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ScienceDirect
Molecular Immunology 44 (2007) 3653–3660
Molecular Immunology
www.elsevier.com/locate/molimm

Cloning, expression and characterization of mugwort pollen allergen Art v 2, a pathogenesis-related protein from family group 1

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Received 13 February 2007; received in revised form 12 April 2007; accepted 13 April 2007

Abstract

Mugwort (*Artemisia vulgaris*) belongs to the Compositae family, and is one of the main causes of allergy in late summer and autumn. The aim of the study was to characterize the allergen Art v 2 from mugwort pollen. Skin prick tests, performed in 19 patients allergic to mugwort and 10 control patients, showed an Art v 2 sensitization prevalence of 58%, whereas none false-positives were detected among control patients. Art v 2 was purified by standard chromatography and binding to Concanavalin A column and had an apparent molecular mass of 33 and 20 kDa, calculated by gel permeation and SDS-PAGE under denaturing conditions, respectively, showing that the allergen is composed of two identical subunits. Art v 2-encoding cDNA was amplified by PCR using degenerate primers based on reported partial amino acid sequences. Cloned cDNA encoding Art v 2 contains 140 bp that codify for a polypeptide of 15.8 kDa, with a predicted p*I* value of 5.2, and one potential *N*-glycosylation site. Protein homology search demonstrated that Art v 2 share 55-42% identical residues with pathogenesis-related protein PR-1 of tomato, potato, rape, wheat and rice. Homology was also found to Ves v 5 (41% identical residues). Bacterial-expressed recombinant Art v 2 was recognized only by 21% of mugwort-allergic patients. In conclusion, Art v 2 from mugwort is the first weed pollen allergen that belongs to the pathogenesis-related protein PR-1 and its recombinant form could help molecular diagnosis of mugwort associated allergy.

Keywords: *Artemisia vulgaris*; Art v 2; Recombinant allergen; Gene expression; Pathogenesis-related protein; PR-1

1. Introduction

Type I (IgE-mediated) allergy is a genetically determined clinical disorder that affects almost 25% of the population in development countries (Akdis and Blaser, 2000). *Artemisia vulgaris* (mugwort), included in the family Compositae or Asteraceae (one of the largest families of flowering plants), is a weed widely spread in temperate regions and subtropics of Europe, North America and Asia (Charpin and Surinyach, 1974). Its pollen is considered an important elicitor of type I allergy during late summer and the beginning of autumn (Spieksma et al., 1980). *A. vulgaris* pollen has allergenic structures that are recognized by cross-reactive IgE antibodies to different allergen sources and therefore, it is involved in several pollen-food syndromes (Egger et al., 2006). Up to 15 allergic proteins with variable clinical importance have been described in *A. vulgaris* pollen extracts (Nilsen et al., 1991a). Art v 1 is a 24–28 kDa glycoprotein encoded by an open-reading frame of 108 amino acids with a defensin-like domain (Himly et al., 2003). Art v 1 prevalence varies depending on the studies, it has been described that between 50 and 90% of the mugwort-allergic patients had specific IgE to Art v 1 (Himly et al., 2003; Lombardero et al., 2004), and from 50 to 70% had positive skin prick test (SPT) to the same allergen (Lombardero et al., 2004). Art v 3 is a major allergen with an apparent molecular weight of 9.7 kDa. It belongs to the lipid-transfer-protein (LTP) family and its N-terminal amino acid sequence has been published (Díaz-Perales et al., 2000). Around 70% of mugwort sensitized patients had positive SPT and specific IgE to natural Art v 3 (Lombardero et al., 2004). Mugwort profilin (Art v 4), which is an important cross-reactive panallergen for patients suffering from multiple food and pollen allergies, reacted to IgE antibodies of 36% of mugwort-allergic patients (Wopfner et al., 2002). Art v 2 has been identified as a

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0161-5890/$ – see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.molimm.2007.04.022

glycoprotein of about 35 kDa that consisted of at least six isoforms and reacted to 45% of sera from mugwort-allergic patients (Nilsen and Paulsen, 1990). Although the NH_2-terminal amino acid sequence and cyanogen bromide fragments of deglycosylated Art v 2 has been described (Nilsen et al., 1991b), its complete molecular structure had not been identified up to this work.

Pathogenesis-related proteins (PRs) are proteins encoded by the host plant but induced specifically in pathological or stress situations (Van Loon and Van Strien, 1999). Plant-derived allergens have been identified with sequence similarity to PR families 2–5, 8, 10 and 14 comprising β-1,3-glucanases, three types of chitinases, thaumatine-like proteins, Bet v 1-like proteins and lipid transfer proteins (Hoffmann-Sommergruber, 2002). Recently, a Bermuda grass pollen allergen, Cyn d 24, has been described as new member of PR-1 type proteins (Chow et al., 2005).

In this study, we report for the first time the cloning and identification of Art v 2 from *A. vulgaris* pollen as member of the pathogenesis-related proteins PR-1. The allergen was expressed in *Escherichia coli* as a non-fusion protein and the allergenic reactivity of the recombinant protein compared with the natural counterpart in relation to their capacity to bind specific IgE and to elicit skin prick test (SPT).

2. Materials and methods

2.1. Patients

Clinical data of 19 patients (age 10–55 years; 9M/10F) used in this study are shown in Table 1. Patients showed immediate hypersensitivity to *A. vulgaris* according to a clinical history of seasonal or perennial rhinitis, positive SPT response to commercially available *A. vulgaris* extract (Bial-Arístegui, Bilbao, Spain) and specific IgE serum level to *A. vulgaris* extract as determined by using the ImmunoCAP System (Phadia, Uppsala, Sweden). Ten control subjects, three no atopics, and seven with other no-related allergies (olive and grass pollen, mites and cat dander) were also included. All patients and control subjects provided oral and written informed consent.

2.2. Purification of natural allergens and experimental rabbit antisera

Fifteen grams of *A. vulgaris* pollen was extracted with 150 mL PBS for 2 h, centrifuged at 5000× *g* for 15 min and the pellet extracted again. The supernatants were dialyzed against 20 mM phosphate pH 7.0 and subjected to cationic exchange chromatography in a High Flow SP column (GE-Healthcare, Uppsala, Sweden) in order to purify Art v 1 as previously described (Schmid-Grendelmeier et al., 2003). Flow through fraction was adjusted to 1 M ammonium sulfate and applied to a Phenyl Sepharose 16/20 column (GE-Healthcare). Bound proteins were eluted with water and dialyzed, adjusted to 20 mM Tris pH 8.0; 0.5 M NaCl; 1 mM $CaCl_2$; 1 mM $MnSO_4$ and injected into a HiTrap Concanavalin A-Sepharose (GE-Healthcare). Bound glycoproteins were eluted with 0.5 M methyl-α-D-glucopyranoside and concentrated by ultrafiltration (Amicon Ultra 4, Millipore, Bedford, MA, USA). Final purification was achieved with a Superdex 75 gel permeation column fitted in a Smart system (GE-Healthcare). Art v 4 was purified following the modified method of Lindberg previously described (Vallverdú et al., 1997).

Polyclonal antibodies were obtained in New Zealand rabbits by immunizing with five boosts of 200 μg of natural Art v 2 every 2 weeks, emulsified in complete Freund's adjuvant (Difco, Detroit, Michigan). Sera, collected 10 days after the last injection, were tested by ELISA and stored at −80 °C.

Table 1
Sensitization data of patients allergic to mugwort included in the study

Patient	Gender	Symptoms	Mugwort IgE reactivity (U/mL)	Minimal concentration of allergen with positive SPT (μg/mL)			
				nArt v 2	rArt v 2	nArt v 1	nArt v 4
1	F	RC	91.81	5	–	5	50
2	F	RC, A	62.57	–	–	–	–
3	F	A	45.36	5	5	5	50
4	F	RC	54.59	100	–	50	–
5	F	R	79.47	–	–	5	5
6	M	RC, A	35.51	100	–	100	–
7	M	RC	71.59	–	–	5	–
8	M	R	52.57	–	–	–	5
9	M	RC	50.75	–	–	–	–
10	M	RC, A	43.13	100	–	–	–
11	M	RC	122.50	5	5	5	5
12	F	RC	48.37	50	50	5	5
13	F	RC, A	97.14	5	–	5	50
14	F	RC, A	73.95	–	–	5	–
15	F	RC	65.17	100	–	5	5
16	M	R	67.18	–	–	5	–
17	F	RC	46.90	–	–	50	–
18	M	RC	64.46	50	50	5	–
19	M	RC	49.86	5	–	–	5

(RC) Rhinoconjunctivitis, (A) asthma, (R) rhinitis, (–) no SPT response at any of the concentrations tested.