

Oral Allergy Syndrome to Chicory Associated with Birch Pollen Allergy

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Key Words

Birch pollen · Chicory · Cross-reactivity · Food allergy · Oral allergy syndrome

Abstract

Background: A few cases of IgE-mediated chicory allergy with oral, cutaneous, and/or respiratory symptoms are reported. We present 4 patients with inhalant birch pollen allergy and oral allergy syndrome to chicory. IgE-binding proteins in chicory and cross-reactivity with birch pollen were studied. **Methods:** Chicory extract was prepared and immunoblotting was used to study IgE reactivity and cross-reactions with birch pollen. **Results:** The pattern of IgE binding to chicory was variable among the patients, with protein bands recognized at 18, 21, 40, 52 and 71 kD. Bet v 1-like proteins were detected in chicory by monoclonal antibody binding. Chicory-birch pollen cross-reactivity, as studied in 2 patients from whom enough serum was available, could be demonstrated but did not involve the Bet v 1 protein family. In one of these cases, a 51-kD protein of birch pollen was found to be responsible for cross-reactivity. **Conclusions:** Chicory should be added to the list of foods that can cross-react with birch pollen and cause the birch pollen-associated oral allergy syndrome.

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Introduction

Chicory (*Cichorium intybus* ssp. *sativum*), also known as Belgian endive, or witloof, is a member of the Compositae (Asteraceae) family, grown for its leaves and roots. The etiolated leaves can be eaten raw or cooked, while the roots are used as a coffee substitute after they have been roasted. Although pollen of mugwort (*Artemisia vulgaris*) and ragweeds (*Ambrosia* spp.), both of the same family as chicory, are well-known allergen sources, the edible members of that family, like lettuce (*Lactuca sativa*), endive (*Cichorium endivia*), artichoke (*Cynara scolymus*), and salsify or oyster plant (*Tragopogon porrifolius*), rarely elicit IgE-mediated allergic reactions.

Chicory was first reported to induce asthma in a chicory grower [1], but firm evidence of an IgE-mediated mechanism was missing at the time. We described in 1996 a case of occupational allergy to chicory with oral, cutaneous and respiratory symptoms [2], and we demonstrated cross-reactivity of antichicory IgE with endive and lettuce but with none of the pollen tested. A 48-kD protein, confined to the nonilluminated parts of the plants, was held responsible for the IgE-mediated reactions in this patient. Two other cases of chicory allergy have been reported since then. One case involved a patient sensitized to grass pollen and nonoccupationally exposed to chicory [3]. He displayed a variety of symptoms after the ingestion of chicory and urticaria after contact, but did

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Table 1. Clinical data of the 4 patients

Patients	Sex	Age	Positive ST ¹	Total IgE Ua/ml	CAP scores ²	Respiratory clinical symptoms	Oral allergy syndrome to	Other symptoms
BC	F	35	TP, GP	450	weed mix: neg rBet v 1: >100 rBet v 2: neg	rhinoconjunctivitis in early spring	apple, carrot	contact urticaria to tomato plant, strawberry plant, and raw potato
MD	F	36	GP, TP, MW, cat	304	birch pollen: 51 cat: 6 mugwort pollen: neg rBet v 1: 34 rBet v 2: neg	seasonal rhinoconjunctivitis, especially in May to August and upon contact with cat, asthma once in June	apple, kiwi, nuts (not peanuts), stone fruits, raw celery	contact urticaria to raw potato and strawberry plant
GK	F	44	TP	90	weed mix: neg GP: neg apple: 15 rBet v 1: >100 rBet v 2: neg	seasonal rhinoconjunctivitis, especially in May to September, sporadic mild asthma	apple, nuts, stone fruits	dyspnea after ingestion of strawberry
LVD	F	31	GP, TP, MW, HDM, cat, dog	143	birch pollen: 27 ryegrass pollen: 27 latex: 1.2 rBet v 1: 12 rBet v 2: 0.57	rhinoconjunctivitis and strong asthma in March to May and in contact with cat or dog, mild asthma in the rest of the year	nuts, kiwi, melon, cherry, paprika, peach, banana	conjunctivitis on contact with asparagus, beans, carrot and parsley

ST = Skin tests; TP = tree pollen; GP = grass pollen; MW = mugwort pollen; HDM = house dust mite; neg = negative; Ua = arbitrary unit.

¹ The standard battery of allergens has been used in all patients, namely tree pollen, grass pollen, weed pollen, cat fur, dog fur, house dust mite, and moulds (all from HAL, Haarlem, The Netherlands). Only the positive tests are given.

² CAP scores were obtained with the Pharmacia CAP system. Only the tests mentioned were performed.

apparently not react with the botanically related lettuce. Experiments investigating possible cross-reactions between grass pollen and chicory were not performed. The second case involved an occupational allergy to chicory and lettuce, with rhinitis, asthma and food allergy as IgE-mediated symptoms. There was no association with any pollen allergy [4].

We report here on nonoccupational chicory allergy with the oral allergy syndrome (OAS) in 4 patients with birch pollen allergy. Birch pollen-chicory cross-reactivity could be demonstrated by immunoblotting.

Patients, Materials and Methods

Patients

We present 4 patients with OAS to chicory, mainly characterized by itching in the mouth. All patients had rhinoconjunctivitis and positive skin tests to tree pollen. The complete clinical characteristics of the patients besides OAS to chicory are presented in table 1. The immediate adverse reactions to the offending food were determined

only by case history, except for patient LVD, in whom a skin prick test with fresh chicory was carried out. This reaction was clearly positive. A peculiarity of this patient is that she started to complain of OAS to chicory 6 months after she had started a desensitization procedure to house dust mite, grass pollen, tree pollen, mugwort and *Alternaria*. Six months later, she still reported OAS to chicory, but also to peach and banana, and in addition also conjunctivitis to carrot and parsley. A blood sample was taken at that time.

Serum Samples

In patients MD and GK, two samples were taken at an interval of 2 years. Patient MD received injections for desensitization to birch pollen between the two blood samples. Only one sample was available for patient BC and patient LVD. The latter had been on immunotherapy for 1 year when the sample was taken.

Allergens and Antibodies

Chicory extract was prepared according to the method described by Björkstén et al. [5]. Chicory leaves were cut into small pieces before they were suspended in extraction medium (75 g/50 ml), consisting of 2% polyvinylpyrrolidone, 2 mM ethylenediaminetetraacetic acid, 10 mM diethyldithiocarbamate, and 1 M sucrose. The mixture was homogenized with a mixer for 1 min, stirred for 30 min at room temperature, centrifuged for 5 min at 12,000 g, filtered

through a Whatman No. 1 filter paper; centrifuged again for 30 min at 30,000 *g* and 4 °C, and concentrated 2-fold by membrane filtration (cut-off value of 10 kD). The resulting extract was aliquoted and stored at –70 °C after filtration through a 0.45-µm membrane.

An aqueous extract of birch pollen was prepared at pH 7.5, as already described [6]. Recombinant Bet v 1a and Bet v 2 were purchased from Biomay (Linz, Austria). Anti-Bet v 1 monoclonal antibody 5H8 was obtained as described [7]. Recombinant Der p 2 is a kind gift of J.-M. Saint-Rémy (Catholic University of Leuven).

Proteinase K Digestion

An amount of 750 µg of birch pollen proteins was digested by 30 µg of proteinase K (Roche Diagnostics, Brussels, Belgium) overnight at 50 °C. Digestion yield reached 99%, as measured with Bradford's Coomassie blue assay (Bio-Rad, Richmond, Calif., USA). The enzyme was heat-denatured after digestion.

SDS-PAGE and Immunoblotting

SDS-PAGE was carried out in a 5% polyacrylamide stacking gel and a 13% separating gel. An amount of 30 µg of chicory protein was loaded per centimeter of gel, for birch pollen, 100 µg was loaded. Electrophoresis was performed at 350 V, until the bromophenol blue band reached the bottom of the gel. Afterwards, proteins in SDS-PAGE gel were electroblotted onto a polyvinylidene difluoride membrane (Immobilon, Millipore, Bedford, Mass., USA). The membrane was then immersed in 0.2% defatted dry milk phosphate-buffered saline solution (PBS-NFDM 0.2%) for 1 h at room temperature. Strips cut from this membrane were each incubated overnight at 4 °C with 300 µl of patient's serum diluted in 1 ml PBS-NFDM 0.1%. Bound IgE was detected with a mouse monoclonal anti-human IgE antibody (CLB, Amsterdam, The Netherlands) diluted 1/1,000, followed by peroxidase-conjugated rat anti-mouse kappa chain antibody (Biosource Europe, Nivelles, Belgium), diluted 1/20,000. PBS-NFDM 0.1% was used for dilutions and washings. Bands were visualized by incubation in tetra-methyl-benzidine (TMB peroxidase membrane substrate, KPL, Gaithersburg, Md., USA).

For inhibition experiments, the serum was first preincubated with either 200 µg of proteins when complete extracts were used or 50 µg of recombinant protein per strip. To perform inhibition with the proteinase-K-digested birch pollen extract, the same volume of inhibiting extract was used as that used with the nondigested extract, namely 133 µl. Inhibition was also carried out as follows: a first SDS-PAGE immunoblot with birch pollen extract (100 µg/cm) in an 8-cm slot was performed. The part of the blotting membrane corresponding to between 45 and 55 kD, where a unique 51-kD birch pollen protein reacts with patient GK's serum, was located, cut all along the 8 cm, and blocked in PBS-NFDM 0.2%. Patient serum was then incubated overnight at 4 °C with that piece of membrane to deplete the serum of antibodies to the 51-kD protein before it is used on chicory immunoblots.

Results

Immunoblotting

Strip E on figure 1 demonstrates binding of anti-Bet v 1 monoclonal antibody 5H8 to chicory protein bands in the 17- to 18-kD molecular mass range. To exclude a pos-

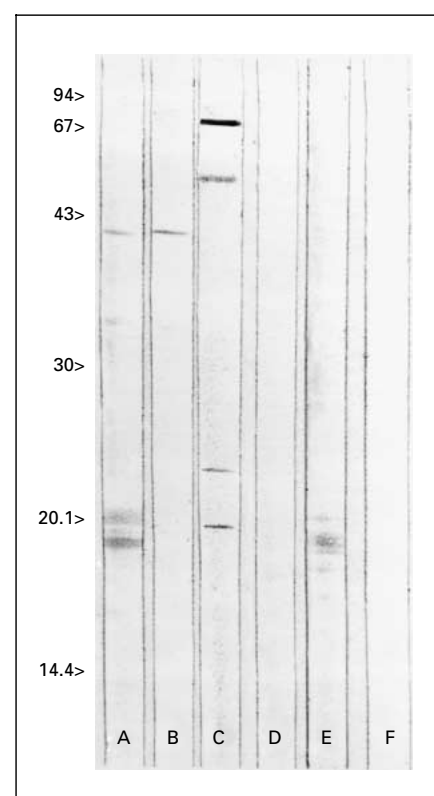


Fig. 1. IgE-binding patterns to chicory proteins in patients with oral allergy to chicory. Immunoblot strips from SDS-PAGE gel are shown. Strips were probed with serum of patient BC (A), patient MD (B), patient GK (C), house dust mite-monospecific patient serum (negative control) (D), 5H8 monoclonal antibody (E), and anti-CD44 monoclonal antibody (F). Molecular masses are indicated on the left in kilodaltons.

sible aspecific binding of 5H8, a control strip was made using a monoclonal antibody of the same immunoglobulin subclass (IgG1) but with a totally different specificity (anti-CD44, Biosource Europe) (strip F in fig. 1). The IgE recognition patterns of the patients BC, MD, and GK are also shown in figure 1. The three patterns were different from each other. Patient BC had IgE-reactive chicory protein bands at 17 to 18 kD, which might correspond with a Bet v 1-related protein in chicory. Patient BC, in addition, showed IgE binding to a 40-kD band. A similar 40-kD band was the only one to be clearly recognized by the patient MD. The serum from patient GK showed binding to four bands at 71, 52, 21 and 18 kD. With a second blood sample of GK, taken 2 years later, a similar binding profile was detected (fig. 2). The IgE-binding pattern of patient LVD to chicory is shown in figure 3. This serum recognizes two bands in the range of 15–17 kD.

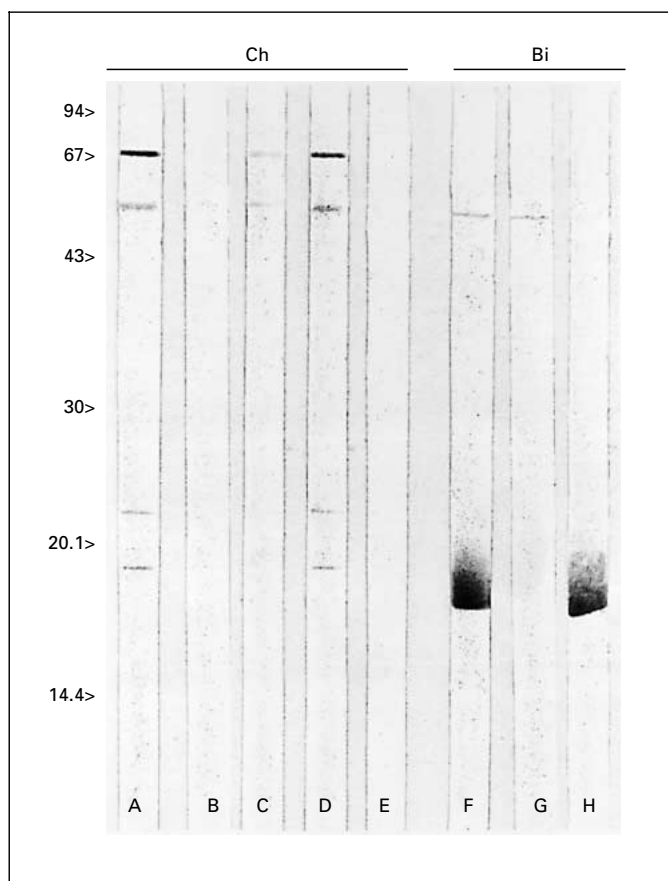


Fig. 2. Cross-reactivity between chicory and birch pollen allergens in patient GK. IgE immunoblots from SDS-PAGE protein patterns of chicory (Ch) and birch pollen (Bi) are shown. Strips were probed with serum of patient GK without inhibition (A, F), preincubated with complete birch pollen extract (B), proteinase-K-digested birch pollen extract (C), rBet v 1a (D, G), 51-kD band of birch pollen (E), or complete chicory extract (H). Molecular masses are indicated on the left in kilodaltons.

Figure 2 shows the results of cross-reactivity studies performed with serum GK. On a birch pollen immunoblot profile, patient GK recognized an 18-kD band that corresponds with Bet v 1, as proven by immunoblot inhibition with preincubation of the serum with rBet v 1a (strip G in fig. 2), and a thin band at around 51 kD (strip F in fig. 2). Preincubation of serum of patient GK with a total birch pollen extract eliminated all reactions to chicory (strip B in fig. 2). Preincubation with rBet v 1a was not able to inhibit IgE reactivity to any chicory band (strip D in fig. 2), but preincubation with the 51-kD birch protein band inhibited the reactions to all chicory bands (strip E in fig. 2). Preincubation with proteinase-K-digested birch

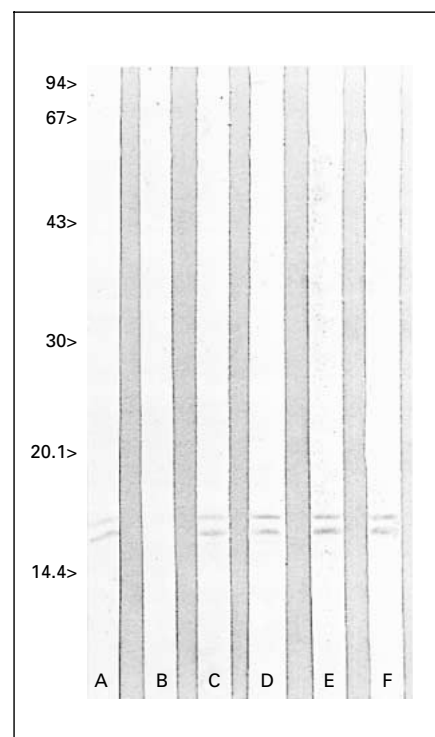


Fig. 3. Cross-reactivity between chicory and birch pollen allergens in patient LVD. IgE immunoblots from an SDS-PAGE protein pattern of chicory are shown. Strips were probed with serum of patient LVD without inhibition (A), preincubated with complete birch pollen extract (B), rBet v 1a (C), rBet v 2 (D), rDer p 2 (E), or proteinase-K-digested birch pollen extract (F). Molecular masses are indicated on the left in kilodaltons.

pollen extract evidenced complete inhibition of the two lower-molecular-mass bands in the chicory extract, and partial inhibition of the two bands at 71 and 52 kD (strip C in fig. 2).

In addition, we performed inhibition of the binding of patient GK serum to a birch pollen protein pattern by preincubation with chicory extract. This resulted in the disappearance of the reactivity to the 51-kD band only (strip H in fig. 2).

Immunoblot inhibition experiments were also carried out with serum of patient LVD (fig. 3). Preincubation with complete birch pollen extract could inhibit the binding to the two bands at 15–17 kD, but rBet v 1a and rBet v 2 were not able to inhibit such binding (strips B, C and D, respectively, in fig. 3). Recombinant Der p 2, used as negative inhibition control, did not inhibit the binding (strip E in fig. 3). Proteinase-K-digested birch pollen extract did not inhibit the binding either (strip F in fig. 3).

Discussion

We report here on four cases of nonoccupational chicory allergy. All 4 patients had clear OAS to chicory, for which they showed different IgE-binding profiles on SDS-PAGE immunoblots. No double-blind placebo-controlled food challenge was performed in these patients. However, other authors have already shown that the diagnosis of OAS by means of case history is reliable [8, 9], and a positive skin test with fresh chicory in patient LVD and a demonstration of IgE binding in immunoblots further corroborates the OAS reactions that the patients had reported.

Two serum samples, taken at an interval of 2 years, were available for 2 patients (MD and GK). Patient GK still showed reactivity to chicory on IgE immunoblots with the same pattern as 2 years earlier. Patient MD had avoided eating chicory in this interval, and had been receiving, in addition, injections for desensitization to birch pollen. At the second visit, she did not report allergic reactions to chicory any more. To corroborate this, an immunoblotting experiment with the second serum sample of this patient showed no longer an IgE-reactive chicory band (not shown). Whether this reflects the effects of the elimination diet or those of immunotherapy can only remain speculative.

A common characteristic of our 4 patients is that they showed symptoms of birch pollen allergy, either isolated or together with other inhalant allergies, which distinguish them from all previously published chicory allergy cases [2–4]. Because there are no reports on birch pollen-associated chicory allergy, we investigated whether there might be a cross-reactivity between chicory and birch pollen. Using serum of patient GK, we found that a complete birch pollen extract was able to inhibit the IgE-binding reactions to the chicory proteins. For several reasons, we initially suspected Bet v 1 to be at least partly responsible for cross-reactivity. However, preincubation with rBet v 1a did not influence the binding to any chicory protein band, although the same molecule could totally inhibit the binding to the Bet v 1 band in birch pollen immunoblot with serum of the same patient. We then investigated whether the thin 51-kD band of the birch pollen IgE-binding pattern could be responsible for some cross-reactivity with chicory. To our surprise, after depletion of the antibodies to that protein from the patient serum, all IgE binding disappeared from the chicory immunoblot. The 51-kD protein thus seems to cross-react with four different proteins of chicory. However, inhibition with proteinase-K-digested birch pollen extract, in which protein cores

but not sugar moieties have been degraded, indicated that the reactivity to the two bands at 21 and 18 kD probably was due to cross-reactive carbohydrate determinants. The two bands at 71 and 52 kD were only partly inhibited, which implies the involvement of carbohydrate moieties in the cross-reactions but also of the protein cores. If the reactions to a 52-kD protein of chicory and a 51-kD protein of birch pollen suggest similar proteins in both species, the cross-reaction with the 71-kD protein is more difficult to interpret when not only carbohydrates are involved. The simplest explanation is that the 71-kD protein is a precursor of the 52-kD protein in chicory. Another explanation implies that epitopes of the 51-kD protein of birch could be found on two different proteins of chicory. Although this is theoretically possible, but has not been previously observed, we find it less likely. We also inhibited the reactivity to birch pollen by preincubating serum of patient GK with chicory extract. This led to the disappearance of the thin 51-kD band in the birch pollen pattern, but not of the Bet v 1 band, further corroborating in this patient the involvement of this protein and not of Bet v 1 in the chicory-birch cross-reactions.

Immunoblot inhibition experiments with LVD serum showed that cross-reactivity between birch pollen and chicory also applied to this patient. A proteinase-K-digested birch pollen extract did not inhibit the binding to the two chicory bands, which excludes the involvement of cross-reactive glycans in this case. We did not find, however, a candidate protein to be held responsible for this cross-reactivity. Neither rBet v 1a, nor rBet v 2, which we assayed because the CAP test for Bet v 2 was more than 0.35 Ua/ml, could inhibit the binding to the two chicory-reactive bands at 15–17 kD.

The case of patient LVD is somewhat different from the three other cases, because complaints about chicory appeared during desensitization. Cross-reactivity with birch pollen was ascertained by immunoblot inhibition experiments. The occurrence of chicory reactivity might have been a consequence of the birch pollen desensitization. That inhalant allergen injections might induce IgE to cross-reactive food allergens has already been reported, especially in the context of the mite-snail syndrome [10–12]. It could also be that this reactivity simply reflects the initial rise in serum IgE during the first months of desensitization. Indeed, this could exacerbate a nondeclared preexisting reactivity to chicory up to a clinical level. However, no rise in IgE to rBet v 2 could be observed to further support this hypothesis. There is, therefore, no clear explanation for the emergence of the chicory allergy in this patient.

The first message of this report, when analyzing our 4 patients together with previously published cases [2–4], is that chicory allergy is not characterized by a typical sensitization profile. However, reactivity in the range 45–52 kD has been described in all the above-mentioned cases [2–4]. In addition, IgE reactivity between 40 and 50 kD has up to now been found in all cases of allergy to the botanically related lettuce [4, 13, 14]. These might be independent descriptions of a same major allergen of the Compositae family.

The second message of our report is that there can be true IgE cross-reactivity between birch pollen and chicory. Our two more extensively investigated patients were polysensitized and it might be that other cross-reactivities could have been found between pollen and other foods. However, due to serum limitations we had to restrict the

study to chicory-birch cross-reactivity. This has led us to the finding that a yet unknown 51-kD birch protein can be involved in food-pollen cross-allergies. Although our present examples do not involve Bet v 1, cross-reactions involving this molecule cannot be excluded in other patients with this syndrome, especially as bands in the chicory pattern were specifically recognized by the anti-Bet v 1 monoclonal antibody 5H8. This cross-reactivity should be kept in mind, especially in the countries where chicory is widely consumed.

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