

Isolation and Characterization of cDNA Clones Coding for Mugwort (*Artemisia vulgaris*) Pollen Allergens

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Key Words

Mugwort pollen · *Artemisia vulgaris* · Allergen · cDNA clones · Recombinant

The family *Compositae* or *Asteraceae* is known to include several allergenic plants, and among them, *Ambrosia* (ragweed), *Parthenium* (feverfew) and *Artemisia* (mugwort) are of major importance. Pollen of mugwort (*Artemisia vulgaris*) is one of the main causes of allergic reactions in late summer and autumn in Europe. It is widely spread, but is predominantly found in the temperate and humid zones of the northern hemisphere and along the Mediterranean basin [1]. In central Europe, mugwort pollination occurs at the end of July and throughout August, whereas in the Mediterranean areas, it mainly occurs in September and the beginning of October [2]. Among patients suffering from pollinosis, the incidence of allergic disease caused by mugwort pollen is between 10 and 14% [2, 3]. No information about the complete molecular structure of any mugwort pollen allergen has been published so far. In the present study, we report the isolation and characterization of cDNA clones

coding for mugwort pollen allergens. For this purpose, a cDNA expression library from mugwort pollen was constructed in lambda ZAP and used for immunoscreening with patients' IgE and monoclonal antibodies raised against mugwort pollen proteins. Figure 1 shows the reactivity patterns of 22 monoclonal antibodies (MUP1–MUP22) tested with mugwort pollen extract. Monoclonal antibodies MUP1–3, MUP5–9, MUP15 and MUP17–19 were used for screening the mugwort pollen cDNA library. Table 1 summarizes the results of screening with the monoclonal antibodies. MUP7, MUP9 and MUP18 all recognize a small protein (90 amino acids long) belonging to the group of plant cystatins (phytocystatins), which are cysteine proteinase inhibitors [4]. MUP5 and MUP15 recognize a 14-3-3 protein, which is involved in signal transduction pathways (protein phosphorylation) and protein-protein interactions regulating the activity of some key enzymes in eukaryotic cells [5]. MUP6 recognizes the glycolytic enzyme triose phosphate isomerase (E.C. 5.3.1.1), and MUP17 a plant-specific Ca²⁺-dependent protein kinase. The recombinant proteins recognized by monoclonal antibodies MUP7, MUP9, MUP18, MUP5, MUP15 and MUP6 showed no IgE-binding activity when tested with sera from mugwort pollen-allergic

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Fig. 1. Immunoblot of mugwort pollen extract using 22 monoclonal antibodies raised against mugwort pollen proteins. Antibodies marked in bold (MUP1–3, MUP5–9, MUP15 and MUP17–19) were used to screen the mugwort pollen cDNA library.

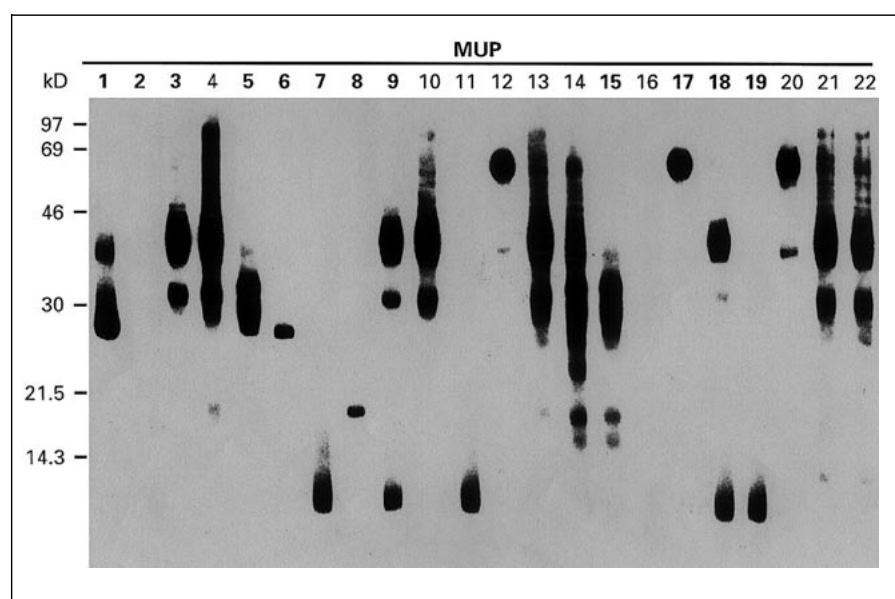


Table 1. Monoclonal antibodies used to screen a mugwort pollen cDNA library and the corresponding identified antigens

Monoclonal antibody	Isolated cDNA clones
MUP7, MUP9, MUP18	cystatin (proteinase inhibitor)
MUP5, MUP15	14–3–3 protein
MUP6	triose phosphate isomerase
MUP17	Ca ²⁺ -dependent protein kinase
MUP1	unknown protein
MUP2, MUP3, MUP8	no immunopositive cDNA clones
MUP19	sunflower pollen-specific protein

patients. Postsynthetic modification(s) seems to be important for the formation of epitopes recognized by MUP2, MUP3 and MUP8, as no positive signals were obtained in the screening.

IgE immunoscreening was performed using a serum pool from 16 mugwort pollen-allergic patients. Thirteen of the 42 IgE-positive clones coded for the panallergen profilin [6]. DNA sequence analysis of seven cDNA clones revealed three different profilin isoforms, differing between 1 and 17 amino acids. Mugwort profilins showed sequence similarities with other plant profilins; among them, profilin from sunflower pollen [7] showed the highest degree of homology. The cDNAs coding for the two most divergent mugwort profilin sequences were expressed in *Escherichia coli* and the recombinant pro-

teins were tested for IgE reactivity. Approximately 30–40% of the mugwort pollen-allergic patients tested displayed IgE antibodies reacting with profilin. All sera recognizing natural profilin also showed IgE reactivity with the recombinant isoforms. However, no significant differences were observed for the IgE-binding properties of the two profilin isoforms.

An IgE-positive clone, designated Art21, showed sequence similarities with calmodulin and contained four EF-hand calcium-binding domains. However, the EF-hand domains of Art21 are not able to bind calcium, as demonstrated by blot overlays with ⁴⁵Ca and Ca²⁺-dependent mobility shift. About 3% of the mugwort pollen-allergic patients showed IgE reactivity with recombinant Art21 protein produced in *E. coli*. Other IgE-positive clones that were isolated are presently being characterized. So far, none of the isolated cDNA clones coded for mugwort pollen allergens that were previously isolated and partially characterized at the protein level [8–11].

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