
Component-Resolved Diagnosis to Optimize Allergen-Specific Immunotherapy in the Mediterranean Area

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■ Abstract

Allergen-specific immunotherapy (SIT) is the only allergen-specific treatment for allergy. It can prevent progression of the disease and has a long-lasting therapeutic effect. Since SIT is allergen-specific, the identification of the disease-eliciting allergen is an essential prerequisite for the accurate prescription of treatment. Diagnostic tests based on allergen extracts consist of mixtures of various allergens of which some are specific for the allergen source and others occur as cross-reactive allergens in various unrelated allergen sources. It may therefore be difficult and sometimes impossible to identify the disease-causing allergen with such tests, particularly in patients who are sensitized to more than one allergen source. Sensitization to pollens from olive, grasses, and *Parietaria* in the Mediterranean area is frequently treated with SIT. Here, we describe allergen molecules from these sources that can be used for component-resolved diagnosis of allergy to facilitate the selection of patients for SIT and monitor the immunological effects of treatment.

Key words: Component-resolved diagnosis. Mediterranean area. Specific immunotherapy. Pollen allergy. Recombinant allergens.

■ Resumen

La inmunoterapia específica (ITE) es el único tratamiento contra las alergias específico para un alérgeno. Puede evitar el avance de la enfermedad y tiene un efecto terapéutico a largo plazo. Dado que la ITE es específica para un alérgeno, la identificación del que provoca la enfermedad es un requisito esencial para la prescripción de un tratamiento preciso. Las pruebas diagnósticas basadas en extractos de alérgenos consisten en mezclas de varios alérgenos de los cuales algunos son específicos para la fuente alérgica y otros ocurren por efecto de la reactividad cruzada en diversas fuentes alérgicas no relacionadas. Por tanto, puede ser difícil (y a veces imposible) identificar el alérgeno causante de la enfermedad con este tipo de pruebas, particularmente en el caso de los pacientes sensibilizados a más de un alérgeno. La sensibilización a los pólenes de olivo, gramíneas y *Parietaria* en la región mediterránea se trata frecuentemente mediante ITE. En este documento describimos las moléculas del alérgeno de estas fuentes, que se pueden usar para el diagnóstico basado en componentes de la alergia para facilitar la selección de los pacientes para la ITE y para controlar los efectos inmunológicos del tratamiento.

Palabras clave: Diagnóstico basado en componentes. Región mediterránea. Inmunoterapia específica. Alérgenos recombinantes.

Introduction

IgE-mediated allergies affect more than 25% of the world's population [1]. The symptoms of allergy may be mitigated by pharmacological treatment, but only allergen-specific immunotherapy (SIT) is allergen-specific, can prevent disease progression, and has long lasting effects [2-4]. Since the introduction of SIT in 1911, numerous studies have demonstrated its clinical efficacy, but several factors limit its broad application [5]—frequent administration of injections, drops, or tablets; possible side effects; and the need for identification of the disease-eliciting allergen in order to

prescribe the allergy vaccine. The selection of patients for SIT must take into consideration clinical parameters (eg, documentation of a clinically relevant sensitization to a given allergen source, exclusion of contraindications for SIT) and confirmation of the presence of an IgE-mediated allergic sensitization by the demonstration of allergen-specific IgE antibodies [5]. Diagnostic tests based on natural allergen extracts are composed of relatively ill-defined mixtures of non-allergenic materials, major allergens, and cross-reactive allergens [5], with the result that it is difficult and often impossible to precisely identify the disease-eliciting allergen, particularly in patients sensitized to more than

Sequence identities of Ole e 1-related allergens.*

	Ole e 1 (P19963)	Fra e 1 (Q7XAV4)	Syr v 1 (X76541)	Lig v 1 (O82015)	LAT52 (P13447)	Zm 13 (P33050)	Cro s 1 (Q29W25)	Che a 1 (Q8LGR0)	Pla l 1 (P82242)	Phl p 11 (Q8H6L7)	Lol p 11 (Q7M1X5)
Ole e 1	100%	89%	89%	87%	38%	38%	38%	38%	38%	33%	33%
Fra e 1		100%	87%	91%	38%	38%	39%	39%	40%	37%	36%
Syr v 1			100%	84%	39%	37%	38%	38%	37%	33%	33%
Lig v 1				100%	38%	37%	37%	37%	39%	33%	33%
LAT52					100%	36%	45%	46%	37%	40%	42%
Zm 13						100%	39%	39%	32%	44%	45%
Cro s 1							100%	97%	33%	48%	49%
Che a 1								100%	34%	47%	47%
Pla l 1									100%	29%	30%
Phl p 11										100%	94%
Lol p 11											100%

* The sequence identities have been determined for the Ole e 1-related allergens as displayed in the left column and top line of the table and are listed for each match. Ole e 1 indicates olive tree (*Olea europea*); Fra e 1, ash (*Fraxinus excelsior*); Syr v 1, common lilac (*Syringa vulgaris*); Lig v 1, common privet (*Ligustrum vulgare*); LAT52, tomato (*Solanum lycopersicum*); Zm 13, maize (*Zea mais*); Cro s 1, saffron crocus (*Crocus sativus*); Che a 1, white goosefoot (*Chenopodium album*); Pla l 1, plantain (*Plantago lanceolata*); Phl p 11, timothy grass (*Phleum pratense*); Lol p 11, perennial ryegrass (*Lolium perenne*).

one allergen source. In such cases it would be important for the clinician to know whether a patient is co-sensitized to several allergen sources and needs SIT for each, or whether a patient is sensitized to several sources due to sensitization to cross-reactive components in each of the suspected allergen sources (ie, cross-sensitization) [7]. Advances in allergen characterization (molecular techniques to identify allergens and recombinant DNA technology for the production of recombinant allergen molecules) have provided us with new tools, such as recombinant allergens, that can improve the diagnosis of allergy [8].

Component-Resolved Diagnosis

The term component-resolved diagnosis has been coined to designate diagnostic tests based on pure allergen molecules which are either produced by recombinant expression of allergen-encoding cDNAs or by purification from natural allergen sources [6]. These molecules are designated according to the Allergen Nomenclature and the prefix “r” indicates that the molecule is the result of recombinant expression whereas “n” indicates that the molecule has been purified from the allergen source. A detailed list of allergen molecules together with a description of their biological function may be obtained from databases, review articles, and book chapters [reviewed in 9].

Component-resolved diagnostic tests include marker allergens to diagnose the genuine sensitization of patients towards a given allergen source or cross-reactive molecules that point to cross-sensitization to several allergen sources [10-12]. These “gatekeeper” tests enable the accurate prescription of SIT for birch pollen [10, 11], grass pollen [10], house dust mites [13], and cat [14,15], and include marker allergens for important Mediterranean pollen sources, including olive [16] and *Parietaria* [17].

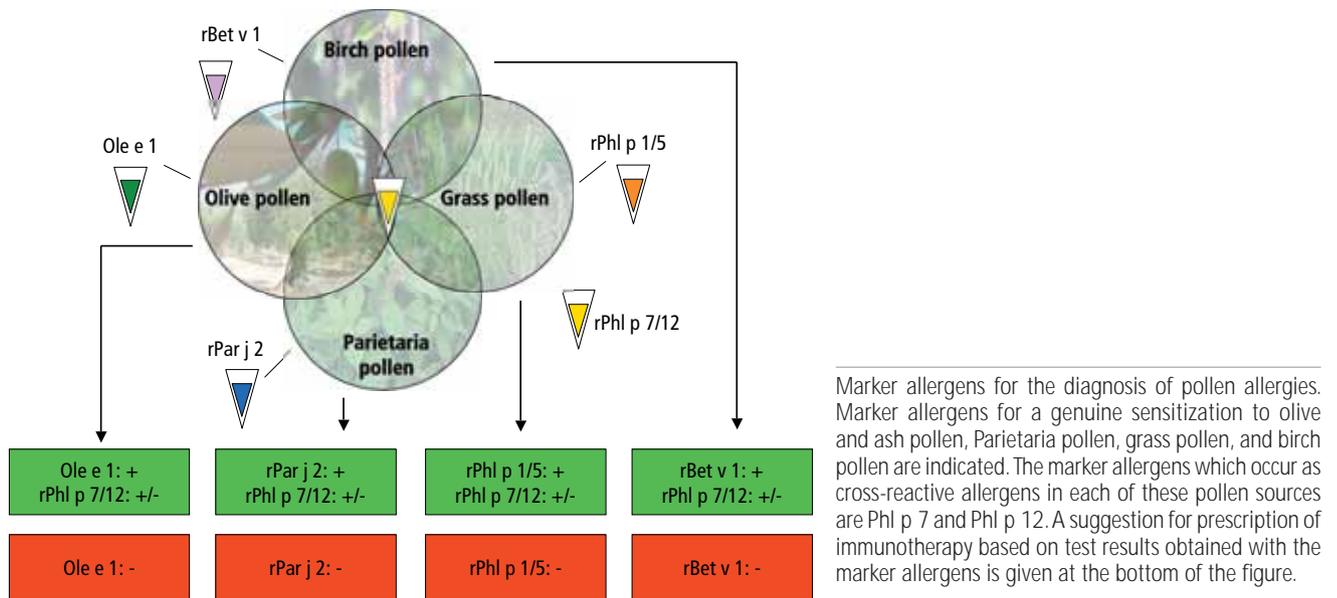
Important Pollen Allergies and Marker Allergens for the Mediterranean Area

Pollen from grasses, olive, weeds, in particular *Parietaria*, cypress, and cedar are important allergen sources in the Mediterranean area. The allergenic components of these allergens have been summarized in review articles and are designated according to the Allergen Nomenclature [reviewed in 18-22]. A continuous update of allergens can be obtained from various databases [23]. SIT for pollen allergies is mainly prescribed for grass, olive, and *Parietaria*, although other pollen allergies can also be treated [5].

cDNAs coding for major pollen allergens have been isolated and recombinant allergens for diagnosis and treatment have been produced [reviewed in 9]. Successful clinical studies have been performed with recombinant allergens and vaccines may soon be available for clinical use. However, SIT still uses allergen extracts in clinical routine. In order to optimize the selection of patients for extract-based treatment and to monitor the effects of treatment on the immune system, component-resolved diagnostic tests have been developed for major allergen sources [10,12].

Optimal Selection of Pollinosis Patients for Immunotherapy in the Mediterranean Area Using Component-Resolved Diagnosis

In northern and central Europe, grasses and birch are the most important pollen allergen sources [11,18]. Diagnostic tests for the selection of patients for SIT with birch and grass pollen allergens use the major birch pollen allergen Bet v 1 and a combination of the two major timothy grass pollen allergens, Phl p 1 and Phl p 5 [10]. Allergens which



can be found as highly cross-reactive allergens in the pollen of birch, grasses, trees, and weeds include calcium-binding allergens with 2 calcium binding domains, known as 2 EF-hand allergens or polcalcins [24]. This group also includes the cytoskeletal protein profilin, which can be found in pollen and in somatic plant tissues and is a cross-reactive allergen in plant-derived food [25-28]. Cross-inhibition studies performed with 2 EF-hand allergens and profilins from various plants have demonstrated extensive cross-reactivities, but most IgE epitopes seem to reside in the grass pollen-derived allergens Phl p 7 and Phl p 12 [29-31]. Therefore, Phl p 7 and Phl p 12 can be used as markers for cross-reactivity between birch, grass, olive, and *Parietaria* pollen. A genuine sensitization to grass pollen can be diagnosed with a test consisting of a combination of the major grass pollen allergens Phl p 1 and Phl p 5. IgE reactivity to Phl p 1 without IgE reactivity to Phl p 5, Phl p 2, and Phl p 6 indicates sensitization to the Bermuda grass subfamily [32]. The major *Parietaria* allergen Par j 2 is a marker allergen for a genuine sensitization to *Parietaria* pollen [17]. It confirms *Parietaria* sensitization and may help to discriminate *Parietaria*-sensitized patients from those who are sensitized to other weed pollens such as mugwort or ragweed [17]. The major olive pollen allergen, Ole e 1, shares extensive sequence similarity with allergens from the *Oleaceae*, including olive, ash, and privet, and there is extensive cross-reactivity between these allergens (table). IgE-reactivity to Ole e 1 can therefore be used to confirm a genuine olive pollen sensitization in the Mediterranean area and helps to identify patients with a genuine sensitization to ash in other areas [16]. Sequence identity between Ole e 1 and the corresponding grass pollen allergens Lo p 11 and Phl p 11 is low and, accordingly, there is no relevant cross-reactivity (table) [33]. Diagnostic testing with the marker allergens Ole e 1, Par j 2, Phl p 1, Phl p 5, and Bet v 1 allows the clinician to diagnose and confirm genuine sensitization to the corresponding allergen sources as a basis for the accurate

prescription of immunotherapy. Lack of or very low IgE reactivity to the genuine marker allergens indicates that the patient is not sensitized to the corresponding allergen sources and is therefore not suitable for specific immunotherapy with an extract of this allergen source (figure). Diagnostic tests containing Ole e 1, Par j 2, and Phl p 1/5 should therefore make it possible to confirm sensitization to olive, *Parietaria*, and grass pollen in the Mediterranean area, and provide more patients with the benefits of specific therapy.

Component-Resolved Diagnosis for Monitoring the Effects of Immunotherapy

The accurate diagnosis of a sensitization is a prerequisite for the correct prescription of SIT. However, the success of SIT depends on other factors such as vaccine quality and degree of immune response to treatment. It is well established that SIT induces allergen-specific IgG responses, but it has been difficult, if not impossible, to measure the development of IgG responses against the major allergens with allergen extracts because they consist of a mixture of allergenic and nonallergenic components. Therefore, it is not possible to use extract-based tests to measure whether the immune system responds to SIT by production of IgG against the major allergens [reviewed in 34]. However, component-resolved diagnosis based on the major allergens now enables us to measure whether the immune system responds to SIT. Several SIT studies performed with allergen extracts and with purified allergens, recombinant allergens, or hypoallergenic recombinant allergen derivatives also suggest that the success of treatment is associated with the development of allergen-specific IgG antibodies [35-45]. The allergen-specific antibodies induced by SIT belong to the IgG1, IgG4, and IgG2 isotypes, whereas only very few, if any, allergen-specific IgA antibodies are induced. Depending on the

quality/dose of the vaccine, allergen-specific IgG antibodies appear after two months of treatment or after prolonged treatment. They can be measured with the marker allergen tests for Ole e 1, Par j 2, Bet v 1, and Phl p 1/5 by comparing their levels in the pretreatment serum sample with those of serum samples obtained during the course of treatment. The clinician can therefore use these tests to determine whether SIT has induced an allergen-specific IgG response and whether the immune system has responded.

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