for practical purposes, be grouped into five subfamilies [5]. There is a close degree of homology between allergenic proteins of the grass subfamilies, with the molecular relationships reflecting their phylogenetic proximity. To date, 11 groups of grass pollen allergens eliciting specific IgE responses have been identified. The group 1 and group 5 allergens are the most widely recognized pollen allergens with 90% and 65–85% of allergic patients having IgE to these, respectively. Within each group, allergens from the various subfamilies exhibit a high degree of homology in terms of their amino acid sequence composition, which translates into significant cross-reactivity in terms of antibody (IgE and IgG) as well as T cell responses. Nevertheless, for a given allergen group, there is also evidence of both interspecies differences (due to different amino acid sequences) and intraspecies molecular variability (driven by multigenes, post-translational modification, mRNA splicing, or editing) [6]. These variations probably explain the clinically observed cross-reactivities, and hence understanding more about this may be useful in designing hypoallergenic proteins and safer vaccines [7].

Grass pollen-allergic patients are naturally exposed and sensitized to multiple pollens from various grass species. The question arises as to whether such patients need to be desensitized with extracts based on all the pollens to which they may be sensitized or whether a single pollen extract will suffice. Because of the cross-reactivity between pollens, neither conventional serological diagnosis based on IgE reactivity nor pollen counts enable the accurate identification of which grass species are involved in an individual patient's condition.

Allergy to taxonomically related species is a common phenomenon caused by the same antibody or T cell

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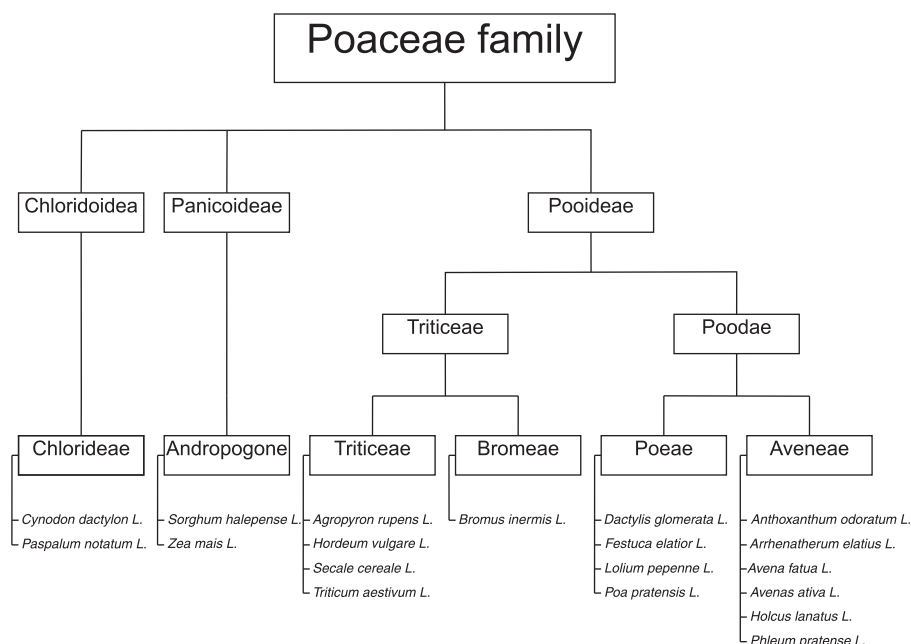


Fig. 1. Phylogenetic tree of common grasses and cultivated cereals involved in pollen allergy.

receptor recognizing homologous allergens from different species. Knowledge of patterns of cross-reactivity is crucial for the selection of optimal products for diagnosis and for SIT [8]. Investigating this *in vitro* has been difficult, because allergen-specific cells are present in very low frequency in peripheral blood of humans, and differ in function in allergic and non-allergic individuals, in particular in their requirement for *ex vivo* expansion or in the TCRs they use, which are often distinct from those of allergic individuals [9].

Nevertheless, it can be shown that pollen allergens also contain species-specific T cell and B cell epitopes, and there is evidence that at least 50% of allergic patients are sensitized to such distinct epitopes. In addition, substantial quantitative differences exist in the amount of each allergen group in pollen grains obtained from distinct grass species. Some would therefore argue that desensitization vaccines should contain pollens from each of the main grass subfamilies. Five-grass allergen mixtures have shown to be effective, and it has been argued that they reflect better the natural exposure and sensitization conditions at the molecular level than vaccines based on a single pollen [10].

On the other hand, it is easier to standardize vaccines based on a single grass species. In Northern Europe *Phleum pratense* is usually chosen, as most patients with grass pollen allergy react to *P. pratense*, sometimes alone and sometimes together with other grass species.

Clinical trials with single pollen vaccines have confirmed efficacy, and while no direct comparisons have been made, the general level of efficacy seems at least as good. Data published hitherto suggest that the immune

system does not appear to distinguish at the IgE level between the different species of Pooideae grasses. This extensive IgE cross-reactivity towards the allergens suggest that any of the Pooideae species could be used for diagnostic as well as therapeutic purposes [8].

In this issue, Chabre et al. [11] report on their molecular analysis of pollen allergens from different grass species. Their key findings were that there is more variability in allergens than recognized previously, with up to 50 isoforms of each major group five allergen in each species. Some of this variability is due to differences in amino acid sequence, and some of it reflects variability in glycosylation. From modelling studies, it seems that at least some of the amino acid variability is visible on the protein surfaces, and hence may offer different structural conformations affecting epitope recognition. This heterogeneity in the allergens goes some way to explain the variability in IgE responses between patients, but in addition many patients have IgE responses confined to one (or only a few) grass pollen species. This heterogeneity of recognition also extends to the T cell epitopes, which are thought to be critical for the efficacy of SIT of 14 MHC-restricted epitopes found in group 1 allergens, and only one was conserved between the different grass species.

Overall this work argues, from a molecular perspective, that if we want to desensitize patients to grass pollen effectively, we ought to use a vaccine that contains pollens from more than one grass species. This molecular vision has to be set against clinical experience demonstrating that treating with a single grass species seems to be sufficient to confer protection against the European grass pollen season [12]. There are several possible

explanations. It could be that the relevant epitopes show greater cross-reactivity *in vivo* than is apparent from the *in vitro* molecular analysis. Alternatively, patients may effectively be exposed only to one main pollen. There is no evidence of any bystander effect, and hence it is unlikely that SIT with a single pollen renders direct protection against unrelated antigens.

At its heart, this paper provides a molecular validation for the concept of using a five-grass vaccine rather than a single pollen vaccine for grass pollen SIT. The fact that a clinical advantage has not yet been shown may be because the concept is flawed, or perhaps because the five-grass vaccines available do not adequately cover the full range of isoform heterogeneity revealed here. It is unlikely that anyone will ever perform a clinical trial to address this, as it is notoriously difficult to show superiority over an active comparator. For now, we should recognize the valuable and painstaking work that has been performed to demonstrate the biological heterogeneity of pollen allergens and once we have digested it, perhaps we should revisit some of our sacred cows and consider carefully how valid they remain.

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