Tobacco allergy: demonstration of cross-reactivity with other members of Solanaceae family and mugwort pollen

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Background: Tobacco is a plant belonging to the Solanaceae family. This plant is usually used as a contact insecticide for several infestations in some areas, such as the Canary Islands. Allergy induced by inhalation of this plant is unusual. Identification of the potential allergen in growing areas is essential.

Objective: We report a patient with occupational sensitivity to an aqueous solution of cut tobacco whose clinical manifestations were rhinoconjunctivitis and urticaria. Past medical history was significant for seasonal allergic rhinoconjunctivitis to mugwort pollen and oral allergy syndrome with avocado.

Methods: Green tobacco and cured tobacco leaf extracts were prepared, skin prick tests were performed with green tobacco, cured tobacco leaf extracts, and certain aeroallergens. Conjunctival challenge test was carried out with green tobacco and cured tobacco leaf extract. Serum-specific IgE against tobacco leaf was performed by commercial CAP. CAP inhibition experiments were carried out with tobacco and *Artemisia vulgaris*.

Results: Skin prick tests and conjunctival challenge tests with green tobacco and cured tobacco leaf extracts were positive, as well as serum-specific IgE by CAP, indicating an IgE-mediated sensitization. CAP inhibition experiments were carried out and it was found that tobacco, mugwort pollen, and tomato extracts inhibited the binding of the patient's serum to solid-phase tobacco leaf. No inhibition was observed when Alternaria, *D. pteronyssinus*, and potato were used as control inhibitors. Inhibition of immunoCAP to mugwort was obtained with mugwort and tobacco extracts and no cross-reactivity to *D. pteronyssinus* was shown.

Conclusion: The results suggest that tobacco can induce IgE-mediated reactions that are mediated by the existence of common antigenic epitopes between tobacco and mugwort pollen. This allergy can be a hazard of employment in the agricultural areas.

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INTRODUCTION

Tobacco is a plant of the Nicotiana genus, native to Central America. It belongs to the Solanaceae family, along with tomato, potato, eggplant, and green pepper. Tobacco is cultivated in tropical areas such as the Canary Islands. Fruit and vegetable crops are also fumigated with an aqueous solution of cut tobacco. The tobacco leaf is placed in a boiling water bath, after this the juice is sprayed on the plant. Nicotine, an alkaloid of tobacco plants, is used for eliminating the eggs of the insects.

Tobacco smoke represents one such environmental agent that has the potential for inducing neoplasm and widespread respiratory effects including aggravation of asthma. Tobacco sensitivity has not been the subject of many studies because it is a rare cause of allergic reactions. To our knowledge only one case of tobacco allergy has been described in the literature review. Gleich et al[1] reported a patient who experienced rhinitis and asthma while working in a cigarette factory and who was sensitive to allergens in tobacco leaf.

In the present paper, we report one patient with occupational sensitivity to an aqueous solution of tobacco leaf and we describe his clinical characteristics and associated sensitivities.

CASE REPORT

We describe a 21-year-old male, nonsmoker, whose past medical history was significant for seasonal allergic rhinoconjunctivitis and oral allergy syndrome with avocado. Previous skin prick tests were positive to mugwort pollen, *Alternaria tenuis*, and raw avocado. He worked as a gardener for 3 years and for the last 3 months of those years he had experienced nasal itching, sneezing, rhinorrhea, watery eyes, and generalized urticaria while fumigating orange trees with an aqueous solution of cut tobacco; these symptoms occurred within 30 to 40 minutes of starting work and disappeared within 2 hours after work. He usually improved during weekends, and became asymptomatic when he stopped fumigating for 2 weeks. He also remained asymptomatic during the months the orange trees are pollinating. Since the onset of this illness he had noted that exposure to cigarette smoke particularly in crowded rooms where many people were smoking, induced lacrimation and rhinorrhea but not urticaria. The results of a physical examination were normal (in spring). The white blood cell count was 5.760/mm^3 with 120 eosinophils/mm^3. Chest and sinus radiographs were within normal limits.

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Basal spirometry disclosed FEV_1 of 102% and FVC of 93% of predicted. Suspecting occupational rhinoconjunctivitis and urticaria, an etiologic study with our patient's informed consent was initiated.

MATERIAL AND METHOD

Tobacco Extract

Green tobacco and cured tobacco leaf were supplied by the patient. He cut a leaf of green tobacco the day before we prepared the extracts which we used. The cured tobacco leaf was cut by the patient from the tobacco plant about 6 months ago. We prepared extracts of green tobacco and cured tobacco leaf in our laboratory. Each raw material was extracted in 10% wt/vol of phosphated buffered saline (PBS) (pH 7.3). After stirring for 30 minutes, solutions were passed through a filter paper and dialyzed overnight in PBS using Spectrapor dialyzed membrane (molecular weight cut-off, 10 kDa) (Spectrum Medical Industries Inc, USA). After centrifugation at 3,000 *g* for 15 minutes, supernatants were filtered through Watman 1 filter paper and were passed through a 0.22-μm Millipore filter (Millipore, USA) for sterilization. The supernatants were frozen until use, and stored at −20°C. Protein content was determined by the Bradford method (Bio-Rad, USA).[2]

All the other allergen extracts used were biologically standardized extracts that were provided by a pharmaceutical laboratory for aeroallergens (Abello SA, Madrid, Spain) and from foods (Bencard, Worthing, UK).

In Vivo Tests

Skin Tests

Cutaneous tests were carried out by means of a skin prick tests, and were performed by the method of Dreborg.[3] Available commercial skin prick tests, extracts were done with a battery of commercial aeroallergens (mites, fungi, pollens, and animal dander from dog and cat) and foods from the Solanaceae family (potato and tomato). In-house extract from green tobacco (1 μg/mL to 20 μg/mL, wt/vol) and cured tobacco leaf (1 μg/mL to 10 μg/mL, wt/vol), at decreasing dilutions, were tested on the patient.

Histamine phosphate at 10 mg/mL was used as positive control and physiologic saline as negative control. Lancets (Prick-Lancetter, Dome Hollister-Stier, Miles Laboratories Ltd, UK) were used to puncture the skin. The results of the skin test were read at 15 minutes. Wheal diameters equal or greater than 3 mm were considered as positive in the absence of a response to the saline control. Twenty healthy volunteers and 20 atopic patients were used as the control group for skin prick test.

Conjunctival Challenge

Conjunctival challenge test was performed with green tobacco (1:50, wt/vol) and cured tobacco leaf (1:50, wt/vol) according to the procedure of Möller.[4] Allergen extracts were applied in the lower conjunctival sac at intervals of 20 minutes, beginning with the most diluted. Criteria for a positive test were congestion of the conjunctival mucous and itching of the eyes. Conjunctival challenge tests to tobacco were performed in 10 control subjects.

In Vitro Tests

Total IgE

Total serum IgE was measured by the Delfia method (Pharmacia Diagnostics, Sweden), according to the manufacturer's instructions.

Specific IgE

Serum specifics IgE to *Dermatophagoides pteronyssinus*, *Alternaria tenuis*, *Artemisia vulgaris*, tobacco leaf, potato, and tomato were measured by Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden). The results were expressed in kU/L. A CAP level equal or greater than 0.7 kU/L were considered to be positive.

CAP Inhibition

We carried out an immunoinhibition assay in liquid phase in the CAP system. Aliquots (50 μL) of the patients serum were incubated for 2 hours at room temperature with 50 μL of 10-fold extract of: *Dermatophagoides pteronyssinus*, *Alternaria tenuis*, *Artemisia vulgaris*, potato, tomato, and with a solution of green tobacco [1 μg/mL, wt/vol dilutions (100 μg/mL to 100 nag/mL) on phosphate buffer, pH 7.4, containing 0.03% (wt/vol) human albumin]. With all the immunodistributed samples incubated with the different dilutions of the allergens in liquid phase, ImmunoCAP to tobacco leaf and artemisia (Pharmacia Sweden) (solid phase) were then performed in duplicate, according to Pharmacia's directions for use. The degree of inhibition was measured in percentage, the zero level being defined as the uptake to the solid phase when allergen is replaced with phosphate buffer.

RESULTS

Our patient's skin prick tests to *Dermatophagoides pteronyssinus*, animal dander (dog and cat), and potato were

Table 1. Results of In Vivo and In Vitro Tests

	Prick Test, Mm	CAP, kU/L	Conjunctival Challenge
Der p	3	<0.35	
Alternaria	6	4.1	
Mugwort pollen	9	3	
Dog, Cat	1	<0.35	
Potato	1	2.68	
Tomato	3	1.42	
Raw avocado	4	2.59	
Green tobacco	3	n.d.	Positive
Cured tobacco	4	n.d.	Positive
Tobacco leaf	5	3.44	

n.d. = not done.

negative. Skin prick tests, to *Alternaria tenuis*, *Artemisia vulgaris*, and tomato were positive. The solutions of green tobacco (10 μg/mL, wt/vol) and cured tobacco leaf (1 μg/mL, wt/vol) were strongly positive with the allergen wheal areas being larger than wheal areas of histamine (Table 1). Skin prick tests to solutions of green tobacco and cured tobacco leaf were negative in 40 controls subjects.

The patient had a positive conjunctival provocation test with the lowest concentration of tobacco administered. Conjunctival injection, nasal obstruction, and rhinorrhea occurred in the patient during the provocation test. It was negative in the 10 controls subject.

Our patient's total IgE was 710 kU/L and serum-specific IgE results for *Alternaria tenuis*, *Artemisia vulgaris*, potato, tomato, and tobacco leaf were positive (Table 1). Protein content was 20 μg/mL for green tobacco and 11.4 μg/mL for cured tobacco leaf. During tobacco leaf CAP inhibition, the preincubation of the patient's serum with dilutions of green tobacco extract 1:10 resulted in 100% inhibition; the tobacco leaf CAP inhibition by tomato extract in liquid phase was 67% and by *Artemisia vulgaris* was 63%. The addition of *Dermatophagoides pteronyssinus*, *Alternaria tenuis*, and potato at concentrations as high as 1:10 yielded no inhibition (Fig 1). Finally, during artemisia CAP inhibition with increasing concentration of green tobacco extracts in liquid phase, a progressive inhibition was obtained, reaching 61% at the maximum concentration, on incubation with artemisia, the inhibition was 100%; and with *Dermatophagoides pteronyssinus*, 23%.

To exclude the possibility that the observed cross-inhibition was due to nonspecific binding of IgE, similar experiments (with ovalbumin as solid phase) were performed with a serum pool with a known level of specific IgE to ovalbumin. None of these IgE reactions were affected by preincubation of the sera with the allergens of the study (data not shown).

DISCUSSION

Lehrer et al[5] found that IgE antibodies against crude tobacco leaf were present in smokers, non-smokers, and ex-smokers and that atopic individuals were far more likely to show such responses than nonatopic individuals. It has also been established that IgE antibodies against at least three specifics tobacco leaf allergens could be detected, but these IgE antibodies did not correlate with any type of clinical tobacco smoke sensitivity. We report a case of immediate hypersensitivity to tobacco. This plant can induce IgE-mediated reactions with various clinical manifestations, varying from rhinoconjunctivitis to urticaria. We consider that the clinical history together with a positive skin prick test, and conjunctival challenge is sufficient for correct diagnosis and that the tobacco extract was not acting merely as irritant in a man with IgE antibodies against tobacco. We did not find control subjects who were both agricultural workers and who would have been exposed to tobacco extract. It would be helpful to have more information about this disease.

It is interesting to observe that the presence of symptoms to tobacco were accompanied by positive skin prick tests, CAP and CAP-inhibition to tomato, correlated on the basis of botanically related vegetables. This clinically significant association indicates that crossreacting allergens of different kinds are present in these vegetables. Our patient, however, experienced an IgE-mediated rhinoconjunctivitis to tobacco but he did not report symptoms after eating tomato and other vegetables of the Solanaceae family. Perhaps tobacco leaf allergy may exist before the appearance of food allergy to tomato or other members of the same family. Future, clinical assessments of this patient will be necessary to detect any new food allergy.

In our study, however, allergy to mugworth pollen appeared associated with the sensitivity to tobacco. We found the CAP-inhibition to tobacco and the converse study done (tobacco inhibiting IgE from to mugwort in solid phase) confirmed crossreactivity with mugwort pollen, suggesting the presence of at least some epitopes to both allergens. Since only one patient was studied, general conclusions cannot be drawn, but the existence of vegetable panallergens such as profilins[6,7] (ubiquitous proteins present in plants, in pollen, fruits, leaves, and roots) or other common antigens could explain these reactions. There are a few studies about the crossreactivity between mugwort and Solanaceae family. Crossreactivity among *Artemisia vulgaris* and vegetables belonging to the Compositae and Umbilliferae families is known.[8–10] In these cases it can be speculated that the sensitivity to vege-

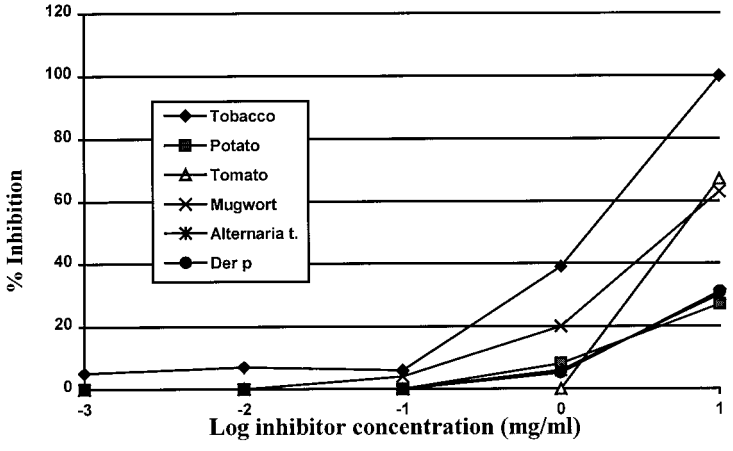

Figure 1. Cap inhibition studies performed with tobacco leaf (solid phase) on incubation with tobacco, potato, tomato, *Artemisia v, Alternaria t, Dermatophagoides p* extracts (liquid phase).

tables and fruits are a consequence of a primary sensitivity induced by crossreactive antigens present in mugwort pollen. From a general point of view, the pollinosis almost always preceded the appearance of food allergy, suggesting it may be a factor favoring allergy to fruit and vegetable plants.[10,11]

Tobacco is an allergen that rarely has been identified the development of occupational allergy among farmers. There are other substances, however, which may be found in the workplace, that cause problems such as storage mites, molds or pollens, all of which have allergenic capacity and might be causative agents of occupational allergy.[12] We believe these allergens should always be considered as etiologic agents in the diagnosis of occupational allergy. Other substances besides these can act as occupational allergens among farmers as a result of repeated exposure. Farmers become sensitized through an IgE-mediated mechanism. This patient was sensitive to mugwort pollen and Alternaria as well as to tobacco.

Finally, our results confirm reports in the literature that allergy to tobacco can be an occupational hazard. This study suggests that tobacco leaf is able to induce respiratory and cutaneous IgE-mediated allergy. The lack of tobacco allergy reports in the medical literature review could be due to poor recognition of this disorder by the medical community.[12] The high tobacco use in the Canary Islands may also increase the incidence of tobacco sensitivity in this geographic area.

We concluded that tobacco leaf is a potential cause of IgE-mediated sensitization. We have not found any reports of patients allergic to aqueous solutions of cut tobacco. Epidemiologic studies are necessary to assess the importance of tobacco allergen among exposed gardeners and among the general population in tobacco-growing areas.

REFERENCES

1. Gleich GJ, Welsh PW, Yunginger JW, et al. Allergy to tobacco: an occupational hazard. N Engl J Med 1980;13: 617–619.
2. Bradford M. A rapid and sensitive method for the quantification of microgram qualities of protein utilizing the principle of protein-dye binding. Ann Biochem 1976;72:248–254.
3. Dreborg S, ed. Skin test used in type I allergy testing. Position paper. Allergy 1989;44(Suppl 10):1–59.
4. Möller C. The Precision of the conjunctival provocation test. Allergy 1984;39:37–41.
5. Lehrer SB, Stankus RP, Salvaggio JE. Tobacco smoke sensitivity: a results of allergy? Ann Allergy 1986;56: 369–377.
6. Valenta R, Duchene M, Ebner C, et al. Profilins constitutes a novel family of functional plant pan-allergens. J Exp Med 1992;175:377–385.
7. Pauli G, Oster P, Deviller P, et al. Skin testing with recombinant allergens rBet v1 and birch profilin, rBet v2: diagnostic value for birch pollen and associated allergies. J Allergy Clin Immunol 1996;97:95.
8. Pauli G, Bessot JC, Braun PA, et al. Celery allergy: clinical and biological study of 20 cases. Ann Allergy 1988; 60:243–246.
9. Pauli G, Bessot JC, Dieterann-Molard A, et al. Celery sensitivity: clinical and immunological correlations with pollen allergy. Clin Allergy 1985;15: 273–279.
10. Ortolani C, Ispano M, Pastorello EA, Bigi A, et al. The oral allergy syndrome. Ann Allergy 1988;61:47–52.
11. Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen and allergy. Allergy 1983; 38:167–172.
12. Bernstein DI, Malo JL. High molecular weight agents. In: Bernstein IL, Chan-Yeung M, Malo JL, Bernstein DI, eds. Asthma in the workplace. New York: Marcel Dekker, 1993: 373–398.

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