Amaranthaceae Pollens: Review of an Emerging Allergy in the Mediterranean Area

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Abstract

The Amaranthaceae family is composed of about 180 genera and 2500 species. These common weeds have become increasingly relevant as triggers of allergy in the last few years, as they are able to rapidly colonize salty and arid soils in extensive desert areas. The genera *Chenopodium, Salsola*, and *Amaranthus* are the major sources of pollinosis from the Amaranthaceae family in southern Europe, western United States, and semidesert areas of Saudi Arabia, Kuwait, and Iran. In Spain, *Salsola kali* is one of the most relevant causes of pollinosis, together with olive and grasses.

To date, 9 Amaranthaceae pollen allergens from *Chenopodium album, Salsola kali*, and *Amaranthus retroflexus* have been described and are listed in the International Union of Immunological Societies allergen nomenclature database. The major allergens of Amaranthaceae pollen belong to the pectin methylesterase, Ole e 1–like, and profilin panallergen families, whereas the minor allergens belong to the cobalamin-independent methionine synthase and polcalcin panallergen families. These relevant allergens have been characterized physicochemically, and immunologically at different levels. Recombinant forms, allergenic fusion recombinant proteins, and hypoallergenic derivatives of these allergens have been expressed in bacteria and yeast and compared with their natural proteins from pollen.

In this review, we provide an extensive overview of Amaranthaceae pollen allergens, focusing on their physicochemical, and immunological properties and on their clinical significance in allergic patients. We also review studies where these recombinant allergens and their hypoallergenic derivatives have been used in clinical diagnosis and their potential use in personalized therapy.

Key words: Pollinosis. Amaranthaceae. Recombinant allergen. Cross-reactivity.

Resumen

La familia Amaranthaceae se compone de alrededor de 180 géneros y 2500 especies vegetales. En los últimos años, el polen de estas malezas está adquiriendo una relevancia cada vez mayor como inductor de alergia, ya que estas plantas son capaces de colonizar rápidamente los suelos salinos y áridos de zonas desertificadas.

El polen de los géneros *Chenopodium, Salsola* y *Amaranthus* es el causante del mayor número de casos de polinosis asociados a la familia Amaranthaceae en países del sur de Europa, oeste de Estados Unidos, y en las zonas semi-desérticas de Arabia Saudí, Kuwait o Irán. En España, el polen de *Salsola kali* es una de las causas más relevantes de polinosis junto con los pólenes de olivo y gramíneas.

Hasta la fecha, se han descrito un total de nueve alérgenos del polen de *Chenopodium album, Salsola kali* y *Amaranthus retroflexus*, los cuales se han depositado en la base de datos de nomenclatura de alérgenos IUIS. Los alérgenos principales del polen de la familia Amaranthaceae pertenecen a las familias pectin metilesterasa, Ole e 1, o a la familia de panalérgenos -profilina-, mientras que los alérgenos secundarios descritos pertenecen a la familia de panálergenos -polcalcina-, o bien, corresponden a la metionina sintasa independiente de cobalamina. Estos relevantes alérgenos se han caracterizado fisicoquímica e inmunológicamente en mayor o menor profundidad. Las formas recombinantes, y sus variantes recombinantes o derivados hipoalergénicos fusionados a un tag, se han expresado en bacteria o levadura y se ha comparado su funcionalidad con sus correspondientes homólogos naturales presentes en el polen.

En esta revisión, ofrecemos una extensa descripción de los alérgenos del polen de la familia Amaranthaceae, centrándonos en sus propiedades físico-químicas e inmunológicas, y en su importancia clínica en los pacientes alérgicos. Por otra parte, también hemos revisado aquellos estudios en donde se han utilizado estos alérgenos recombinantes y sus derivados hipoalergénicos en el diagnóstico clínico, o bien, en donde se describe su potencial uso en la terapia personalizada.

Palabras clave: Polinosis. Amaranthaceae. Alérgeno recombinante. Reactividad cruzada.

1. Introduction

Weeds are difficult to classify in a specific botanical group or family. They are generally of little commercial or ornamental value and are known for their undesirable effect of invading cultivated environments and thus affecting the growth of other species [1]. Weeds are highly adaptive, especially in regions where the autochthonous vegetation has been damaged by drought and other adverse weather conditions. The high stability of weeds in unfavorable situations makes them major sources of pollens that cause allergic manifestations in increasing numbers of people [2-4].

The Amaranthaceae family has recently been extended to include the Chenopodiaceae family based on morphological and phylogenetic analyses [5]. Most of the members are annual and perennial plants whose growth is perfectly adapted to salty soils and arid environments, from which they draw high nitrogen concentrations. Although the family includes species of agricultural interest, such as *Spinacia oleracea* and the now popular *Chenopodium quinoa* and ornamental plants such as *Amaranthus caudatus*, a number of species are considered invasive [6,7]. The ability of these species to survive in hostile habitats does have advantages, namely, abundant seed production (often reaching 50 000 seeds per plant) and a fruit-dispersal mechanism that involves the whole plant (eg, tumbleweed). The tolerance of some species to toxic substances such as arsenic with no effect on growth contributes to their use as fixatives in highly polluted soils [8].

Members of the Amaranthaceae family share a number of morphological features in their pollen grains that make them indistinguishable using standard microscopy: the grains are spherical with a polypantoporate aperture arrangement that is similar to the surface of a golf ball [9-11]. Pollen production and release tend to be even lower than in other anemophilous species [12], thus reflecting optimal use of resources by xerophytic plants in adverse environments. Perhaps in an attempt to counteract this low pollen production, Amaranthaceae bloom from June to October [13], once the main pollination period of most species in temperate latitudes has finished [14].

Of all the species in the Amaranthaceae family, the genera *Chenopodium*, *Salsola*, and *Amaranthus* are the main triggers of summer allergy in countries with a temperate and dry climate, such as western United States and Australia and semidesert areas of Saudi Arabia, Kuwait, India, and Iran [15-18]. Desertification in extensive areas of the Mediterranean basin has increased the incidence of these weeds, causing symptoms in sensitized individuals, even at low concentrations [19-23]. The ability of Amaranthaceae weeds to resist drought, the ornamental use of several species in hedges and as irrigated crops, as well as their presence as contaminant weeds in cultivars at the expense of productive species such as olive tree, have increased their clinical relevance in the Mediterranean area. Since Mediterranean countries make up one of the world's largest surface areas of olive groves and olive pollen is the major local cause of allergy [24], an increase in polysensitizations to these pollen types is expected [25]. Different taxonomically related species of Amaranthaceae can induce allergic sensitization and could be used in diagnosis and treatment.

From the allergological point of view, *Salsola kali* (also known as Russian thistle) and *Chenopodium album* (also known as lamb's quarters) are considered the most representative pollens of the family in terms of incidence of allergy in Spain [26]. However, other species of the Amaranthaceae family, such as *Salsola pestifer*, *Amaranthus retroflexus*, and *Salsola oppositifolia*, have also been reported as allergenic species [27,28]. *S kali* is highly abundant in the southeast of Spain. *Salsola vermiculata* grows in swamps and marshy areas exposed to the sun, and *Salsola oppositifolia* can be found at roadsides near the sea in dry and hot areas. To date, the major allergens have been described and analyzed only in *S kali, C album,* and *A retroflexus*. A high degree of IgE cross-reactivity between allergens of the Amaranthaceae family has been reported since more than 2 decades ago [29].

The high number of allergenic molecules identified in these and other weed pollens (eg, ragweed, mugwort, pellitory, and annual mercury) are restricted to a few main families of proteins: enzymes, especially those involved in pectin metabolism (eg, pectate lyases, pectin methylesterases, and polygalacturonidases), defensin-like proteins, Ole e 1–like proteins, the nonspecific lipid transfer proteins, and the panallergens profilin and polcalcin [3]. These families include the allergens detected in *C album* (Che a 1, Che a 2, and Che a 3), *S kali* (Sal k 1, Sal k 3, Sal k 4, and Sal k 5), and *A retroflexus* (Ama r 2) [17,30-48].

From a clinical perspective, and considering the dramatic rise in Amaranthaceae pollinosis in temperate countries, the qualitative and quantitative differences in allergenic content between extracts of various species, and the inaccurate diagnosis of patients due to the overlapping of allergenic weed pollens [28], Amaranthaceae pollens are expected to play an increasingly important role in the allergic response. Consequently, Amaranthaceae are expected to become one of the primary causes of pollen sensitization in the Mediterranean areas, as is the case in some areas of the Iberian Peninsula [23,49].

2. Allergens of the Amaranthaceae Family

Knowledge of the allergenic profile of a biological source is a prerequisite for improved diagnostic protocols and accurate immunotherapy. This knowledge is particularly important in the case of pollens, since the geographical distribution of plant species is strongly influenced by geophysical and climatic conditions. The use of purified allergens rather than currently available extracts has made it easier to identify the sensitizing allergen. The enormous efforts made in past decades to obtain the most significant allergens of the different pollen species have been essential for our understanding of the course to be followed by a given patient. Nine Amaranthaceae pollen allergens have been described to date (Table).

The Amaranthaceae family is composed of about 180 genera and 2500 species. In Spain, these common weeds are increasingly relevant as triggers of allergy and are especially abundant in several regions of the southeast and the Ebro Valley. In the dry areas of the southeast (provinces of Alicante

Table 1. Allergens From the Amaranthaceae Family That Have Been Identified, Isolated, Characterized, and Expressed as Recombinant Proteins

Species	Allergen	Accession Number[a]	Protein family	Natural Protein[b]	Source of Recombinant Protein Expression	Reference[c]
Chenopodium album						
	Che a 1	Q8LGR0	Ole e 1–like	[37]	*Pichia pastoris*	[39,47]
	Che a 2	Q84V37	Profilin	[36,38]	*Escherichia coli*	[30,40]
	Che a 3	Q84V36	Polcalcin	[36,38]	*Escherichia coli*	[42,48
Salsola kali						
	Sal k 1	Q17ST4, P83181	Pectin methylesterase	[35]	*Escherichia coli*	[34]
	Sal k 2	Q8L5K9	Protein kinase homologue	Not isolated	*Escherichia coli*	
	Sal k 3	C1KEU0	Cobalamin-independent methionine synthase	Not isolated	*Escherichia coli*	[33]
	Sal k 4	C6JWH0	Profilin	[43]	*Escherichia coli*	[32,43]
	Sal k 5	E2D0Z0	Ole e 1–like	[41]	*Pichia pastoris*	[41
Amaranthus retroflexus						
	Ama r 2	C3W2Q7	Profilin	Not isolated	*Escherichia coli*	[46]

[a]Accession number as it appears in the UniProt database.
[b]Reference for isolation of the natural allergenic protein from pollen.
[c]Reference for expression of the allergen in a heterologous system.

and Murcia), levels of Amaranthaceae pollen range between 100 and 250 grains/m^3 from April to October, with a peak in August (www.polenes.com). Although these weeds release reduced numbers of pollen grains during the pollination season, around 40% of allergic individuals with symptoms at the end of summer are allergic to these aggressive pollens. In Spain, *Salsola* pollen, together with olive and grasses, is one of the main causes of pollinosis [26], both in regions where it is a major cause of sensitization (eg, Zaragoza and Murcia) and in regions where *S kali* grows wild in olive tree cultivars [50]. *Salsola* pollen has also been extensively associated with a high degree of IgE cross-reactivity between members of the Amaranthaceae family for decades [29].

In the Mediterranean area, the most relevant members of Amaranthaceae are *S kali* and *C album* [26,50]. Indeed, in southeastern and central areas of Spain, *S kali* pollen has been reported to produce pollinosis at almost the same level as olive and grass pollen [26,50,51].

2.1 Chenopodium

Chenopod pollen is one of the best-characterized allergenic pollens of the Amaranthaceae family. Chenopod (*C album*) is a perennial plant. Although it can be found in all kinds of soils, it is mostly observed in arid and salt-rich soils [28,52]. Chenopod pollination takes place from June to October.

Areas of high clinical incidence have been reported in the south of Spain, western USA, Iran, Kuwait, and Saudi Arabia [22,26,28,44,52-55]. In countries with large areas of desert, such as Kuwait or Iran, chenopod pollens are the main sensitizing agents in patients with allergic rhinitis or asthma, and prevalence values are higher than those for dusts or molds owing to the use of this plant in greening programs [53-57].

Interestingly, polysensitization is usually observed in patients affected by chenopod pollinosis, and several authors have demonstrated pronounced cross-reactivity with related and nonrelated pollen extracts [29,58].

The 3 major allergens that have been described to date in *C album* pollen are Che a 1 (Ole e 1–like protein), Che a 2 (profilin), and Che a 3 (polcalcin) [36-38,44,53], which have been expressed in their recombinant form in yeast or bacteria [39,42,44,47,48,53] and were recently used for the diagnosis of *C album* allergy [44].

2.1.1 Che a 1

The first major allergen of chenopod pollen, Che a 1, was identified, characterized, and cloned in 2002 [37]. Using sera of olive- and chenopod-allergic patients and Ole e 1–specific rabbit antibodies, Barderas et al [37] identified an Ole e 1–like allergen of about 20 kDa using SDS-PAGE. Che a 1 was isolated from pollen and shown to be a glycoprotein of 17 088 Da and 143 amino acid residues in length. After cloning, its amino acid sequence exhibited 27%-45% identity with the sequences of several members of the Ole e 1–like protein family and only 30% identity with Ole e 1, thus explaining the limited cross-reactivity between both molecules [37]. Remarkably, using a population of patients sensitized to chenopod and other pollens, the authors demonstrated that more than 70% had IgE against this molecule.

The same authors subsequently produced Che a 1 as a recombinant allergen in *Pichia pastoris* yeast [39]. Although the glycosylation pattern of recombinant Che a 1 differed from that of the natural protein, equivalent results were found for recognition of both natural and recombinant proteins by IgE [39]. More recently, expression of Che a 1 fused to a histidine tag has been reported in *Escherichia coli* [47]. Although the protein was isolated from inclusion bodies, on-column refolding of the recombinant protein enabled the authors to obtain a soluble protein able to bind IgE from

chenopod pollen–sensitized patients by either Western blotting or ELISA [47].

Unlike Ole e 1 and its recombinant form, the nonsignificant contribution of the glycan moiety to IgE recognition was shown in Che a 1 through the use of recombinant proteins expressed in both bacteria and yeast. The IgE binding properties of these proteins seem to be similar to those of nChe a 1 [39,47], in that the former does not contain any glycan and the latter has a different glycosylation pattern.

2.1.2 Che a 2

In 2003, it was suggested that the panallergens Che a 2 (profilin) and Che a 3 (polcalcin) played an important role in allergy to *C album* pollen [36]. This role was confirmed when the relevance of profilin and polcalcin in the allergenic properties of *C album* was also shown to be associated with polysensitization in chenopod pollen–sensitized patients [36,38].

In 2004, both panallergens were further studied using 104 sera from individuals with *C album* allergy [38]. Natural Che a 2 was purified to homogeneity from *C album* pollen extract and used to investigate the relevance of profilin in chenopod allergy. Indeed, *C album* profilin was reported to be a major allergen in *C album* allergy, since 55% of the 104 sera tested showed positive IgE responses by ELISA [38]. A similar finding was observed in 2011 by Nouri et al [44], who showed that Che a 2 was a major allergen.

Barderas et al [38] reported the cDNA-encoding sequence of Che a 2 and investigated its amino acid sequence identity with other allergenic profilins deposited in databases. The 3 cDNA clones coding for Che a 2 ranged between 131 and 133 amino acids in length for a molecular mass of about 14 kDa and an acidic isoelectric point (pI) of about 5 [38]. Remarkably, Che a 2 showed higher amino acid sequence identity with Hev b 8 (the profilin from latex) and plant-derived food profilins (Mal d 4 [apple] or Ara h 5 [peanut]) than with pollen profilins (Ole e 2 [olive], Bet v 2 [birch], and Phl p 12 [grass]) [38].

Later in the same year, the heterologous expression of Che a 2 as a nonfusion protein was reported in *E coli* [40]. Recombinant Che a 2 was produced as a soluble protein, purified to homogeneity, and characterized in detail using antiprofilin polyclonal antiserum and sera from *C album*–pollen allergic patients. rChe a 2 showed similar immunological properties to those of the natural allergen isolated from pollen, as determined by immunoblotting, ELISA, and inhibition experiments [40].

The cross-reactivity of Che a 2 was investigated in detail using ELISA inhibition experiments with the recombinant form and inhibitors (pollens, plant-derived foods, and latex extracts) [40]. Inhibition levels higher than 80% were reported with pollen and latex allergens and between 10% and 95% with plant-derived food extracts, thus further demonstrating the importance of Che a 2 in cross-reactivity [36,38,40].

2.1.3 Che a 3

The third panallergen from *C album* pollen is the polcalcin Che a 3 [36,38]. In the report by Barderas et al [38], Che a 3, which is a 2-EF-hand calcium-binding protein, was also addressed in detail. Che a 3 was isolated from pollen and shown to react with 46% of 104 *C album*–sensitized patients by ELISA [38]. Interestingly, similar recognition of IgE binding (41%) was observed in Iranian sensitized patients [44].

The cDNA-encoding sequence of Che a 3 was reported by Barderas et al [38] in 2004, and its amino acid sequence identity was compared to that of other reported polcalcins. The Che a 3 cDNA clone was 86 amino acids in length for a theoretical molecular mass of 9.5 kDa and a pI of 4.43 [38]. Remarkably, Che a 3 had an amino acid sequence similarity of up to 90% with Bet v 4 (birch polcalcin), and 89% with Ole e 3 and Aln g 4, polcalcins from olive and alder pollen, respectively, thus indicating the highly potent cross-reactivity of Che a 3 with other polcalcins [36,38].

The ability of Che a 3 to cross-react was investigated in detail using its recombinant form produced as a nonfusion protein in *E coli* [42,48]. After purification to homogeneity, Che a 3 was able to inhibit up to 50% of IgE binding to grass pollen polcalcin (Phl p 7), while Phl p 7 inhibited 76% of the IgE binding to immobilized Che a 3 [42]. The 3D structure of Che a 3 was solved by x-ray crystallography [48]. The comparison of the Che a 3 structure with that of polcalcin allergens from grass pollen (Phl p 7) and birch pollen (Bet v 4) showed that all 3 allergens displayed almost equivalent folding but different oligomeric behavior [48]. Che a 3 and Phl p 7 are structured as dimers, while Bet v 4 is found mainly as a monomer [48].

2.2 Salsola

Salsola is the genus from the Amaranthaceae family whose allergenic properties have been best characterized. *Salsola* comprises several allergenic species, including *S kali*, *S pestifer*, *S vermiculata*, and *Salsola soda* [16,59-62].

The only species whose allergens have been identified is *S kali* [31-35,41,43, 59]. The other allergenic species have barely been characterized using immunochemical assays, and their IgE-reactive bands are grouped according to molecular mass. As with most Amaranthaceae weeds, *S kali* is a typical plant of salty soils and is found mainly in dry habitats [63]. *S kali* is a summer-flowering, annual weed, which matures to form spherical bushes (tumbleweeds).

The first cases of sensitization to *S kali* pollen were reported in Arizona, USA [64]. Since then, multiple sensitizations have been described worldwide, as the plant is found extensively in dry soils. In the USA, *S kali* can be found from the northeast to the west, and in Europe, from the Baltic coast to the Mediterranean coast. It is also observed in North Africa and some Arab countries [26,52]. In Spain, *S kali* is very common in Aragón, Andalucía, Murcia, Levante, and Zaragoza (the area with the second highest sensitization rate) [65]. Indeed, in some southeastern and central areas of Spain, sensitization to *S kali* is almost as frequent as sensitization to olive and grass [26,50,51]. In addition, *S kali* constitutes the main cause of pollinosis in Iran [60].

Extensive cross-reactivity has been reported between *S kali* pollen and Amaranthaceae pollens and other, nonrelated pollen extracts [29,58]. Interestingly, in contrast with *C album*–sensitized patients, most of whom are polysensitized, monosensitization to *S kali* is common [65].

The allergens present in *S kali* pollen have been studied extensively in recent years. Despite the complex allergogram of the pollen protein extract (Figure 1), only 5 allergens have been identified and characterized to date, and 4 have been produced as recombinant proteins [31-35,41,43].

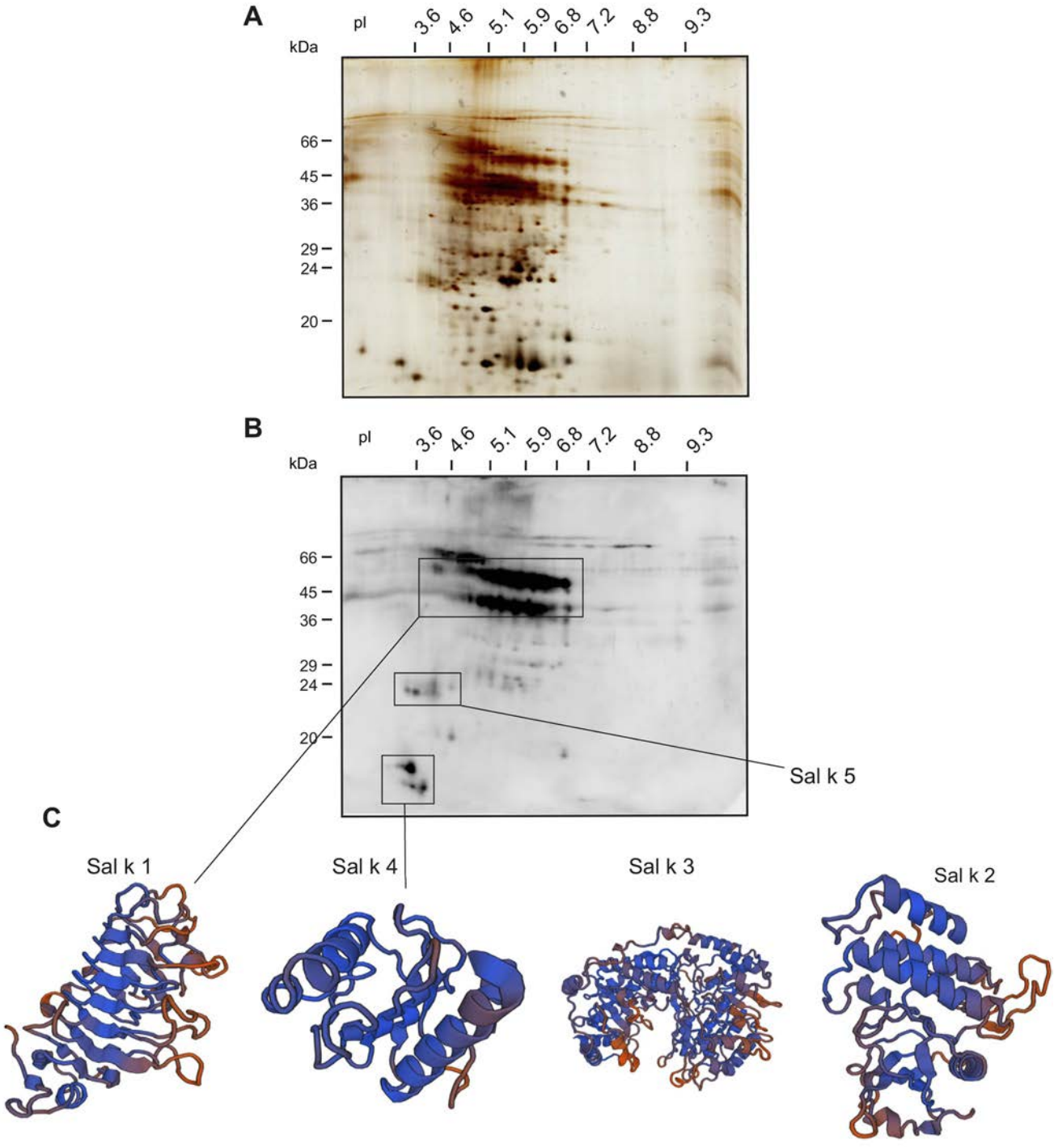

Figure 1. Allergogram of *Salsola kali* pollen protein extract. A, Protein profile of the *S kali* pollen extract after 2D electrophoresis and staining with silver. B, IgE reactive bands using sera from *S kali* pollen–sensitized patients. IgE-reactive spots corresponding to Sal k 1, Sal k 4, and Sal k 5 can be identified in the figure. C, 3D modeling of the allergens Sal k 1, Sal k 2, Sal k 3, and Sal k 4 from *S kali* pollen. The amino acid sequences of these allergens are deposited in the protein data bank and are homologous with previously defined 3D structures. The models were obtained from the Swiss PdbViewer and visualized with PV-JavaScript Protein Viewer. pI indicates isoelectric point.

2.2.1 Sal k 1

Sal k 1 was the first allergen identified from *S kali* [35,59]. It is a very polymorphic protein with a molecular mass of around 37 kDa and more than 20 isoforms with isoelectric points ranging from pI 4 to 9.5. Sal k 1 belongs to the pectin methylesterase protein family [35,59] and can be considered a major allergen, since more than 50% of sensitized patients are allergic to it [34,35,59]. Sal k 1 is responsible for up to 80% of cases of sensitization to *S kali*. Indeed, Sal k 1 was proposed as the marker of sensitization to *S kali* pollen, since it was shown to be the main reason for the differences in sensitization to *S kali* and *C album* pollen [35].

Expression of Sal k 1 in *E coli* was reported in 2010 [34]. The recombinant protein was produced as a soluble protein tagged with thioredoxin to increase the solubility of recombinant fusion and with histidine to ease purification [34]. Immunoblotting, ELISA, and inhibition experiments using sera from *S kali*–sensitized patients showed that the fusion protein was able to bind specific IgE to the same extent as the natural molecule isolated from pollen [34]. In the same report, the authors described a low IgE-binding variant of Sal k 1 modified by mutation of predicted B-cell epitopes and produced using the same approach [34]. Indeed, the rSal k 1 mutant exhibited reduced IgE-binding capacity against the wild-type rSal k 1 both in vitro and in vivo. Accordingly, the authors suggested using this molecule for immunotherapy. Barderas et al [35], who first cloned the major allergen of *S kali* pollen, recently produced Sal k 1 in bacteria and yeast (personal communication) and demonstrated the scant involvement of the glycan moiety in sensitization to Sal k 1. The authors also demonstrated the potential use of recombinant Sal k 1 in clinical diagnosis, since it spans all the immunological properties of the most representative isoforms in the natural pollen protein.

2.2.2 Sal k 2

The nucleotide sequence of Sal k 2 was deposited in the EMBL/GenBank/DDBJ databases (accession number: Q8L5K9) in 2002. Sal k 2 was identified as a protein kinase homologue because of its conserved catalytic core. The protein is composed of 320 amino acids and has a molecular mass of about 36 kDa. However, other than the reported sequence, no additional data on Sal k 2 or its allergenic relevance are available.

2.2.3 Sal k 3

Sal k 3 was first identified in 2011 by Assarehzadegan et al [33] through fractionation of *S kali* pollen extract by SDS-PAGE and IgE-immunoblotting. The authors confirmed the identification using mass spectrometry. The 45-kDa protein was identified as a fragment of a cobalamin-independent methionine synthase of about 85 kDa [33]. The authors produced the recombinant allergen in the 2 fragments of the wild-type protein according to the fragment of the 85-kDa protein in the 45- and 40-kDa protein subunits. The recombinant proteins were fused first to a thioredoxin tag and then to a histidine tag [33]. After extensive purification of the recombinant protein, Sal k 3 was characterized by IgE-immunoblotting, inhibition assays, and skin prick tests. Both fragments of Sal k 3 possessed IgE-binding ability and were able to inhibit the natural protein of the pollen extract [33].

2.2.4 Sal k 4

Sal k 4, the profilin from *S kali* pollen was first characterized in 2010 by Arilla et al [32], who cloned 2 different isoforms of Sal k 4 and expressed 1 of them fused to thioredoxin and histidine tags. The authors also showed the high IgE cross-reactivity of *S kali* profilin with other profilins from chenopod and olive pollen [32].

A new report on *S kali* profilins published in 2011 [43] revealed the presence of a low IgE-binding profilin isoform among isoforms identified by 2D electrophoresis. Two different isoforms were cloned and expressed as nonfusion proteins in bacteria. Modeling the 3D structure of both isoforms and their different IgE-binding ability (which suggested a hypoallergenic character for 1 of them) and the findings of previous epitope mapping studies enabled the authors to propose the amino acid residues involved in the loss of IgE-binding ability. Of particular interest, the authors produced a hypoallergenic derivative identified from *S kali* pollen, which might be a good candidate for desensitization protocols using cocktails of recombinant allergens [43], as in the clinical diagnosis of sensitization to *C album* pollen [44,53].

2.2.5 Sal k 5

In 2009, an Ole e 1–like protein, Sal k 5, was detected in the pollen of *S kali*. Moreover, a high level of IgE cross-reactivity was detected between Sal k 5 and its homologue in *C album* pollen, Che a 1, although cross-reactivity with Ole e 1 was very low.

Data on the identification and characterization of Sal k 5 were reported by Castro et al [41] at the end of 2013, when the authors also published their report on the recombinant expression of the protein in *P pastoris* yeast. The purified pollen allergen was a glycoprotein measuring 151 amino acid residues in length for a molecular mass of about 17 kDa. Its amino acid sequence exhibits 68% and 32% identity with Che a 1 and Ole e 1, respectively. The frequency of sensitization to Sal k 5 was between 30% and 40% based on 2 different populations of *S kali*–sensitized patients in the center and on the east coast of Spain [41]. Moreover, the recombinant protein was shown to behave similarly to the natural pollen protein in terms of its physicochemical, and immunological properties [41].

2.3 Amaranthus

The genus *Amaranthus* is found worldwide as short-lived perennial plants. *Amaranthus* comprises about 60 species with inflorescences and foliage ranging in color from purple and red to green and gold.

Amaranthus mainly contains weeds, some of which are used as ornamental plants. Others are highly valued as leaf vegetables or cereals. There are several reports on the allergenic potential of *Amaranthus* pollen [27,29,58,65,66], and the amaranth grain (*Amaranthus paniculatus*) has been reported to produce food allergy [67].

The pollen of *A retroflexus*, *Amaranthus viridis*, and *Amaranthus spinosis* has been described as allergenic [27,66],

although the only pollen characterized through identification of allergenic proteins is *A retroflexus* [46]. In fact, this weed is a major trigger of allergic reactions in Iran, where about 69% of allergic patients are sensitized. Cross-reactivity with several species of the Amaranthaceae family has been widely confirmed using different immunological techniques [22,27,29,58,65].

2.3.1 Ama r 2

The protein profile of crude *A retroflexus* extract showed at least 9 IgE-reactive bands ranging from 10 kDa to 70 kDa, with main bands at 10, 15, 18, 25, 39, 45, 50, 66, and 85 kDa [46].

The profilin Ama r 2, which is the only allergen of *A retroflexus* pollen detected to date, was first reported in 2011 [46]. Ama r 2 was detected by immunoblotting and its sequence was cloned and then expressed as a soluble His-tagged protein in *E coli* [46]. Four of 12 *A retroflexus*–sensitized patients (33%) had sIgE against Ama r 2 [46]. The authors also showed that recombinant Ama r 2 was able to induce a wheal in skin prick testing, thus confirming its allergenic potential [46]. Finally, high cross-reactivity of Ama r 2 with profilins from other Amaranthaceae species—*S kali* (Sal k 4) and *C album* (Che a 2)—has been reported [46].

3. Recombinant Allergenic Derivatives for Diagnosis and Therapy of Amaranthaceae Allergy

IgE-mediated diseases are currently one of the most common health problems worldwide. About 25% of the world's population has some type of allergy [68-70], and children are the most affected segment of the population [70,71]. In recent decades, prevalence has increased and is notably higher in developed countries [72].

Amaranthaceae pollen has become one of the most common allergic triggers because climatic and environmental changes have produced extensive desert areas that are rapidly colonized by this family [19-23,26,50,51], thus increasing the risk of sensitization to Amaranthaceae pollen.

Rapid and effective diagnosis of the different sources to which a patient is sensitized is essential if we are to reduce exposure to allergenic molecules, symptoms of allergy, and visits to the emergency department because of allergic reactions. We will also be in a better position to evaluate prognosis. Triggering of allergy symptoms can be avoided with drugs such as corticosteroids and antihistamines. Other drugs that can be administered to relieve symptoms after onset include anticholinergics and decongestants. However, these drugs are palliative. The only currently available treatment that modulates the course of the disease and has a long-lasting therapeutic effect is specific allergen immunotherapy (SIT) [73,74], which consists of subcutaneous or sublingual administration of increasing doses of crude extracts of the natural source until the patient is able to tolerate the allergen. Treatment lasts 3 years or even longer [75].

Biological extracts contain several allergens and nonallergenic molecules. The amounts of the most relevant allergens vary depending on the source material, and major variations in the quality of allergenic extract batches have been observed [76]. Natural extracts are also affected by the low levels of specific allergen components and contamination with other allergens or bacterial components. The advent of recombinant allergens has resolved these issues [77].

During the last 3 decades, hundreds of allergenic molecules have been identified and characterized in depth, and many have been produced as recombinant proteins (Figure 2). Recombinant production of allergens makes it possible to obtain high amounts of protein, thus facilitating characterization of the molecule and application in clinical practice. In most studies, in-depth physicochemical and immunological characterization of allergens has been performed to assess the quality of the recombinant protein and determine its allergenicity [78], thus making it possible to distinguish between relevant allergens and those with IgE-binding ability but minor clinical significance [79-81]. Moreover, the comparison of the recombinant allergen with its natural counterpart has facilitated investigation not only of the properties that make a molecule an allergen, but also the use of allergens in the clinical diagnosis of allergy (eg, component-resolved diagnosis and microarray-based diagnosis) [79-84].

Clinical diagnosis by identification of the components of an allergen to which a patient is sensitized using natural or validated recombinant allergens can optimize desensitization

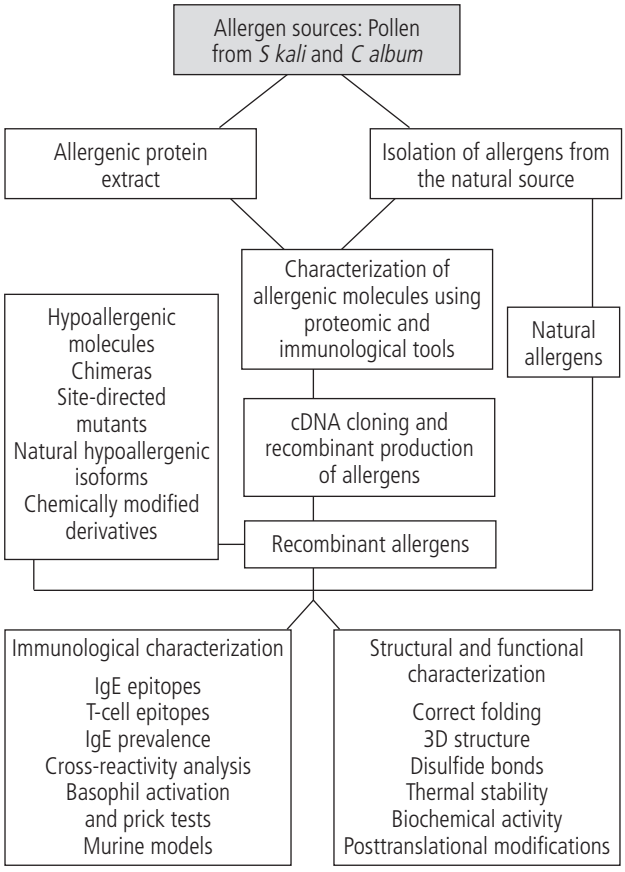

Figure 2. Design and production of recombinant allergenic derivatives with allergenic properties (naïve or altered) and potential use in clinics for diagnosis and therapy of allergic diseases.

protocols based on SIT [79-84]. In this sense, component-resolved diagnosis has become essential in patients whose routine diagnosis is unclear and who therefore cannot receive successful treatment [79-84]. As for Amaranthaceae allergy, Sal k 1 is the only allergen used to date in component-resolved diagnosis by ImmunoCap (Phadia), since it is the sensitization marker of *S kali* pollen. However, its inclusion in component-resolved diagnosis based on Che a 1 or Sal k 5, both of which are markers of Amaranthaceae allergy, will enable firmer diagnoses.

The large body of knowledge on validated recombinant allergens has facilitated the design of molecules with altered allergenic properties. Hypoallergenic derivatives or peptides used instead of unmodified allergens have been boosted to overcome the risk of adverse reactions during desensitization [80]. These derivatives have been proposed as a means of improving SIT by ensuring shorter regimens and enhanced safety profiles. In recent years, several reports have demonstrated the feasibility of these molecules as a replacement for whole allergen extracts in SIT [81-85]. In addition, studies reporting data from clinical trials have demonstrated the feasibility and safety of recombinant allergens and hypoallergenic variants for desensitization by SIT [81,85-90]. In this sense, 2 recombinant allergenic derivatives used as vaccines can induce allergen-specific IgG while reducing allergen-specific IgE in sensitized patients [81,85].

Recombinant hypoallergenic derivatives of Amaranthaceae have also been produced [43,45], and a cocktail of recombinant allergens has proven safe and more accurate for the clinical diagnosis of *C album* sensitization by skin prick test [44,53]. Furthermore, a fusion-based hypoallergenic derivative composed of 3 recombinant allergens (Che a 1, Che a 2, and Che a 3) is now being tested in clinical trials for specific desensitization in patients who are allergic to *C album* [45]. This fusion protein was expressed in bacteria as a soluble His-tagged protein. The recombinant trimeric allergenic molecule was shown to have reduced IgE-binding properties (probably as a consequence of secondary structural alterations) compared with effectiveness of the mixture of the 3 independent recombinant allergens, whose low tendency to aggregate and preserved T-cell activation properties led the authors to consider it a candidate for immunotherapy in patients allergic to *C album* [45].

Remarkably, more and more hypoallergenic derivatives are now ready for preclinical and clinical studies, indicating that in the near future, both clinical diagnosis of components and personalized immunotherapy could be based on recombinant allergens.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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