

Specific Immunotherapy for Common Grass Pollen Allergies: Pertinence of a Five Grass Pollen Vaccine

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Key Words

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Abstract

Patients throughout Europe are concomitantly exposed to multiple pollens from distinct Pooideae species. Given the overlap in pollination calendars and similar grain morphology, it is not possible to identify which grass species are present in the environment from pollen counts. Furthermore, neither serum IgE reactivity nor skin prick testing allow the identification of which grass species are involved in patient sensitisation. Due to their high level of amino acid sequence homology (e.g., >90% for group 1, 55–80% for group 5), significant cross-immunogenicity is observed between allergens from Pooideae pollens. Nevertheless, pollen allergens also contain species-specific T or B cell epitopes, and substantial quantitative differences exist in allergen (e.g., groups 1 and 5) composition between pollens from distinct grass species. In this context, a mixture of pollens from common and well-characterised Pooideae such as *Anthoxanthum odoratum*, *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense* and *Poa pratensis* is suitable for immunotherapy purposes because (1) it has been validated, both in terms of safety and efficacy, by established clinical practice; (2) it reflects natural

exposure and sensitisation conditions; (3) it ensures a consistent and well-balanced composition of critical allergens, thus extending the repertoire of T and B cell epitopes present in the vaccine.

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Introduction

In Europe, grass pollen immunotherapy accounts for one third of specific immunotherapies against type I allergies [1, 2]. It involves common grasses belonging to the Pooideae subfamily, which represents a coherent phylogenetic group distinct from other subfamilies [3]. Pollens from Pooideae grasses exhibit a similar morphology [4]. Thus, neither pollen counts nor microscopic analyses can discriminate pollens originating from any given grass species. Common grasses also exhibit an overlapping geographical distribution and similar pollination calendars (fig. 1). As a consequence, no data relating to exposure to a specific grass pollen are available.

Pooideae pollen extracts demonstrate significant IgE cross-reactivity [5–12]. Neither in vitro diagnosis nor skin prick tests carried out with such extracts can incriminate a given grass species in sensitisation. Nevertheless, all patients encounter a variety of species during a grass

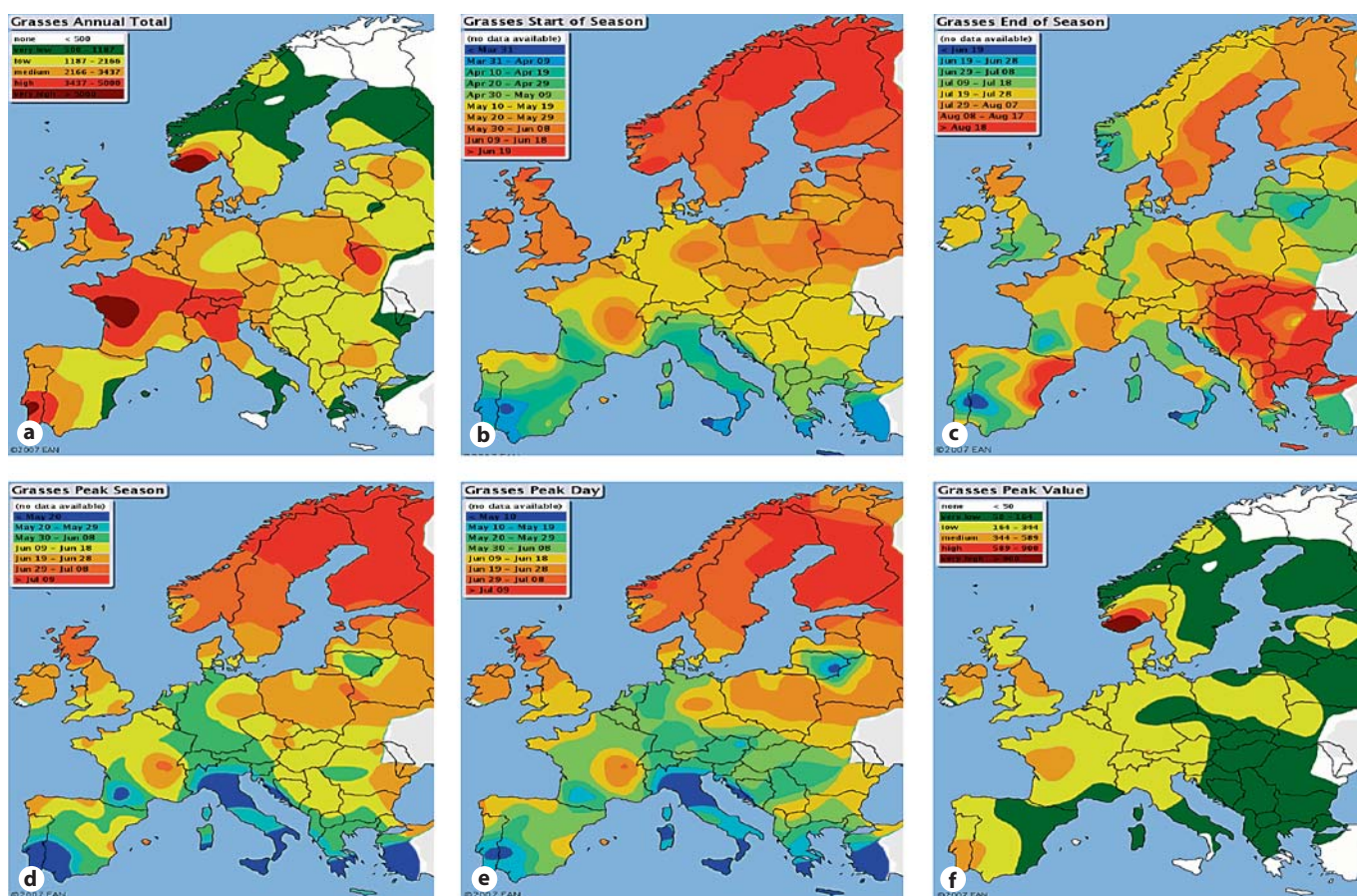


Fig. 1. Pooideae pollen exposure in Europe. Data over the past 10 years (1997–2006) from the European Aeroallergen Network (EAN) was used, compiling 2,569 annual cycles from 497 monitoring sites. **a** Relative intensity of grass pollen seasons in various areas by plotting the average cumulative grass pollen counts. **b** Distribution of the median start of the average grass pollen season. The start of season was defined as the day on which the daily pollen count exceeded 1% of the annual total grass pollen count for the first time under the condition that not more than 6 con-

secutive days without data or with zero grass pollen count followed. **c** Distribution of the median end of the average grass pollen season. **d** Average temporal progression of the grass pollen season from the South to the North. The median of the peak season has been chosen as an indicator of the peak season (50% of the annual cumulative grass pollen count, with 25% of the annual total cut-off on both ends). **e** Median peak day distribution of the average grass pollen season. **f** Average cumulative grass pollen count during the peak season.

pollen season, with such polyexposure leading to polysensitisation. In this context, in order to desensitise grass pollen-allergic patients, the question arises as to whether the extract should be made from a single pollen or from multiple grass pollens.

Arguments in Favour of a Mixed-Pollen Vaccine

A working assumption to design an ideal vaccine is that natural exposure conditions and sensitisation mechanisms should be taken into account. Regarding the for-

mer, a vaccine should recapitulate natural exposure conditions. With respect to the latter, patients are not sensitised to pollens *sensu stricto* but rather to selected molecules (i.e. pollen allergens) and derived epitopes. Thus, the rationale to choose the right vaccine should be established at the molecular level.

Most grass pollen-allergic patients are sensitised mainly to group 1 and group 5 major allergens, whereas a few are also sensitised to group 2, 3, 4, 6, 7, 10 and 13 allergens [7–11]. There is ample evidence that both group 1 and group 5 allergens are well conserved among Pooideae, with an amino acid sequence homology in the range

of 90 and 55–80%, respectively [13–15]. There is some level of conservation of other pollen allergens besides groups 1 and 5 among Pooideae, with homologies at the amino acid level ranging from 40 to 60% (i.e. less than for group 1 and 5 allergens).

However, differences have been reported regarding the allergen content of pollens from distinct grass species. For example, alignment of known group 5 allergen sequences from various Pooideae reveals significant variability, in the form of stretches of amino acids specific to a given group 5 allergen (data not shown). Quantitatively, Pooideae pollens contain varying amounts of group 1 and group 5 allergens (with differences in the ratio between 5a and 5b isoforms) depending on the species considered (fig. 2, noteworthy results are expressed using a log scale). Both quantitative and qualitative differences have also been described between Pooideae species regarding group 2, 4, 10 or group 13 [16–18] allergens.

Importantly, there is evidence that a patient's immune system can detect these differences. As shown in figure 3, amino acids which vary between known group 1 molecules from distinct grass species are exposed at the surface of the molecule. Thus, such differences are likely to affect IgE, IgG or T cell recognition. Furthermore, using purified group 1 or group 5 allergens from various Pooideae species, approximately 10–20% of grass pollen-allergic patients exhibit IgEs specific to allergens originating from a given grass species (manuscript in preparation). This is in agreement with reports in the literature for T and B cell responses to epitopes restricted to a given species within group 1 or group 5 allergens [19, 20].

Altogether, an analysis of exposure conditions to grass pollens at the molecular level reveals the existence of a broad epitopic repertoire of both major and minor allergens. Using an extract based on a single pollen for desensitisation would restrict this repertoire, both qualitatively and quantitatively. Thus, we conclude that a grass pollen mixture better reflects natural exposure conditions.

Which Mixture of Pooideae Grasses Should We Use?

The most widely prescribed combination (representing 85–90% of grass pollen immunotherapy in France, Italy, Germany and Spain; our unpublished data) is a pollen mixture from *Anthoxanthum odoratum*, *Dactylis glomerata*, *Lolium perenne*, *Poa pratensis* and *Phleum pratense*. Clinical trials, including a double-blind placebo-controlled phase III efficacy study, have demonstrated that this 5 grass pollen vaccine is both safe and effica-

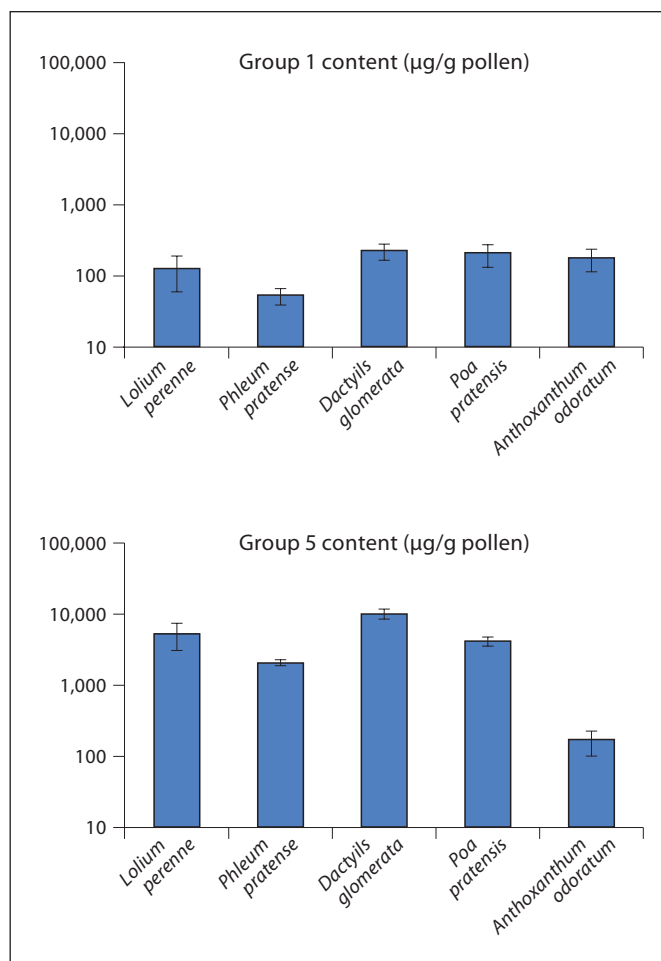
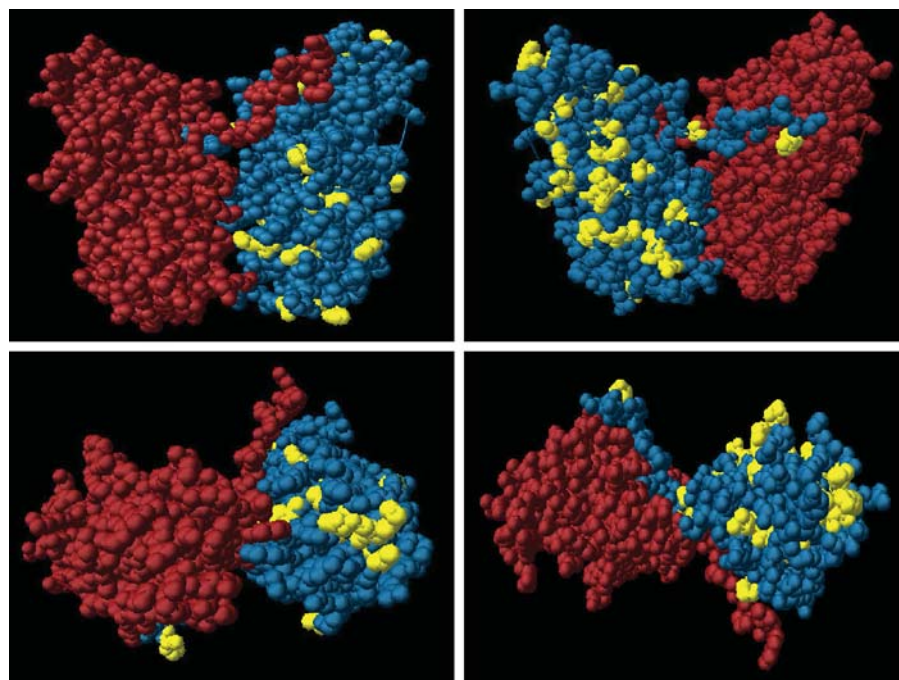


Fig. 2. Quantitative variations in group 1 and group 5 content among grass pollen species. The content of group 1 and group 5 major allergens was analysed in 6 batches of pollen obtained from 2 different suppliers (Allergon and Pharmallerga) for each of 5 grass species. Group 1 and group 5 major allergens were measured by sandwich ELISA in aqueous extracts obtained at 1/20 w/v in ammonium bicarbonate 4 g/l for 24 h at 4°C. ELISA assays were performed using Stal 19/Stal 11 (anti-group 5) and Tim 22b/Tim 03b (anti-group 1) pairs of monoclonal antibodies. These cross-reactive antibodies were obtained after immunising mice with a *D. glomerata* pollen extract, and further selected based on their reactivity with either rPhl p 1 or rPhl p 5 recombinant allergens. Results are expressed as micrograms/gram pollen on a log scale.

cious in patients with allergic rhinoconjunctivitis: a daily 300 IR (index of reactivity) tablet of this sublingual vaccine throughout the pollen season leads to a 37% reduction in symptoms associated with a 46% decrease in the requirement for symptomatic medications, when compared with placebo ($p < 0.0001$) [21]. The safety and efficacy of a vaccine based on a single (timothy) grass pol-

Fig. 3. Three-dimensional localisation of intra- and interspecies sequence variations among group 1 grass pollen allergens. The 3-dimensional structure of the Phl p 1 homodimer has been resolved on the Phl p 1.0102 isoform (PDB ID code 1N10). Amino acid sequences of group 1 isoforms from timothy grass (Phl p 1.0101 and 1.0102), cocksfoot grass (Dac g 1.0101 and 1.0102), rye grass (Lol p 1.0101 and 1.0103) and Kentucky blue grass (Poa p 1.0101) group 1 allergen isoforms were aligned using CLC Combined Workbench Software. Residues which differ from the Phl p 1.0102 reference in either of those sequences are highlighted on a monomer. Variable amino acids tend to cluster on the molecular surface. A left and right side view, and a top and bottom view are shown in the upper and lower panels, respectively.



len extract has been documented in humans, both via the subcutaneous and sublingual routes [22, 23]. Assessing the benefit of a single versus a mixed pollen vaccine in a comparative way is not possible, in that it would require a dedicated clinical study enrolling a very large number of patients. Nevertheless, based on the arguments listed above, we conclude that a mixed pollen vaccine is more appropriate to treat patients sensitised to multiple (i.e. both shared and species-specific) epitopes from Pooideae allergens.

Besides its broad historical utilisation for desensitisation purposes by clinicians, the 5 grass combination can be justified based on (1) the widespread distribution in Europe and North America of these Pooideae, which belong to 2 distinct tribes; (2) a well-characterised content of both major and minor allergens [3, 5, 10–15].

Conclusions

Grass pollen immunotherapy currently relies on biological extracts prepared from a pollen mixture rather than from a single pollen. This practice is recognised by the European Medicines Agency and other health agencies in Europe and North America [24]. The most widely prescribed combination is a pollen mixture from *A. odoratum*, *D. glomerata*, *L. perenne*, *P. pratense* and *P. pra-*

tensis. These species are widely distributed and their allergen content has been well characterised. Importantly, this 5 grass vaccine was shown to be safe and effective in a double-blind, placebo-controlled phase III study [21].

Such a 5 grass pollen extract better represents natural exposure and sensitisation conditions encountered by grass pollen-allergic patients. Specifically, a 5 grass pollen vaccine offers the advantage of balancing the content of both major and minor allergens, while extending the repertoire of T and B cell epitopes. Based on these environmental and molecular arguments, we conclude that a mixture of pollens from 5 Pooideae species is a valid active ingredient for specific immunotherapy.

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