CASE REPORT

Occupational Rhinoconjunctivitis and Asthma Caused by Chicory and Oral Allergy Syndrome Associated With Bet v 1–Related Protein

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Abstract

We report the case of a patient working in a factory producing inulin from chicory who developed rhinoconjunctivitis and asthma to the dust of dry chicory roots and oral allergy syndrome to raw fruits and vegetables.
Nonspecific bronchial hyperresponsiveness was diagnosed. A provocation test with dry chicory induced acute rhinoconjunctivitis and an immediate asthmatic response with no further clinical symptoms. Skin prick test results were positive to birch pollen and fresh/dry chicory, and negative for inulin. Specific immunoglobulin (Ig) E was >100 kU_A/L for rBet v 1. Specific IgE were detected by immunoblotting chicory extract with the patient's serum, but not with a control serum. The main immunoreactive band corresponded to a protein with a molecular weight of approximately 17 kDa, like Bet v 1, and this immunoreactivity was effectively inhibited by preincubating serum with purified Bet v 1.
This case documents occupational rhinoconjunctivitis and asthma due to IgE sensitization to inhaled chicory allergens, including one identified for the first time as a 17-kD Bet v 1 homologous protein, with secondary oral allergy syndrome to related foods.

Key words: Chicory. Allergen. Specific IgE. Birch allergy. Occupational allergy.

Resumen

Comunicamos el caso de un paciente trabajador de una fábrica productora de inulina de la achicoria, que desarrolló rinoconjuntivitis y asma al polvo de las raíces secas de la achicoria y síndrome de alergia oral a frutas y vegetales crudos.
Fue diagnosticado de hiperreactivad bronquial inespecífica. Un test de provocación con achicoria seca indujo rinoconjuntivitis aguda y respuesta asmática inmediata sin otra clínica asociada. Los resultados de las pruebas cutáneas Prick fueron positivos para el polen de abedul y achicoria fresca/seca, y negativa para la inulina. La inmunoglobulina (Ig) E fue >100 kU_A/L para el rBet v 1. Se detectó IgE específica mediante un Inmunoblot de extracto de achicoria con el suero del paciente, pero no con suero control. La principal banda inmunoreactiva se correspondió con una proteína, Bet v 1, con un peso molecular de aproximadamente 17 kDa. Esto se inhibía preincubando el suero con Bet v 1 purificado.
Este caso documenta rinoconjuntivitis y asma ocupacional debido a sensibilización de IgE por alérgenos de achicoria inhalada, incluyendo uno identificado por primera vez como una proteína homóloga de Bet v 1 de 17-kD, con síndrome de alergia oral secundario a alimentos relacionados.

Palabras clave: Achicoria. Alérgeno. IgE específica. Alergia a abedúl. Alergia ocupacional.

Introduction

Chicory belongs to the Asteraceae family (Compositae). The different species include *Cichorium* endivia and *Cichorium intybus*. *Cichorium intybus* var. *sativum* is grown for its roots, which are used to produce inulin and roasted to produce coffee substitute. The white leaves of *Cichorium intybus* var. *foliusum*, also known as witloof or Belgian endive, are eaten raw or cooked.

Chicory-induced asthma was first reported in 1989 in a chicory grower [1]. In 1996, a case of occupational allergy to chicory was reported, with oral, respiratory, and cutaneous manifestations [2], and there has also been a report of contact urticaria and food allergy to chicory in a patient sensitized to grass pollen, with no cross-reaction to lettuce [3]. More recently, Cadot et al [4] described 4 new cases with respiratory symptoms to birch pollen and oral allergy syndrome (OAS) to chicory in nonoccupational settings. Finally, Morita et al [5] described a case of occupational contact urticaria with chicory in a ragweed-sensitized patient who experienced anaphylactic reactions after washing lettuce.

We report a case of occupational allergy to the dust of chicory roots in a patient who was sensitized to birch pollen, experienced seasonal work-related asthma and rhinoconjunctivitis, and later developed OAS to Bet v 1–related foods.

Case Description

A 32-year-old man had been working for a few months as an engineer in a Belgian factory producing inulin from chicory

Table. Results of Skin Testing and Specific Immunoglobulin E Antibodies

A

Commercial Aeroallergens (Prick Tests)	Size of Wheal, mm	Fresh Extracts (Prick-to-Prick)	Raw: Wheal Size, mm	Cooked: Wheal Size, mm
Dermatophagoides pteronyssinus	2×2	Soy milk	14×12	
Dermatophagoides farinae	0	Apple	6×5	0
Cat dander	0	Peach	10×5	0
Dog dander	0	Strawberry	13×6	
Alder pollen	15×16	Pear	0	
Birch pollen	8×9	Plum	4×4	
Hazel tree pollen	12×11	Cherry	0	
Grass pollen	0	Celery	5×5	
Mugwort	0	Parsley	18×10	
Latex	0	Carrot	15×7	2×3
Aspergillus mix	0	Potato	0	
Alternaria	0	Dry chicory root	7×7	
Cladosporium	0	Fresh chicory root	10×10	
Penicillium	0	Na metabisulfite	0	
Codeine phosphate, 9%	8×7	Inulin	0	
Negative control	0	Wheat	0	

B

Serum Specific Immunoglobulin E, kU_A/L				
rBet v 1	> 100	Carrot	2.03	
rBet v 2	<0.35	Celery	23.7	
Bromelain	0.81	*Phleum pratense*	1.07	
Soy	0.67	Total IgE	896	

Abbreviation: Ig, immunoglobulin.

roots when he developed acute asthma and rhinoconjunctivitis in the presence of dust from dry chicory roots (processed between March and June). Both were reported as more intense in the late evening at home, although they improved at the weekend. Five months later, the patient developed OAS when eating raw apple, pear, and carrot, and rhinoconjunctivitis when peeling carrots, potatoes, and zucchini. Between September and March, when raw chicory is processed, the respiratory symptoms disappeared. He had never experienced symptoms of respiratory allergy to birch pollen or food-related cross-reactions.

Nonspecific bronchial reactivity to histamine and specific pulmonary challenge were performed according to published recommendations [6,7]. In the specific provocation test, the patient had to mix dry chicory roots in a bowl without direct contact with the skin or clothes. The patient was monitored and spirometry was performed every 10 minutes till the end of exposure (determined by a 20% reduction in forced expiratory volume in 1 second [FEV_1]), then every 30 minutes during the next 2 hours, and every 60 minutes till the end of the test (7 h).

Skin prick tests (SPT) were performed to common aeroallergens using standardized extracts (Stallergènes SA, Antony, France) and to both fresh and cooked fruits and vegetables, inulin, and chicory used in the factory (by prick-by-prick testing). A skin test was considered positive if the mean wheal diameter at 15 minutes was ≥3 mm greater than the negative test, while 9% codeine phosphate was used as a positive control.

A serum sample was obtained from the patient, and from nonatopic individuals (controls). Specific immunoglobulin (Ig) E antibodies were assessed using the ImmunoCAP System (Phadia, Uppsala, Sweden), with a value >0.35 kU_A/L considered as positive.

Chicory extract was prepared from dry chicory roots according to Relyveld et al [8] and incubated in phosphate-buffered saline (PBS), pH 7.2, overnight at 4°C with stirring. The solution was centrifuged at 3000 rpm and supernatant proteins were precipitated by the addition of 4.5 M ammonium sulphate (Sigma Aldrich, St Louis, Missouri, USA). After centrifugation, the protein pellet was resuspended in PBS and the protein concentration was determined at 1.6 mg/mL using the bicinchoninic acid assay (Pierce, Rockford, Illinois, USA). Reactivity to the leaves of *Cichorium intybus* var. *sativum* was not tested, because the patient had not been exposed to or eaten them.

Chicory proteins and birch/grass extracts (Stallergènes, Paris, France) were loaded (5 µg of protein) into a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-12% PAGE) and run at 180 V for 1 hour. Proteins were transferred onto a nitrocellulose membrane (Hybond-C, Amersham Biosciences, Little Chalfont, UK) at 70 mA for 1.5 hours at room temperature. The membrane was blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline with 0.1% Tween 20 (TBST) for 1 hour at room temperature, before being washed and probed with the patient's serum or control serum (diluted 1:10 in PBS) overnight at 4°C. The membrane was then incubated for 1 hour at room temperature with HRP-conjugated anti-human IgE antibodies (clone LOHE-17, Prof. Bazin, Brussels, Belgium) diluted 1:10 000 in TSBT-1%. Immunoreactive bands were developed using chemiluminescence (ECL, Amersham Biosciences) and detected with the Chemidoc XRS apparatus (Bio-Rad Laboratories, Hercules, California, USA). Inhibition assays were performed by preincubating the patient's serum for 1 hour with 50 µg/mL of purified Bet v 1 (ALK-Abelló, Horsholm, Denmark) before probing the membrane as described above.

Whereas baseline lung function was normal, nonspecific bronchial hyperresponsiveness to histamine was documented at the beginning of June with a reduction in FEV_1 of 640 mL

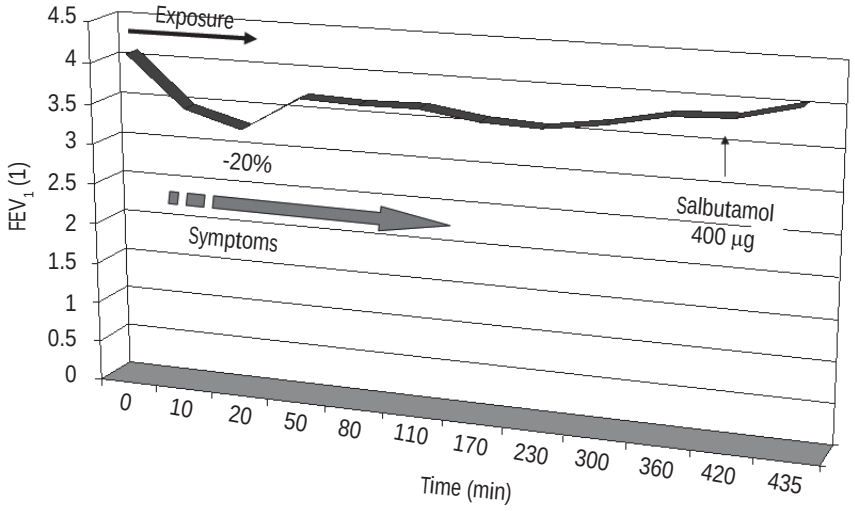

Figure 1. Specific bronchial provocation test with dry chicory roots. A drop in FEV_1 (–820 mL, –20%) was observed after 20 minutes, while facial pruritus and rhinoconjunctivitis appeared within 10 minutes of exposure (and persisted for 2 hours). FEV_1 remained significantly reduced for >4 hours until salbutamol was administered. FEV_1 indicates forced expiratory volume in 1 second.

(–16%) after inhalation (at tidal volume) of 1 mg/mL histamine for 3 minutes. A challenge test was performed under realistic conditions at the end of June with dry chicory. It induced facial pruritus and acute rhinoconjunctivitis after 10 minutes of exposure and a reduction in FEV_1 (–820 mL, –20%) after 20 minutes. Rhinoconjunctivitis persisted for 2 hours, whereas FEV_1 remained low for >4 hours until salbutamol was administered (Figure 1). No respiratory symptoms were reported within the next 24 hours.

Peak expiratory flow rate could not be monitored at work, as the patient changed jobs before the next chicory season.

SPT results were positive for birch pollen, raw carrot, celery, soy, apple, strawberry, peach, and fresh and dry chicory, and they were negative for grass and mugwort pollens, inulin, and cooked Rosaceae (Table, A).

Specific IgE levels were positive for rBet v 1, celery, *Phleum pratense*, carrot and bromelain (Table, B). An immunoreactive band of approximately 17 kDa was recognized by the patient's serum IgE in the chicory extract (Figure 2, A). Weaker signals were also observed for larger proteins (4-5 bands, from approximately 45 kDa to 75 kDa). No signal was observed in the chicory extract or grass and birch extracts with control sera (data not shown). The 17-kDa protein migrated as purified Bet v 1 and was abrogated by preincubating the patient's serum with purified Bet v 1 (lane 3 in Figure 2, B), in contrast with the other (higher molecular weight) bands. The specificity of the inhibition test was controlled by the absence of effect of preincubation with Bet v 1 on IgE to grass.

Discussion

We report a case of occupational IgE-mediated allergy to the dust of dry chicory roots. This allergy progressed with respiratory symptoms (asthma, rhinoconjunctivitis) and OAS after contact with Bet v 1–related foods. One of the IgE-reactive chicory allergens was identified as a 17-kDa protein corresponding to a Bet v 1–homologous protein. Occupational chicory allergy with inhalative, oral, and cutaneous symptoms had already been reported, although the patient was not sensitized to birch pollen and a 48-kDa chicory protein was identified as the IgE binding antigen [2]. Chicory allergy had also been reported in 4 patients sensitized to birch pollen but with no occupational exposure to chicory [4]. These 4 patients had birch pollen–related respiratory symptoms—with or without grass pollen allergy—that manifested as rhinoconjunctivitis, asthma, and OAS with cross-reacting foods. They also developed OAS when eating chicory leaves,

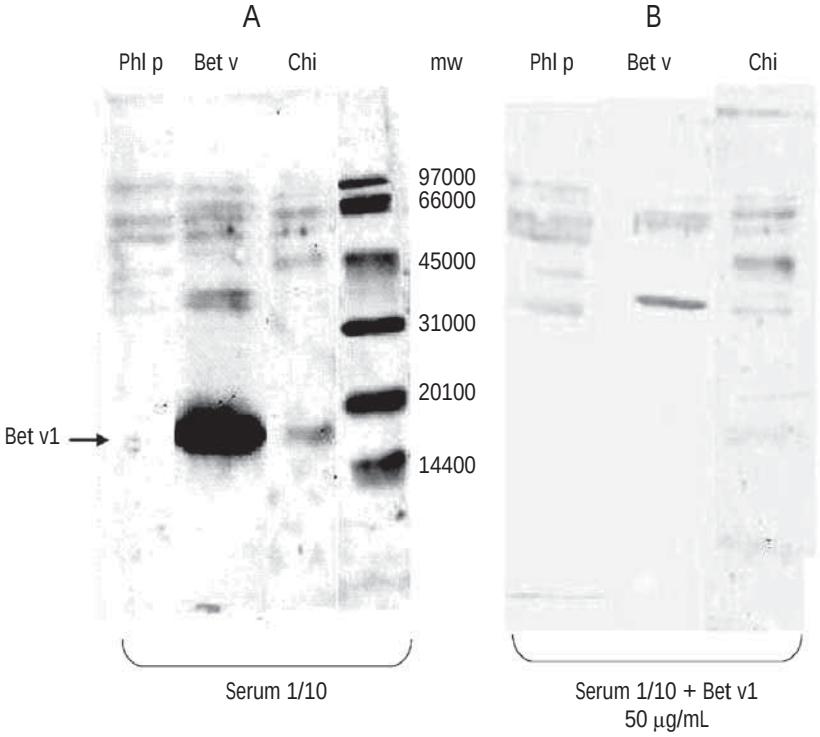

Figure 2. A, IgE immunoblot to grass pollen (Phl p, *Phleum pratense*, lane 1), birch pollen (Bet v, *Betula verrucosa*, lane 2), and chicory (Chi, lane 3). After SDS-PAGE of allergen extracts, membranes were probed with the patient's serum, and IgE-binding proteins were revealed by enhanced chemiluminescence. B, The same membrane was re-probed with patient's serum preincubated with purified Bet v 1 (50 μg/mL). Molecular weight markers (mw) are shown in the medium (in daltons, D). Ig indicates immunoglobulin; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

and the individual patterns of IgE-binding to chicory proteins were different, each serum recognizing more than one protein. The observed birch–chicory cross-reactivity did not involve Bet v 1 but a 51-kDa birch protein.

In contrast to these cases of nonoccupational food allergy to chicory with sensitization to birch pollen, our patient developed respiratory symptoms as a result of occupational exposure to the dust of dry chicory roots of the species *Cichorium intybus* var. *sativum*. He remained able to eat raw or cooked leaves of *Cichorium intybus* var. *foliosum*, but later (5 months after the beginning of work-related respiratory symptoms) developed OAS to raw fruits and vegetables known for their cross-reactivity to Bet v 1. Immunoblotting revealed that a 17-kDa chicory protein was responsible for binding to serum IgE, and inhibition assays indicated that this protein corresponded to a Bet v 1–homologous protein. Other IgE binding proteins (with a higher molecular weight) of chicory have also been identified. Cadot et al [4] reported chicory IgE binding proteins of 18 kDa and 21 kDa (probably related to cross-reactive carbohydrate determinants), as well as more relevant proteins of 52 kDa and 71 kDa. All these chicory proteins cross-reacted with birch pollen. We hypothesize that the 17-kDa chicory root allergen recognized by our patient's IgE consisted of a Bet v 1–related inhaled protein to which the patient became sensitized through the respiratory route. Accordingly, the patient had (respiratory) symptoms only when dry roots were processed and did not display clinical hypersensitivity to fresh chicory roots. Interestingly, we were able to demonstrate that cross-reactivity involved Bet v 1 allergen, in contrast to previous reports of (food) allergy to chicory among patients sensitized to birch pollen [4].

In conclusion, we report a case of occupational IgE-mediated allergy to chicory with unique clinical and immunological features, namely, rhinoconjunctivitis and asthma mediated by IgE recognition of chicory allergens including a Bet v 1–homologous protein, and with OAS secondary to Bet v 1–related foods.

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