

The Spectrum of Allergens in Ragweed and Mugwort Pollen

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Abstract

Ragweed and mugwort are important allergenic weeds belonging to the Asteraceae or Compositae plant family. Pollen of mugwort is one of the main causes of allergic reactions in late summer and autumn in Europe and affects about 10–14% of the patients suffering from pollinosis. Ragweed pollen represents the major source of allergenic protein in the United States, with a prevalence of about 50% in atopic individuals. In Europe, ragweed allergy is now rapidly increasing particularly in certain areas in France, Italy, Austria, Hungary, Croatia, and Bulgaria. Amb a 1 and Art v 1, the major allergens of ragweed and mugwort, respectively, are unrelated proteins. Amb a 1 is an acidic 38-kDa nonglycosylated protein. The natural protein undergoes proteolysis during purification and is cleaved into a 26-kDa alpha chain, which associates noncovalently with the beta chain of 12 kDa. The two-chain form seems to be immunologically indistinguishable from the full-length molecule. Art v 1 is a basic glycoprotein comprising two domains: an N-terminal cysteine-rich, defensin-like domain and a C-terminal pro-

line/hydroxyproline-rich module. The proline/hydroxyproline-rich domain was recently shown to contain two types of glycosylation: (1) a large hydroxyproline-linked arabinogalactan composed of a short β 1,6-galactan core substituted by a variable number (5–28) of α -arabinofuranose residues forming branched side chains with 5-, 2,5-, 3,5-, and 2,3,5-substituted arabinoses, and (2) single and adjacent β -arabinofuranoses linked to hydroxyproline. As described for other pollen, ragweed and mugwort pollen also contain the pan-allergen profilin and calcium-binding proteins, which are responsible for extensive cross-reactivity among pollen-sensitized patients.

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Introduction

The Asteraceae or Compositae family is one of the largest families of flowering plants comprising about 1,100 genera and 20,000 species. However, only a few genera are relevant as allergenic sources, the most important being *Ambrosia* (ragweed) and *Artemisia* (mugwort).

Short ragweed or common ragweed (*Ambrosia artemisiifolia*, *Ambrosia elatior*) is the most widespread *Ambro-*

sia species, giant ragweed (*Ambrosia trifida*) being much less abundant. These two species are the main cause of health problems in the sensitive population. Other species (e.g. perennial ragweed, *Ambrosia psilostachya*; silver ragweed, *Ambrosia tenuifolia*; sea ragweed, *Ambrosia maritima*) have never become a serious problem for allergic individuals. Most of the *Ambrosia* species are native to eastern and central North America. In the USA and Canada more than 15 million people suffer from ragweed pollen allergy with a prevalence of about 45% in atopic individuals [1].

Mugwort (*Artemisia vulgaris*) is spread in temperate and humid zones of the northern hemisphere and along the Mediterranean basin. It pollinates at the end of summer and beginning of autumn. In western and central Europe, the main pollination season occurs at the end of July and through August, while in the Mediterranean areas it takes place mostly in September and beginning of October. In central Europe, the incidence of allergic disease caused by the pollen of *A. vulgaris* is between 10 and 14% among pollinosis patients [2].

Several reports demonstrated that ragweed and mugwort pollen share common allergenic structures, i.e. antigens recognized by cross-reactive IgE antibodies in different allergen sources [3–5]. IgE recognition of such common antigens will trigger allergic reactions in sensitized patients after contact with different allergen sources. Although the geographical distribution of ragweed is more restricted to North America, the possibility of co-sensitization and/or cross-reactivity is becoming increasingly relevant for the European population. In this respect, it is interesting to follow the recent spread of ragweed in Europe and the rapid increase of allergic sensitization to ragweed pollen. *A. artemisiifolia* was imported as ballast weed from North America to Europe at the beginning of the last century and was initially limited to Hungary. In the 1990s, the plant migrated rapidly into new areas of central and southern Europe and it is now abundant in the Rhone valley (France), northern Italy, and eastern parts of Austria, Hungary, Croatia and Bulgaria [6]. In these areas, the sensitization of the population to ragweed is constantly increasing. Long-term observation data from Vienna, Austria, showed that among patients with inhalation allergy, positive RAST to ragweed pollen did rise from 20% in the 1980s to about 30% at the end of the 1990s. These findings correlate well with a 3 times elevated annual ragweed pollen load over the same period of time [7].

Ragweed pollen allergy in northern Italy is also a quite recent phenomenon. Although the presence of ragweed

had been reported in the area of Turin since the beginning of the 1980s [8], it was only at the end of that decade that ragweed allergy began its dramatic increase starting from the surroundings of the Malpensa International Airport, some 30 km north of Milan [9, 10]. At the beginning of the 1990s ragweed pollen was not yet cited as a relevant cause of respiratory allergy in Italy [11], but only 5 years later it had become the second most frequent respiratory allergy in the area of Milan [12], and towards the end of the decade also the European Academy of Allergology and Clinical Immunology (EAACI) recognized that ragweed was abundant in northern Italy [6]. A multicenter epidemiological survey of emerging pollinosis in Italy concluded that ragweed pollen caused asthma much more frequently than other pollens [13]. In 1999 the Lombardia Regional Government promoted a public campaign to fight ragweed spread and keep pollen levels low, but the objective was not achieved, as ragweed pollen did not show any significant reduction during the following years, reaching peak levels of 700 pollen grains/m³ [14]. The de novo exposure of the northern Italian population to a novel major airborne allergen resulted in an interesting and somewhat unique phenomenon: the sensitization of many older people lacking any relevant predisposing factor [15]. Moreover, ragweed was responsible (along with birch pollen) for most new sensitizations in patients that were monosensitized to other airborne allergens in the area of Milan [16]. The claimed preventive effect of allergen specific immunotherapy (SIT) on sensitization to other airborne allergens was not confirmed by a recent study, showing that SIT with airborne allergens other than ragweed did not prevent ragweed sensitization [17]. Finally, in a recent work injection SIT with ragweed pollen extract was equally effective in recently sensitized elderly patients as in younger people [18].

Molecular Characterization and IgE Reactivity

Ragweed Allergens

Presently, six groups of ragweed allergens have been characterized: the Amb a 1/Amb a 2 group, Amb a 3/Amb a 7 group, Amb a 5 homologues, Amb a 6, Amb a 8 (ragweed profilin), and the calcium-binding allergen Amb a 9 (fig. 1, table 1).

Amb a 1 (formerly antigen E) is the most important allergen since 95% of ragweed-sensitive individuals react to the protein in skin tests and show high IgE antibody titers. Approximately 13% of the total serum IgE in ragweed-sensitive individuals is specific for Amb a 1. Three

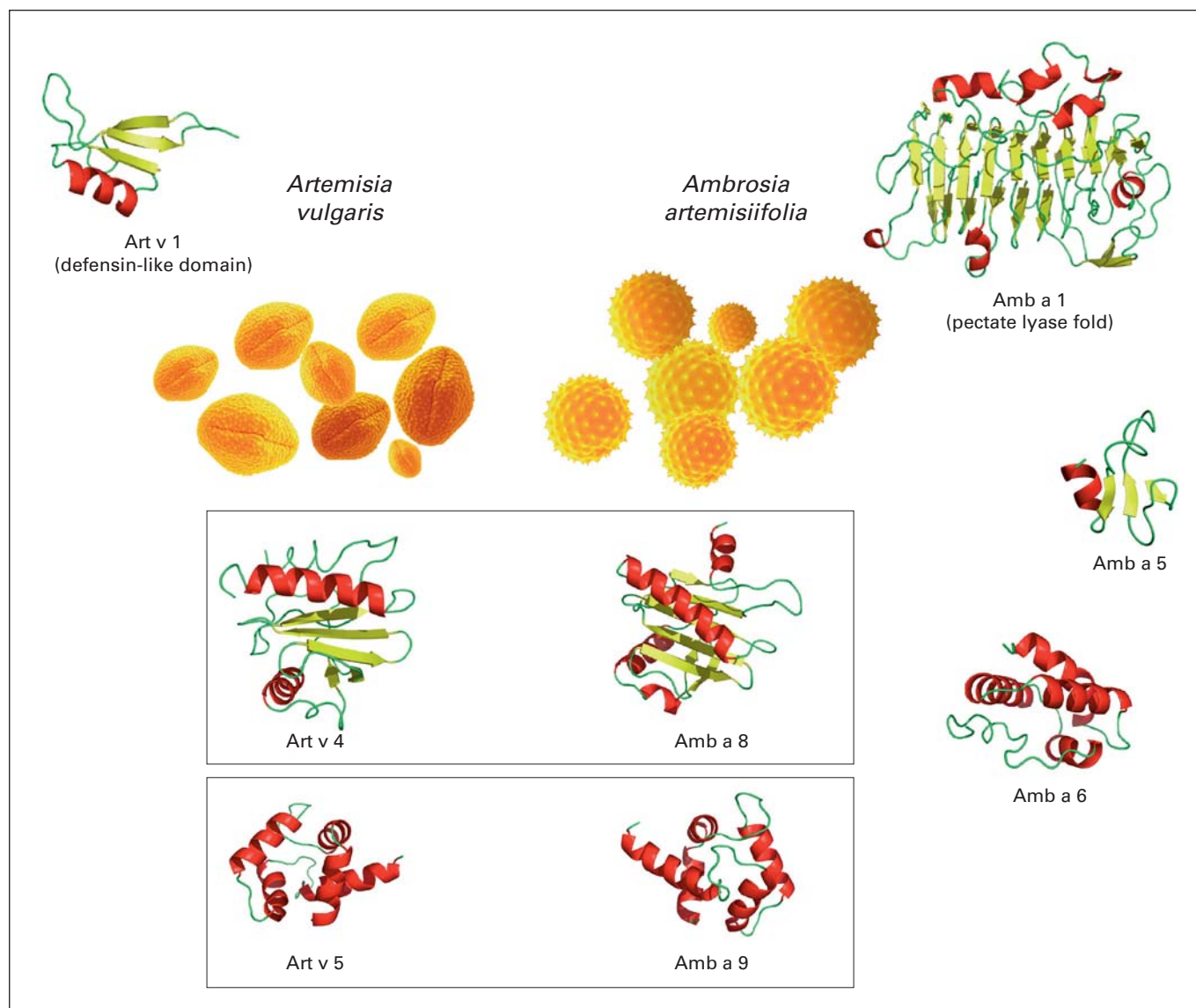


Fig. 1. Three-dimensional structural modeling of ragweed and mugwort pollen allergens. The major mugwort allergen Art v 1 has a defensin-like domain and is unrelated to the major ragweed allergen Amb a 1, which belongs to the pectate lyase family of proteins. 3-dimensional models were generated with the comparative modeling tool 3D-JIGSAW at Expasy (www.expasy.org). The 3D-JIGSAW server builds models based on homologues of known structure. The templates used for model calculations were as follows: Amb a 1, *Juniperus ashei* allergen Jun a 1 (PDB 1PXZ); Amb a 6, mung bean nsLTP (PDB 1SIY); Amb a 8, birch pollen profilin Bet

v 2 (PDB 1CQA); Amb a 9, birch pollen calcium-binding allergen Bet v 4 (PDB 1H4B); Art v 4, latex profilin Hev b 8 (PDB 1G5U); Art v 5, birch pollen calcium-binding allergen Bet v 4 (PDB 1H4B). The defensin domain of Art v 1 was calculated as previously described [36] using the *Aesculus hippocastanum* defensin (PDB 1BK8) as template. The Amb a 5 model was created with the program Modeller 7.7 (www.salilab.org/modeller) using giant ragweed Amb t 5 (PDB 1BBG) as template. Secondary structure elements are displayed in red (helices), yellow (beta sheets), and green (loops and turns).

clones coding for the 398-amino acid Amb a 1 allergen were obtained screening a cDNA library from short ragweed pollen. Sequence comparison showed that they share more than 80% homology, suggesting that Amb a 1

is actually a family of proteins [19]. These three recombinant forms of Amb a 1 (Amb a 1.1, Amb a 1.2, and Amb a 1.3) were expressed in *Escherichia coli* and shown to be specifically recognized by pooled sera of patients allergic

Table 1. Ragweed and mugwort pollen allergens

Species	Allergen	IgE reactivity %	Description/biologic function	MW (SDS-PAGE) kDa	Reference or Accession No.
<i>A. artemisiifolia</i> (short ragweed)	Amb a 1	>90	Pectate lyase family	38	19, 20
	Amb a 2	70	65% identity with Amb a 1	38	20, 55
	Amb a 3	30–50	N-terminal 101 aa sequence	11	24
	Amb a 5	10–20	minor allergen	5	28, 29
	Amb a 6	20–35	nsLTP family	10	33, 34
	Amb a 7	20	N-terminal 38 aa sequence	12	26
	Amb a 8	35	profilin	14	AY894660
	Amb a 9	10–15	polcalcin, Bet v 4 homologue	9	AY894657
<i>A. vulgaris</i> (mugwort)	Art v 1	95	PR-12 protein, defensin-like	24–28	36
	Art v 2	33	N-terminal 30 aa sequence	35	41, 42
	Art v 3	36–40	N-terminal 37 aa sequence, nsLTP family	9.7	43
	Art v 4	36	profilin	14	44
	Art v 5	10–15	polcalcin, Bet v 4 homologue	9	AY904434
	Art v 6	20–26	Pectate lyase family, Amb a 1 homologue	42	AY904433

polcalcin = Pollen-specific 2-EF-hand calcium-binding allergen; aa = amino acid; MW = molecular weight.

to ragweed. A small proportion of the IgE of the patients exclusively reacted with recombinant Amb a 1.1, whereas the IgE of most patients reacted with Amb a 1.1 as well as Amb a 1.2 and Amb a 1.3 proteins. Another family member of Amb a 1 termed Amb a 1.4 was identified, which appears to be only a minor component of the Amb a 1 family [20].

At the protein level, Amb a 1 is highly abundant comprising about 6% of the total protein in neutral aqueous extracts of ragweed pollen. Amb a 1 is an acidic (pI 4–6), nonglycosylated single-chain protein with a molecular weight of approximately 38 kDa. It undergoes proteolysis during chromatographic purification and is cleaved into an alpha chain noncovalently associated with a beta chain of 26 and 12 kDa, respectively. The two-chain form seems to be indistinguishable from the intact molecule concerning its allergenic and antigenic properties. However, it was demonstrated that other chemical modifications of the protein, including reduction and alkylation of disulfide bonds, urea denaturation and renaturation, or succinylation of lysine residues, reduce the IgE reactivity of the molecule [21]. Conjugation of purified natural Amb a 1 with synthetic oligodeoxynucleotides (ODN) containing the hexameric consensus sequence for immunostimulatory sequence (ISS) motifs (central CpG dinucleotide flanked by two purines at the 5' end and by two pyrimidines at the 3' end) also led to reduced IgE reactivity [22].

An average number of four ODN molecules were conjugated to a single Amb a 1 molecule. The Amb a 1-ISS conjugate induced high titers of anti-Amb a 1 IgG antibodies in rabbits and monkeys. While the Amb a 1-ISS conjugate was less allergenic than Amb a 1 alone, as shown by a 30-fold lower histamine release from human basophils of ragweed-allergic patients, mixing of ISS-ODN with Amb a 1 did not cause a reduction of binding to IgE [23].

The complete amino acid sequence of the minor ragweed pollen allergen Amb a 3 (formerly Ra3) has been determined to consist of 101 amino acids and was shown to be a basic glycoprotein containing about 8% carbohydrates. Amb a 3 belongs to the plastocyanin family, which are copper-containing plant proteins involved in electron transport [24]. About 30–50% of ragweed-sensitive individuals were shown to react to Amb a 3 [25].

Amb a 7 (formerly Ra7) was identified as a highly basic, blue protein and was also postulated to be a plastocyanin. About 15–20% of ragweed-allergic individuals were found to have IgE antibodies reacting to Amb a 7 [26]. Although only the N-terminal sequence is available for Amb a 7, both Amb a 3 and Amb a 7 should be considered as isoallergens belonging to the same group of basic glycoproteins with a plastocyanin domain (table 1).

Amb a 5 (formerly Ra5) is a low-molecular-weight (5 kDa) basic protein of short ragweed pollen, 45 amino acids long and containing four disulfide bonds. Two isoforms of Amb a 5 were purified and characterized from ragweed pollen; these two forms were antigenically indistinguishable when using animal antisera. However, small differences in binding to Amb a 5a and Amb a 5b isoforms were observed in 10–15% of the patients reacting with Amb a 5 [27]. The obtained cDNA sequence corresponded to the published sequence of the protein, except that it encodes an extra 10 amino acids at the C-terminus [28]. The 55-residue protein is thought to be enzymatically cleaved at the C-terminal lysine giving rise to isoform a. Isoform b cDNA has a stop codon at the lysine site and is therefore only 44 amino acids long. Nuclear magnetic resonance (NMR) experiments of Amb a 5 revealed that the protein comprises a C-terminal alpha helix, a small segment of antiparallel beta sheet, and several loops. A hydrophobic core exists at the interface of the alpha helix and beta sheet [29].

Amb t 5 (formerly Ra5G) is a low-molecular-weight allergen (40 amino acids) of giant ragweed pollen homologous to short ragweed Amb a 5 [30]. Similarly to Amb a 5, two-dimensional NMR analysis of Amb t 5 showed that the protein consists of a C-terminal alpha helix, a short stretch of triple-stranded antiparallel beta sheet, and several loops. Recombinant Amb t 5 produced in *E. coli* was found to be indistinguishable from native Amb t 5 as determined by NMR spectroscopy [31].

Amb a 6 (formerly Ra6) is a basic protein with a molecular weight of 9.9 kDa [32]. It belongs to the ubiquitous group of plant nonspecific lipid transfer proteins (nsLTP) described as potent allergens in various fruits and as the major allergen of *Parietaria* pollen. A common feature shared by this group of proteins is the presence of eight highly conserved cysteine residues that are presumably involved in four disulfide bonds. It is considered to be a minor ragweed allergen, with 21% of ragweed-hypersensitive patients displaying IgE against Amb a 6. It appears that Amb a 6 is composed of at least four closely related isoforms of similar size but slightly different charges and amino acid sequences [33, 34]. Nevertheless, they seem to have similar antigenic and allergenic properties when tested with hyperimmune animal antisera or IgE and IgG from Amb a 6-sensitized patients. One of the Amb a 6 isoforms was expressed as a secreted protein in *Pichia pastoris* and shown to react with polyclonal and monoclonal anti-Amb a 6 antibodies [32]. A significant association between IgE antibody responsiveness to Amb a 6 and the possession of HLA-DR5 was found in a genetic-epide-

miologic study. Eighty-five percent of subjects with IgE against Amb a 6 were typed as DR5, whereas only 14% of those without anti-Amb a 6 IgE possessed DR5 alleles [35].

cDNA clones coding for two isoforms of ragweed profilin were isolated in our laboratory (table 1). Approximately 35% of the ragweed pollen-allergic patients displayed IgE antibodies against natural and recombinant profilin and no significant differences were observed in the IgE-binding properties of the isoforms (unpubl. observations). Ragweed profilin was designated Amb a 8 by the IUIS Allergen Nomenclature subcommittee (www.allergen.org; www.allergome.org). We also identified nine isoforms of the pollen-specific 2-EF-hand calcium-binding allergens and one isoform of a calcium-binding protein with 3-EF-hands by screening a ragweed pollen cDNA library with IgE of patients. Approximately 10–15% of pollen-allergic patients displayed IgE reactivity to this group of allergens (table 1). The ragweed 2-EF-hand allergen has been designated Amb a 9, according to the IUIS Allergen Nomenclature subcommittee (see www.allergen.org; www.allergome.org).

Mugwort Allergens

The allergens Art v 1, Art v 2, Art v 3, Art v 4, Art v 5, and Art v 6 were characterized to varying degrees (fig. 1, table 1). The cDNA coding for the major mugwort allergen, designated Art v 1, was isolated and the recombinant protein produced in *E. coli* [36]. More than 95% of mugwort-sensitized patients reacted to natural Art v 1, whereas approximately 50% reacted to recombinant Art v 1 as shown by IgE immunoblot and RAST experiments. Art v 1 comprises two domains, the N-terminal cysteine-rich domain, defensin-like with significant similarity also to an anther-specific sunflower pollen protein (SF18) and the C-terminal domain, which is rich in proline residues with an extended left-helical shape. Natural Art v 1 was purified and extensively characterized [36]. It is a glycoprotein rich in hydroxyproline residues and highly heterogeneous in size due to its heavy O-glycosylation, which accounts for 30–40% of its molecular weight. In ELISA inhibition, recombinant Art v 1 showed 30% inhibition of IgE reactivity to natural Art v 1. These data emphasized the importance of the posttranslational modifications of Art v 1 for IgE recognition. A detailed characterization of Art v 1 O-glycans was recently reported [37]. Chemical and enzymatic degradation, mass spectrometry, and 800-MHz ^1H and ^{13}C NMR spectroscopy indicated that the proline-rich domain is glycosylated in two ways: (1) a large hydroxyproline-linked arabinogalactan

composed of a short β 1,6-galactan core substituted by a variable number (5–28) of α -arabinofuranose residues, which form branched side chains with 5-, 2,5-, 3,5-, and 2,3,5-substituted arabinoses, and (2) single and adjacent β -arabinofuranoses linked to hydroxyproline. The large Art v 1 arabinogalactan differs from known type II arabinogalactans and should be named type III arabinogalactan [37]. In vivo, both purified natural and recombinant Art v 1 were able to elicit type I positive reactions as shown by skin and provocation tests, although the recombinant showed lower allergenic activity [38], again pointing to a role of posttranslational modifications in IgE binding.

The Art v 1 cDNA was also used for genetic vaccination, a novel approach for SIT capable of modulating the immune system towards an allergen-specific T helper cell 1 (Th1) response in mice [39]. In order to improve its immunogenicity, an important prerequisite for genetic vaccination, the cDNA of Art v 1, was recoded and thereby the codon usage optimized [40]. Expression analysis in mice revealed impaired versus normal translation of original versus recoded Art v 1 cDNA. The resulting mouse antibodies after immunization with the re-coded Art v 1 cDNA recognized both the purified natural and recombinant Art v 1 molecules. The type of immune response was Th1-biased, as indicated by high levels of IgG2a antibodies.

Natural Art v 2 allergen was described as a 35-kDa dimeric glycoprotein consisting of two identical 17.5-kDa subunits linked by disulfide bonds [41]. The presence of at least seven intra- and interchain S-S bonds was determined by amino acid analysis. Glycoanalysis revealed a high-mannose type of N-glycans [42]. Sequence analysis of 30 N-terminal amino acids showed no significant similarity to known proteins.

Art v 3 was isolated from mugwort pollen and characterized [43]. The N-terminal amino acid sequence of Art v 3 showed about 50% identity with chestnut, apple, peach, and birch nsLTGs.

Two isoforms of mugwort pollen profilin, termed Art v 4, were cloned and sequenced, and the recombinant proteins produced in *E. coli* [44]. Thirty-six percent of mugwort pollen-allergic patients displayed IgE to both recombinant isoforms, as well as to purified natural Art v 4. In solution, recombinant and natural Art v 4 molecules were found to exist as homodimers and tetramers, probably stabilized by sulfhydryl and/or ionic interactions.

A 2-EF-hand allergen was shown to be present in mugwort pollen extracts by IgG immunoblots using rabbit anti-Phl p 7 sera [45]. In our laboratory, the cDNA clones

coding for a 2-EF- and a 3-EF-hand allergen were isolated from a mugwort pollen library by screening with serum IgE from mugwort pollen-allergic patients (unpubl. data). The 2-EF-hand allergen was designated Art v 5, according to the IUIS Allergen Nomenclature subcommittee (see www.allergen.org; www.allergome.org). Finally, we identified the Amb a 1 homologue in mugwort pollen (unpubl. results). This allergen belonging to the family of pectate lyases was termed Art v 6, according to the IUIS Allergen Nomenclature subcommittee (see www.allergen.org; www.allergome.org).

IgE Cross-Reactivity

Patients suffering from pollinosis often display adverse reactions upon ingestion of a wide variety of plant-derived foods. As clinical manifestations are usually restricted to the oral cavity (e.g. oral pruritus, hoarseness, swelling of lips, tongue, and throat, pharyngitis, aphthae, laryngeal edema), the term 'oral allergy syndrome' was coined to describe the association between allergic rhinitis to pollen and the concomitant hypersensitive reactions to certain kinds of fruits, vegetables, and spices [46–51]. However, as some patients experience oral allergy syndrome without pollinosis and as the symptoms are not necessarily limited to the oral cavity but may range from oral and gastrointestinal to severe systemic reactions, such as severe laryngeal edema, urticaria, bronchial asthma, or even food-induced anaphylaxis, the term 'pollen-food syndrome' was introduced [52]. The molecular background of pollen-food syndromes is immunological IgE cross-reactivity, which might be clinically manifest or irrelevant. We previously described cross-reactivity as clinical features of allergic reactions to a source without previous exposure, whereas the term co-recognition should be used for the majority of IgE reactivity to homologous molecules found in different sources without knowledge of the original sensitizer [53]. From the clinical point of view, a clear differentiation between co-recognition and cross-reactivity is hardly possible. Therefore both types will be referred to here as cross-reactivity without further distinction.

Cross-reactive mugwort and ragweed pollen allergens can be divided into two major groups: (1) allergen families with restricted species distribution and (2) pan-allergens [54]. Restricted cross-reactions could be attributed to three families of allergens: (1) the ragweed Amb a 1 family of pectate lyases, (2) the ragweed Amb a 5 group of homologous proteins, and (3) the defensin-like Art v 1

family from mugwort. Extensive cross-reactivity within weeds and between other allergenic plants is likely caused by three families of widely distributed pan-allergens: the profilins, the polcalcins, and the nsLTPs.

The Amb a 2 allergen (formerly antigen K) was shown to have the same molecular weight (38 kDa) and shared antigenic determinants with Amb a 1. The full-length Amb a 2 cDNA encodes a protein of 398 amino acids and was expressed as a fusion protein in *E. coli* [55]. Inhibition experiments revealed cross-reactivity between Amb a 1 and Amb a 2, with Amb a 1 being slightly more potent than the homologous Amb a 2 [21, 25]. Both Amb a 1 and Amb a 2 belong to the family of pectate lyases, which are enzymes that catalyze the eliminative cleavage of pectin, the major component of the primary cell walls of many higher plants. Therefore, Amb a 2 should rather be placed in the group of Amb a 1 isoallergens (table 1). Experiments demonstrating IgE cross-reactivity between Amb a 1/Amb a 2 isoallergens and other homologous proteins (e.g. Art v 6, Cry j 1, Jun a 1) are still lacking.

IgE cross-reactivity among Amb a 5 homologues have been described in some detail. Amb p 5 was purified and characterized from western ragweed (*A. psilostachya*) pollen and shown to be highly cross-reactive with Amb a 5a from short ragweed pollen. In addition, Amb p 5 and Amb a 5a showed similar allergenic potency in histamine release experiments. Several Amb p 5 clones obtained by direct PCR of genomic DNA were classified into two groups. The sequences of the Amb p 5a variants show high sequence identity (90%) to Amb a 5, whereas the Amb p 5b variants share approximately 65% sequence homology with Amb a 5. Inhibition studies using sera from ragweed-allergic patients revealed that Amb t 5 did not inhibit the binding of IgE to Amb a 5. Conversely, Amb a 5 produced some inhibition of the binding to Amb t 5 [56]. This probably reflects the lower level of sequence homology (45%) between Amb a 5 and Amb t 5. Using homology modeling, three putative antigenic sites were found to be conserved between Amb a 5 and most of the Amb p 5 variants. These sites are likely to be responsible for the observed cross-reactivity between Amb a 5 and Amb p 5. Interestingly, these three sites are not conserved in Amb t 5, which may explain the low level of cross-reactivity with Amb a 5 [57]. Amino acids of Amb t 5 which were predicted to be part of the antigenic sites were mutated into the corresponding Amb a 5 amino acids. Sera from different Amb t 5-allergic individuals showed distinct profiles of epitope recognition in the mutated proteins. Loop 3 of Amb t 5 was shown to contain an immunodominant B cell epitope, suggesting that antibody rec-

ognition of Amb t 5 is primarily directed towards a single, immunodominant site [58].

Studies dealing with cross-reactivity of Art v 1 [36] are still lacking. In principle Art v 1-based cross-reactivity could be due to polypeptide-specific IgE antibodies recognizing homologous proteins in other allergen sources or to anticarbohydrate antibodies reacting to related as well as to unrelated proteins in other allergen sources. Preliminary results suggested that the single hydroxyproline-linked β -arabinose residues are able to bind antibodies [37]. Monoarabinylation of Art v 1 hydroxyproline differs from known oligoarabinylation of Ser-Hyp₄ motifs described for other plant hydroxyproline-rich glycoproteins (e.g. extensins, solanaceous lectins). Thus, mono- β -arabinylation of hydroxyproline residues could be a new cross-reactive carbohydrate determinant in plant proteins [37]. Future studies should establish the clinical relevance of the Art v 1 O-glycans.

In the case of pan-allergens, it has been demonstrated by IgE inhibition studies using antisera raised against different plant profilins [4] and by inhibition studies using recombinant and natural Art v 4 [44] that profilin is a cause of cross-reactivity between ragweed and mugwort. Using CAP FEIA measurements and IgE immunoblotting, Stumvoll et al. [45] showed that in mugwort- and/or ragweed-sensitized patients living in the non-Mediterranean areas, IgE reactivity to pellitory pollen is frequently caused by profilin and calcium-binding allergens. Another study showed that antiserum raised against recombinant Aln g 4, the alder (*Alnus glutinosa*) polcalcine, cross-reacted with an allergen from ragweed pollen extract in the range of 6–8 kDa, which was presumed to be a calcium-binding allergen [59].

T Cell Recognition

T lymphocytes are essential in the pathophysiology of type I allergy [60]. Antigen-specific B cells have to interact with allergen-specific CD4⁺ Th cells before they actively produce antibodies. Whereas B cells mainly recognize 3-dimensional structures of native proteins, Th cells recognize linear peptide fragments of an antigen created by intracellular processing in antigen-presenting cells (e.g. dendritic cells, B cells) in context with human leukocyte antigen (HLA) class II molecules on the surface of these cells. In response to allergen activation, Th cells belonging to the Th2 subset produce high amounts of IL-4, which is an important prerequisite for an allergic sensitization, since it induces the Ig class switch to IgE in B cells.

In addition, IL-4 and other Th2 cytokines contribute to the growth and differentiation of the effector cells involved in allergic and inflammatory reactions. Thus, knowledge of the T cell epitopes of allergens and the cytokine production profiles of allergen-specific T cells is valuable in the design of therapeutic strategies.

Only very few weed allergens have been studied in detail for T cell recognition. T cell lines (TCL) derived from 12 ragweed-allergic patients reacted with the three recombinant forms (Amb a 1.1, Amb a 1.2, Amb a 1.3) of Amb a 1 [61]. T cell responses to Amb a 5 were characterized in mice [62, 63] and humans [64]. The T cell response to the major mugwort allergen, Art v 1, was recently characterized in detail. For this purpose, PBMC as well as allergen-specific TCL and T cell clones (TCC) were used. Short-term Art v 1-specific TCL were generated by stimulating PBMC with allergen and expanding this culture with growth factors to enrich the oligoclonal population of allergen-specific T cells. Monoclonal TCC, specific for only one epitope, were obtained by limiting dilution of the TCL. Similar to many other respiratory allergens [65, 66], the majority of Art v 1-specific T cells were CD4⁺CD8[−]TCRαβ⁺⁺ and belonged to the Th2 phenotype, i.e. after allergen-specific stimulation they produced high amounts of IL-4 and IL-5, but low or no IFN-γ [67]. Comparable T lymphocyte responses were observed after activation with natural or recombinant Art v 1, indicating that the presence of carbohydrates on the natural protein plays no role in T cell recognition [36, 38]. Stimulation of Art v 1-specific TCL and TCC with overlapping synthetic peptides spanning the entire Art v 1 sequence revealed that the major mugwort allergen contains only one single immunodominant epitope. Of 113 mugwort-allergic individuals, 96% recognized Art v 1_{22–36}, which is located within the α-helical structure of the defensin-like domain in Art v 1 [67]. Very few epitopes other than Art v 1_{22–36} were recognized in a minority of patients. Thus, Art v 1 strikingly differs from other common major pollen allergens, which contain multiple epitopes that also vary from patient to patient [65, 66].

The predominance of Art v 1_{22–36} as sole T cell epitope in Art v 1 could be due to restricted usage of TCR Vα/β families or HLA molecules. For Amb a 5, which also contains a single T cell epitope, the recognition by a dominant TCR beta chain and its restriction by HLA-DRB1*15 have been described [63, 64]. Obviously the small size of the molecule (45 amino acids) does not allow for a variety of T cell epitopes. For the T cell recognition of the larger weed allergen Art v 1 (108 amino acids), however, we demonstrated the involvement of di-

verse TCR Vα/β families [67, 68]. HLA class II presentation of the immunodominant epitope was shown to be restricted by HLA-DR, but not by HLA-DP or HLA-DQ molecules [68]. Furthermore, a highly significant association of response to Art v 1_{22–36} and the HLA-DRB1*01 phenotype expression in mugwort-allergic patients was observed. In addition, HLA-DRB1*01 was also identified as the main restriction element for presentation of Art v 1_{22–36} [68]. In the past, type I hypersensitivity to certain major allergens such as Fel d 1, Alt a 1 or bee venom has been associated with particular HLA class II phenotypes, but this association was less clear [69, 70] and these publications did not include analyses at the T cell epitope level.

The unique features of mugwort pollen allergy make it a model candidate for a T cell-based SIT: (1) a high prevalence of sensitization to Art v 1 in mugwort pollen-allergic patients, (2) the T cell response to Art v 1 is restricted to a single epitope and (3) is clearly associated with HLA-DRB1*01 expression. Beneficial effects of a peptide-based immunotherapy have been reported in allergy to bee venom or cat dander [71]. In view of the applicability of this therapeutic approach in mugwort allergy, we determined the minimal length and critical amino acid residues of the immunodominant epitope of Art v 1 using mutant peptides for T cell stimulation experiments. A core region, varying from 5 to 10 amino acids within Art v 1_{25–34} (KCIEWEKAQH) and containing 3–6 critical residues mostly located within Art v 1_{26–31}, was defined [68].

Concluding Remarks

Within the last decade, several mugwort and ragweed pollen allergens have been characterized at the molecular level. Although ragweed and mugwort belong to the same plant family and seem to share a number of cross-reactive allergens, their major allergens are unrelated proteins. Amb a 1, the major ragweed allergen, is a 38-kDa protein belonging to the family of pectate lyases, which are enzymes that catalyze the eliminative cleavage of pectin of cell walls in many higher plants. Detailed studies on T and B cell epitopes of Amb a 1 are still needed for the rational development of better reagents to be used in ragweed SIT.

Art v 1, the major allergen of mugwort, is a glycoprotein rich in hydroxyproline residues and highly heterogeneous in size (24–28 kDa) due to O-glycosylation, which accounts for 30–40% of its molecular weight. T cell rec-

ognition of Art v 1 is restricted to a single immunodominant epitope with a clear association with HLA-DRB1*01 expression. Thus, mugwort allergy seems to be well suited for a peptide-based approach in allergen SIT.

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