

Allergenic Extracts for Specific Immunotherapy: To Mix or Not to Mix?

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Key Words

Immunotherapy • Extracts • Allergens • Allergenic molecules

Abstract

Immunotherapy for allergic diseases give rise to questions about when a decision must be taken to define the number of extracts to be used in a single treatment. This represents a long-lasting matter of debate between American and European allergists, which seems to be without real solution. Through the use of extract-based versus molecule-based diagnostic approaches we suggest a possible solution to this controversial issue. We used four model patients previously tested with allergenic extracts and later on selected on the basis of a panel of available allergenic molecules. Their reactivity patterns in term of extracts and molecules were compared and the decision for allergenic extract immunotherapy was made choosing either the 'classical' approach or the molecular approach. From our study it seems that molecules could offer the solution to the 'mixing' issue of allergenic extracts. This innovative approach seems to provide a solution for both the American and the European approach.

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Introduction

Pharmacological approaches have been widely considered to treat allergy symptoms, but specific immunotherapy (SIT) is the only causative treatment [1]. A major limit of SIT is represented by the use of natural allergen extracts constituted by an unpredictable mixture of both allergenic and nonallergenic components. On the basis of the current epidemiology of allergic sensitization it is often necessary to treat subjects with multiple extracts. Most of the immunotherapy trials are based on single allergenic extracts, whereas it is not clear whether multisensitized subjects would benefit from such treatment. There is a limited number of studies considering the effect of immunotherapy on multisensitized subjects [2]. The decision step toward correctly choosing the appropriate selection of allergenic extracts is stated in position papers from leading scientific allergy associations [3, 4]. The limit for a multiple allergenic extract preparation to be used in allergy immunotherapy is still a never-ending matter of debate. During one of the last meetings of the American Academy of Allergy, Asthma and Immunology and of the European Academy of Allergology Clinical Immunology Congress sessions were once more dedicated to discuss the topic [from the final programs: AAAAI 61st Meeting, March 20, 2005, Session 3401, 'Immunotherapy: Single vs. Multiple Extracts (Europe vs. US)';

19th WAO-24th EAACI, June 28, 2005, Sister Society Symposium, Allergen Specific Immunotherapy: State of the Art in North America and in Europe]. Historically the American attitude is much more in favor of mixing allergenic extracts, whereas European immunotherapy courses are much more based on choosing single allergenic extract preparations.

Component-resolved allergy diagnosis employing allergen molecules instead of crude extracts has disclosed the existence of several IgE sensitization profiles [5]. Specific IgE analysis by means of allergenic molecules clearly shows how the use of allergenic extracts could make a correct diagnosis difficult [6]. Furthermore, recent studies with allergenic molecules have confirmed the existence of several scenarios of allergen molecule IgE reactivity [7].

Methods, Results and Discussion

We take advantage of the four case reports shown in table 1 to exemplify how the two diagnostic approaches, extract-based and molecule-based, may lead to different conclusions about immunotherapy decision making. Subjects were skin tested with a panel of extracts as previously reported [6]. IgE detection for extracts and allergenic molecules was performed by ImmunoCAP system (Phadia, Uppsala, Sweden).

The first case reported in table 1 is a 28-year-old man having severe seasonal rhinoconjunctivitis, with a single positive skin test (timothy grass extract, Allergopharma, Reinbeck, Germany) and specific IgE to grasses (timothy grass) and negative tests to a wide panel of other pollen and nonpollen allergenic extracts. A monosensitization to grasses was recorded. This subject represents a typical example where SIT is currently recommended without disagreement between the schools. The analyses of specific IgE toward a panel of grass molecules (Phl p 1, 2, 4, 5, 6, 7, 11, 12) revealed that the patient was only sensitized to Phl p 1. The prescription of a SIT in this case, generally composed by a mixture of several grass species, is accepted with a broad consensus.

The second subject is a 27-year-old man with quite severe seasonal respiratory symptoms (rhinitis and asthma). The in vivo and in vitro reactivity toward allergenic extracts was the same as in the subject described above, but the analysis of specific IgE to the same panel of grass allergens gave positive results for Phl p 1, Phl p 2, Phl p 4 and Phl p 5. As in the first case there is full agreement on the prescription and administration of a grass extract im-

muno-therapy. In this case, a 'natural mixture' of four relevant allergen molecules is used.

The third case is a 43-year-old woman reporting persistent respiratory symptoms for a considerable period of time during the year (from February to September) and an associated oral allergy syndrome. In vivo and in vitro tests were positive for olive, timothy grass, *Parietaria*, birch, hazel, and oak pollen extracts and for several foods (apple, carrot, and pear) tested by the prick-prick technique with fresh foods. In the patient with multiple sensitization, SIT is not currently recommended in Europe, whereas a mixture of extracts is acceptable in the USA. The molecular profile in this case showed the presence of IgE toward four allergens: Ole e 1 for olive pollen (tested by immunoblot extract), Par j 2 for *Parietaria*, Phl p 1 for timothy grass and Bet v 1 for birch. The first three allergens were reasonably the cause of the seasonal respiratory symptoms in spring, whereas Bet v 1 reactivity explained food-related symptoms caused by closely related molecules in fruits (Mal d 1, Pyr c 1, Dau c 1) and contributes to respiratory symptoms with pollen homologues (Cor a 1 from hazel pollen, Que a 1 from oak pollen). Despite the appearance, this case looks very similar to case 2, as the pattern of sensitization is limited to four allergens. The difference is that these allergens are derived from different sources, while in case 2 they are all from the same allergenic source. The debate on case 3 cannot be solved in terms of allergenic extracts (to mix or not to mix?), although a mixture of the four pollen extracts could be very similar to a mix of four grasses often recommended in the first two cases. In the future the patient could receive a SIT composed by a 'laboratory-made' mixture of the four natural allergens [8].

Case 4, a 54-year-old woman with severe seasonal asthma and no food allergy symptoms, showed a spread of reactivity to all the tested pollen extracts. As her exposure to pollen starts as early as January (hazel, cypress) and lasts until October (mugwort, pellitory), her respiratory symptoms are troublesome for an extended period during the year. The extract-based diagnosis classified her as having a multiple pollen sensitization [6]. IgE reactivity to the calcium-binding proteins (CBP), Phl p 7 and Bet v 4, was recorded. Pollen CBP as allergens have been defined as 'panallergens' and 34 CBP have been described in 28 different pollen species so far (www.allergome.org). These patients usually display a broad reactivity to all the pollen species due to the sharing of IgE-reactive epitopes on these highly homologous molecules [9]. No other IgE reactivity was recorded using the available allergen molecule panel. The patient revealed therefore a

Table 1. Allergenic extracts and allergen molecules: comparative evaluation of 4 pollen-allergic patients

	Case			
	1	2	3	4
Age, gender	28, M	27, M	43, F	54, F
Symptoms	C, R	A, C, R	C, R, OAS	A, C, R
Skin test and IgE to extracts ^a	timothy grass	timothy grass	timothy grass, olive, <i>Parietaria</i> , birch, oak, hazel, apple, carrot, pear	MPS
IgE to				
Phl p 1	+	+	+	—
Phl p 2	—	+	—	—
Phl p 4	—	+	—	—
Phl p 5	—	+	—	—
Phl p 7	—	—	—	+
Bet v 1	—	—	+	—
Bet v 4	—	—	—	+
Ole e 1	—	—	+	—
Par j 2	—	—	+	—
Unique allergen molecule sensitization	1	4	4	1

Allergens: Phl p = timothy grass; Bet v = birch; Ole e = olive; Par j = *Parietaria*. A = Asthma; C = conjunctivitis; MPS = multiple pollen sensitization; OAS = oral allergy syndrome; R = rhinitis.

^a A wide panel of allergenic pollen extracts was tested as reported by Mari [6].

‘molecular’ monosensitization, very similar to case 1. In this case the difference resides in the presence of the IgE-reactive epitopes in unrelated botanical species, whereas in case 1 the IgE-reactive epitopes are present on related molecules (grass group 1 allergens) from botanically related species. A molecular perspective for immunotherapy treatment of this subject would result in a rather simple approach with a single component preparation.

There is the need to consider that other allergen molecule sensitization could be present in each of the reported subjects as we used a limited number of molecules in our panel.

Thus, how should these patients be treated? A SIT can at present be recommended in the first two cases but not in the last two in Europe, whereas all the subjects could currently be treated in the USA. In our opinion it is necessary to define an approach as in cases 2 and 4. In a single allergenic extract preparation we use a ‘natural’ mixture of allergens, but a similar mixture of molecules can be achieved using purified native or recombinant allergens to treat patients such as case 3. Conversely, when we give immunotherapy with a mixture of allergenic extracts from unrelated species, as in case 4, we are treating

a ‘monosensitized’ subject as in case 1. This is what the molecular approach could teach us. In our experience, a simple distinction between monosensitized and multi-sensitized subjects is no longer acceptable on the basis of testing allergenic extracts.

The use of allergen molecules in a patient-tailored fashion would allow us to treat allergic subjects with either single molecule preparations or ‘artificial’ mixtures. This could be a way to get out of the never-ending debate between European and American attitudes toward the practical use of immunotherapy.

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