

Patient-tailored Recombinant Allergen Products – Mission Impossible?

Oliver Cromwell, Paolo M. Matricardi, Helmut Fiebig

Patient-tailored allergen specific immunotherapy is not new, although the level of sophistication is substantially less than could be achieved making use of the technology that is now available. Allergologists, particularly in the USA, prescribe or tailor mixtures of aqueous extracts of several different allergenic raw materials to match a patient's sensitization profile. However, this practice is obviously not able to take account of different patterns of sensitization to individual allergenic components of one extract. Furthermore, criticism is often levelled at this approach because of the problems associated with mixing unrelated allergens and in particular the fact that proteolytic enzyme activity from one source can cause degradation of other proteins in the mixture.

Advances in molecular allergology and the availability of increasing numbers of recombinant allergens have made it possible to obtain detailed information on patients' individual allergen sensitization profiles (component-resolved diagnosis) [1]. This in turn raises the possibility of conducting specific immunotherapy with allergen mixtures which match a patient's sensitization profile. In the case of allergen sources such as grass pollen and house dust mites, several different allergens have been identified, thus creating the potential for a considerable number of allergen combinations [1,2]. The position becomes even more complicated if we contemplate combining allergens from different sources. If patient-tailored recombinant products are to become a reality it will be necessary to overcome various obstacles in order to satisfy regulatory requirements and obtain marketing approvals.

The four major issues that have to be addressed are quality, efficacy, safety and dosage. The biotechnology and allergen product guidelines [3,4] clearly lay down the requirements for recombinant proteins concerning quality and experience is showing that these can be fulfilled for recombinant allergens [5]. The guidelines are less helpful in defining requirements for demonstrating preclinical safety. Whilst it may be realistic to conduct limited preclinical safety testing with individual allergens, financial considerations would almost certainly limit more extensive studies

to allergen mixtures from which it should be possible to draw conclusions regarding the individual components, provided that no untoward effects are observed. Conventional dosage-finding clinical studies with every single allergen would not be feasible because of the difficulties of finding suitable patients with appropriate sensitization profiles in sufficient numbers and the enormity of the task. Similar considerations would apply to the evaluation of clinical efficacy and therefore pragmatic approaches would have to be identified.

Against this background the questions arise as to whether the objective of true patient-tailored specific immunotherapy is possible to achieve or indeed if it is necessary.

Component-resolved diagnosis

The availability of purified natural or recombinant allergens has made component-resolved diagnosis a reality. The major focus to date has been on the detection of specific IgE antibodies by *in vitro* methods [2,6], but the potential exists to exploit *in vivo* diagnostic methods, too [7]. Not only does this approach help in the identification of specific sensitizations against allergens from one source, but it also provides a powerful tool for the elucidation of sensitizations associated with cross-reactive allergens. For example, in the case of patients who show a positive result for IgE-reactivity to grass pollen, it is possible to dissect the response by testing with up to 11 different grass pollen allergens [8], usually those from *Phleum pratense* (Timothy grass) which serves to represent the sub-family *Pooideae* from the family *Poaceae/Gramineae*. In a detailed study of 50 grass pollen allergic subjects we were able to examine sensitization patterns to 9 different grass pollen allergens and obtained results very similar to those of Andersson and Lidholm [8] (Fig. 1). The highest frequencies of specific IgE reactivity were found for the group 1 and 5 allergens (Fig. 1), which also show the highest antibody levels [8] and which are recognized as allergens that are associated exclusively with grasses [9]. Various frequencies of sensitization and IgE antibody levels are seen with other grass allergens, including *Phl p 7* (2-EF calcium-binding protein) and *Phl p 12* (profilin) which are allergens that have cross-reactive counterparts in other plant species [7]. For example, a subject showing reactivity to *Phl p 7* as well as cross-reactive *Bet v 4* and other allergens from birch pollen, but without reactivity to other grass allergens, is likely to be sensitized primarily to birch and not grass pollen. The availability of such basic information about a patient's sensitisation profile can pave the way for tailoring a therapeutic vaccine. Possibly more importantly it can serve to ensure the correct diagnosis and choice of vaccine, and in this regard recombinant marker allergens have been referred to as 'gatekeepers' for the treatment of allergy [6].

Do we need patient-tailored immunotherapy?

Allergen specific immunotherapy is currently practiced using whole allergen extracts of natural source materials. Consequently, a patient who is only sensitized to the grass group 1 allergen, for example, is treated with a preparation containing at least 10 additional allergens in various amounts. This has however not been seen as a problem. Theoretically, patients might develop new sensitizations to allergens in

the extract, but there is very little evidence for this and it is difficult to exclude the possibility that natural exposure is a significant contributory factor. One of the very few studies in this regard concerned birch pollen immunotherapy in polysensitized subjects, many of whom showed positive responses to fruits and vegetables. Evidence of new sensitizations on the basis of immunoblot experiments was found for two subjects after 4 to 5 months treatment when total specific IgE was significantly increased by comparison with controls. After 12 or more months 17 of 26 subjects were affected, despite the fact that total specific IgE levels had returned to pre-immunotherapy levels [10]. Specific measurements in respect of *Bet v 2* and *Bet v 4* suggested that 7 of the immunotherapy subjects developed a new sensitization to one of these allergens, but specific IgE levels were generally very low. The allergens implicated in the other new sensitizations were not identified.

On the other hand, an immunotherapy study conducted with a mixture of five recombinant grass pollen allergens, including both *Phl p 5a* and *5b*, found no evidence for induction of group 5 specific IgE antibodies over an 18 month period of treatment in four subjects who tested negative for this allergen at the outset of the study [11]. The subjects did however develop strong group 5 allergen-specific IgG1 and IgG4 responses, indicating either pre-existing immunity to the allergen without class-switching to IgE or induction of immunity. These 'blocking antibodies' could well stand the patients in good stead by providing protection against subsequent sensitization.

The fact that a large part of a patient's specific IgE is often directed against just a few allergens raises the possibility that immunotherapy with these allergens alone would be sufficient to ameliorate most of a patient's symptoms, with little need to consider 'minor' allergens. A further factor which might play a role in successful immunotherapy is the so-called by-stander effect, which is based on the hypothesis that the immuno-modulatory effect induced by treatment with one allergen has a beneficial effect on the response to other allergens. Firm scientific evidence for this is however still lacking, but if proved to be relevant it would provide one argument against the necessity to include every allergen to which a patient was sensitized.

It will only be possible to assess the potential benefits of patient-tailored treatment by conducting comparative clinical studies.

Formulating products for patient-tailored immunotherapy

Evidence indicates that particular allergies, such as those to birch pollen and cat, are largely attributable to one 'major' allergen and that is sufficient for successful treatment. The possible need for tailored preparations would only arise in a minority of patients with significant sensitization to other allergens. The case of grass pollen allergy is another matter. Theoretically, patient-tailored preparations might be formulated to contain any combination of eleven recognized allergens, which is to say in excess of 2,000 different permutations! Is that likely to be necessary?

We examined the sensitization patterns to 9 different grass pollen allergens in 50 patients with grass pollen induced rhinoconjunctivitis. The highest frequency of sensi-

tization was to *Phl p 1* (94%), followed by *Phl p 5* with 88% and lesser frequencies to the other allergens (Table 1). *Phl p 2* specific IgE was detected in 37 (74%) of subjects, only one of which was *Phl p 1* negative, a result consistent with the recognized cross-reactivity between groups 1 and 2. No cross-reactivity has been recognized between *Phl p 1* and *Phl p 5*, to which 44 (88%) of the subjects had specific IgE antibodies. Only one of these was *Phl p 1* negative, indicating that 86% of *Phl p 5* sensitized subjects were sensitized to both group 1 and 5. *Phl p 6* is recognized to be highly cross-reactive with *Phl p 5*, and all 31 subjects with *Phl p 6* specific antibodies were also *Phl p 5* reactive. The frequency of associated sensitizations is shown in Table 1 in descending order, and serves to give an impression of the relative importance of selected allergen combinations for specific immunotherapy. When considering the amounts of specific IgE produced against the various allergens, antibodies to group 1 and 5 clearly predominated [8]. Such data allows us to conclude that the large proportion of the specific IgE to allergens from any one source is accounted for by just a few allergens in most subjects. Similar distinctions can be made on the basis of *in vivo* reactivity to the various allergens, and skin prick testing has shown that the order of potency of five grass pollen allergens was *Phl p 2* > *Phl p 5* > *Phl p 1* > *Phl p 4* > *Phl p 13*, with an approximately eight-fold difference between *Phl p 2* and *Phl p 13* [12].

Similar analyses will be applicable to allergens from other sources. For example, in the case of the house dust mite *Dermatophagoides pteronyssinus* at least 18 different allergens have been described to date, but it is clear that *Der p 1* and 2 are the

Table 1. Frequency of sensitization to nine allergens of *Phleum pratense*

		Frequency of sensitization in total patient collective								
Allergen		1	5	2	6	4	11	13	12	7
Frequency of sensitization		94%	88%	74%	66%	58%	56%	36%	14%	6%
Frequency of associated sensitizations	1	94%	86%	72%	64%	56%	52%	34%	12%	6%
	1+5	-	-	68%	64%	54%	52%	32%	12%	6%
	1+5+2	-	-	-	50%	44%	42%	30%	12%	6%
	1+5+2+6	-	-	-	-	34%	36%	26%	12%	4%
	1+5+2+6+11	-	-	-	-	24%	-	22%	10%	4%
	1+5+2+6+11+4	-	-	-	-	-	-	16%	10%	4%
	1+5+2+6+11+4+13	-	-	-	-	-	-	-	6%	2%
	1+5+2+6+11+4+13+12	-	-	-	-	-	-	-	-	2%

Sera from fifty patients with grass pollen-induced rhinoconjunctivitis were screened for specific IgE antibodies using ISAC allergen arrays (VBC-Genomics, Vienna) with 103 allergens, including 8 from *Phleum pratense*. Antibodies against *Phl p 13* were detected using recombinant *Phl p 13* in a solid-phase ELISA.

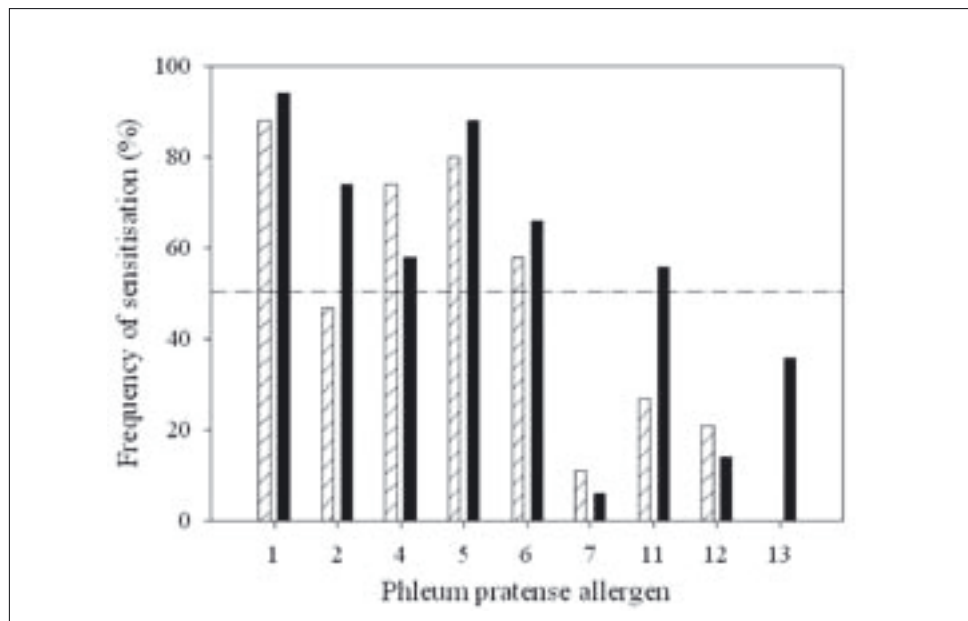


Fig. 1. Frequency of sensitization to nine grass pollen (*Phleum pratense*) allergens. Data from a group of 50 patients with grass pollen induced rhinoconjunctivitis and positive skin test and IgE responses to grass pollen [solid black]; and data for 150 grass pollen-sensitized individuals [hatched] from Andersson and Lidholm [8].

most frequent causes of sensitization [13]. *Der p* 1 and 2 also account for the larger part of the house dust mite specific IgE, with *Der p* 4, 5 and 7 accounting for most of the remaining activity [14].

Demonstrating clinical efficacy

The current CHMP Guideline on the clinical development of products for specific immunotherapy for the treatment of allergic disease requires the demonstration of superiority versus placebo or another comparator, and clearly recommends the use of a combined symptom medication score as the primary end-point [15]. The execution of such demanding clinical studies for numerous different allergen mixtures is clearly unrealistic. This is also the case for dose response studies, and a pragmatic approach will be necessary to define the dosage of each individual allergen. Evidence from various sources shows the necessity for administration of a sufficiently high allergen dose to achieve clinical efficacy, and there is a clear scientific rationale for including all components of an allergen mixture in equimolar concentrations. Therefore a dose response study would test various total protein dosages with a constant concentration relationship.

The first clinical trials with recombinant grass pollen allergens have been conducted using a mixture of the five major allergens *Phl p* 1, 2, 5a, 5b and 6 in approximately

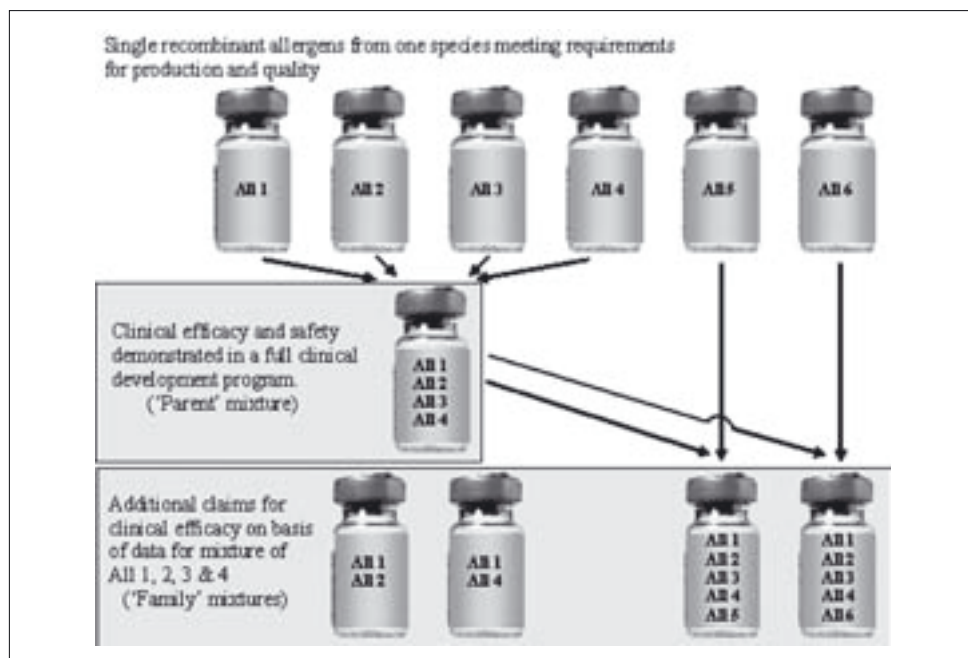


Fig. 2. Possible strategy for claiming clinical efficacy of various allergen combinations.

equimolar concentrations in patients selected on the basis of their reactivity to whole grass pollen [11]. The data in Table 1 support this choice of allergens, and studies have been undertaken with a view to defining a preparation that can be efficacious for all grass pollen allergic subjects. The possibility remains that this mixture might be considered too comprehensive for a small number of patients and inadequate for others. Therefore this basic mixture could be 'tailored' by either removing or adding allergens (Fig. 2), thereby creating a family of allergen mixtures, or products, related to the 'parent' allergen mixture. This raises the question as to how to make a claim for the clinical efficacy of these additional allergen combinations without the need for extensive additional clinical studies.

Allergic rhinoconjunctivitis and allergic asthma can be viewed as single diseases with the same immunological and inflammatory mechanisms, but with many different triggers. Therefore, if clinical efficacy can be demonstrated for one or more allergens it would not be unreasonable to expect to see an effect with other allergens, particularly those from the same source. For example, if the group 1 allergen of grasses can be shown to be clinically efficacious in appropriately sensitive subjects, why should the group 5 allergen not be similarly effective, provided that optimal quality and dosage are achieved?

It has been suggested that orphan drug status might be applicable in cases where it would be necessary to add one or more allergens to a basic mixture of proven clinical efficacy in order to achieve a good therapeutic effect [16]. In the case of grass

pollen allergy, a mixture of groups 1, 2 and 5 might prove to be clinically efficacious in 80% of subjects, but the inclusion of group 4 or group 11, for example, might be deemed necessary to achieve adequate efficacy in subgroups of subjects with these additional sensitizations. Conversely, a similar argument might be used to justify the removal of particular allergens to which a subject had no demonstrable sensitization. This would create a 'product family', with a 'parent mixture' for which a full clinical development program was completed to provide evidence of clinical efficacy for the whole family, and siblings with orphan drug status for which a limited amount of safety data was available.

Possible ways forward

Issues relating to quality, pre-clinical safety and dosage can be resolved, but the major obstacles to developing true patient-tailored immunotherapy are the demonstration of clinical efficacy and the prospect of obtaining marketing authorizations for numerous allergen mixtures. Taking commercial considerations and current regulatory requirements into account, there seems little prospect that such a sophisticated form of treatment will become a reality. The only way that it might be achieved would be to allow the marketing registration of individual allergens which could then be mixed in any desired combination by the manufacturer or a pharmacy to produce named-patient preparations. Such a strategy would run completely contrary to the objectives of current regulations and achieving registered end-products of high pharmaceutical quality. A realistic objective is that of producing a limited range of allergen mixtures which cover the most frequently encountered sensitization profiles. This will nevertheless require willingness on the part of the registration authorities to adopt a pragmatic approach with regard to the demonstration of clinical efficacy with the possible recognition of families of allergen mixtures, with orphan products relating to a 'parent' product for which the most extensive clinical data are presented.

The views and opinions expressed in this article are those of the author alone.

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Discussion

Leibold: I just would like to make a little comment on your rise of IgG4 against *Phl p 5* where the patient had no IgE to. That patient was sensitized on a Th1 dominance so he had already memory cells and therefore he had no allergy and had no IgE. If you would have given him any vaccination you would also have come up with a rise in IgG4, because he already had the memory, a cytokine boost. And for that reason I think it is not very convincing to put additional recombinant allergens into a mixture just in the hope to boost for other possible allergens if the patient is already under an immune control which is Th1-governed.

Cromwell: That was not my argument. My argument was it does not do any harm including it.

Leibold: O yes, that is fine. Because otherwise we could never give a mixture as we are doing now. Otherwise you would be criminal to do it. But basically, I would like to thank you for the very clear presentation. I really hope that the recombinant allergens make much more progress, because then desensitization and specific immune therapy is much more helpful than it is right now with the mixtures.

Robinson: Oliver, can I just question perhaps some of the basis of your assumption that you need to treat with all of the components to which that patient has IgE. Because there you target the level of IgE sensitization, but actually what we are trying to achieve with immunotherapy is immune tolerance. Which maybe quite different or probably is quite different. And if the concepts of linked suppression and other ideas of tolerance are actually true, you may not need to treat with all of the components to achieve tolerance to all of the components. And I wondered in those patients where you were treating with the vaccine that did not cover some of the

components, such as the group 13 allergens, did you see rises in IgG4 to components that you were not injecting in any of the patients?

Cromwell: Unfortunately, we have not looked in that great detail as yet, we have not looked at the IgG4 against the group 13 allergens. I think I am right in saying that. Anyway, that is an interesting line, but I fully agree with you that bystander suppression may be an important factor. However, I would also suspect that in the majority of the patients you can argue that the majority of their symptoms are caused by the major allergens in the preparation. And if they just have a very low level of sensitization to something like group 13 that contribution to their total symptom score, if you like, is probably small.

Robinson: But it may also be possible that you can turn off all of those with just one of the components.

Cromwell: That remains a possibility, yes. That is something we have to look at.

Barber: First of all congratulations for your presentation. But somehow I feel that sometimes we try to make things more complex than they might probably be. In this case, I mean, we have to take care of IgE prevalences for selecting the formulations, because *Phl p 2* is partially cross-reactive with *Phl p 1* and most of the reactivities covered by group 1. In *Phl p 6*, that is specifically present in Phleum and probably *Poa*, practically all the reactivities are covered by *Phl p 5*. And then, with the formulation of the two major allergens, you might probably cover more than 90% of the patients and with that it might be more than enough to start with and to validate the concept. So, if we try to make individual mixtures and change the regulatory framework then probably that might not be realistic at all and you might claim that you will cover those patients with conventional extracts. I think that probably this is not necessarily reflecting the process of my company...

Cromwell: It is clear from the meeting that there is a great deal of interest in the potential of patient-resolved immunotherapy..

Carnes: With the current regulatory framework, it seems feasible that in the coming years we might develop vaccines based on major allergens, without making this more complicated by the inclusion of minor allergens as well for this specific purpose...

Cromwell: Quite possibly. You often have to take a hammer to crack a nut. And part of my objective today was to say to the regulators: I present the problems in the darkest form and then, hopefully, if they take that seriously they may find a pragmatic solution.