

## Identification of obeche wood (*Triplochiton scleroxylon*) allergens associated with occupational asthma

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Obeche (*Triplochiton scleroxylon*), also called African maple or samba, is a West African tree belonging to the Sterculiaceae family. This soft wood is widely used for all purposes for which strength and durability are not very important, and it is suitable for framing, plywood, boxes, planks, model making, and sauna benches. Exposure to obeche wood dust has been shown to cause occupational asthma<sup>1,2</sup> and contact urticaria.<sup>3</sup> The pathogenic mechanism involved in obeche-induced asthma seems to be IgE dependent because positive skin test responses and serum IgE antibodies to obeche have been found in symptomatic exposed subjects.<sup>1-3</sup> The aim of this study was to characterize the obeche wood allergens by means of immunoblotting.

### METHODS

#### Patients

Four carpenters and one framer (4 men and 1 woman) who had symptoms of rhinorrhea, nasal stuffiness, cough, chest tightness, shortness of breath, and wheezing immediately after exposure to obeche wood dust were evaluated. Their ages ranged between 31 and 69 years (mean age, 49.2 years). They had been working in carpentry between 4 and 55 years (mean, 23.8 years). The mean duration of asthma symptoms in these patients was 11.7 years (range, 1.5-25 years). All of them improved on holidays and weekends. Three patients were atopic. Four patients were nonsmokers, and one was an exsmoker. Bronchial challenge tests to obeche extract were performed in 3 patients according to a previously described method,<sup>1</sup> and it elicited isolated early asthmatic responses in all of them. In the remaining two subjects the diagnosis of occupational asthma was confirmed by the significant changes observed in peak expiratory flow monitoring during periods at work and off work.

#### Allergen extract

Obeche wood dust was extracted (5% wt/vol) in ammonium bicarbonate (0.125 mol/L, pH 8.2). After 16 hours of extraction at

4°C, the suspension was centrifuged at 10,000 rpm for 30 minutes, the pellet was discarded, and the remaining solution was filtered through a 0.22- $\mu$ m pore size membrane and dialyzed against distilled water by tangential ultrafiltration in 3000-d cut-off membranes (Millipore, Bedford, Mass). Finally, the extract was lyophilized to obtain a 0.318% wt/wt content from the raw material. A 1% wt/vol obeche extract was prepared for skin prick testing.

#### Specific IgE determinations

The serum levels of specific IgE against obeche were determined in duplicate by using ELISA with a concentration of 20  $\mu$ g/mL and serum dilution series starting from 1:4, as described elsewhere.<sup>4</sup> A result was considered positive when a serum bound 4 times more specific IgE than the mean titer of 5 atopic unexposed control sera.<sup>4</sup> ELISA was also performed with a deglycosylated obeche extract. The deglycosylation treatment was done in nonreducing conditions with a commercial kit (170-6500; Bio-Rad, Hercules, Calif), which enzymatically cleaves all N- and most O-linked oligosaccharides from glycoproteins and glycopeptides.

#### SDS-PAGE and immunoblotting

SDS-PAGE was carried out according to the Laemmli<sup>5</sup> method in reducing conditions. Polyacrylamide concentrations of 15% and 4% were used for separating and stacking gels, respectively, and 120  $\mu$ g of obeche extract were applied per lane. Separated proteins were electrotransferred to Immobilon-P membranes (Millipore), and the membrane was then incubated with the patient's serum diluted 1:5 in 10 mmol/L PBS containing 0.1% polyoxyethylene-sorbitan monolaurate (Tween-20). After washing, the membrane was incubated with anti-human IgE conjugated with peroxidase in the same buffer containing 3% human serum albumin. The protein bands were developed with 3-3', 5-5' tetramethylbenzidine and oxygen peroxide as the substrate.

### RESULTS

The 5 woodworkers had positive skin prick test responses to obeche wood extract at 1% wt/vol. Measurement of obeche-specific IgE by means of ELISA was positive in all the patients: 0.86, 1.19, 1.26, 3.27, and 2.18 ODs at 450 nm, respectively. A negative result was obtained in 5 obeche-exposed, symptom-free, control subjects ( $0.05 \pm 0.01$  OD) and with a serum pool of subjects allergic to grass pollen (0.03 OD). When ELISA was performed with a serum pool from the 5 patients and deglycosylated samba extract, the IgE reactivity was reduced by only 8.6%, indicating that the IgE-binding epitopes on obeche wood were not of a carbohydrate nature.

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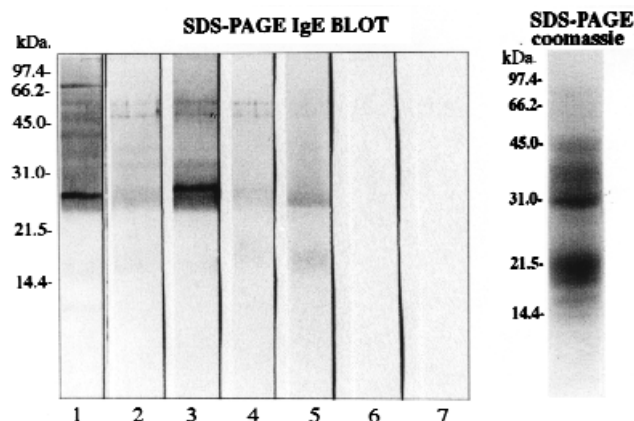


**FIG 1.** SDS-PAGE and IgE immunoblots of obeche wood dust with individual sera from 5 woodworkers (lanes 1-5), a serum pool from control subjects allergic to grass pollen (lane 6), and nonspecific binding without serum (lane 7).

Molecular weight analysis of obeche wood showed that it contains a wide range of protein sizes (Fig 1). Testing the sera of the 5 woodworkers, we could demonstrate IgE reactivity with separated blotted obeche wood proteins. A similar IgE-binding pattern of obeche extract was observed with all reactive sera, although IgE-binding bands were more intense with patients 1 and 3 (Fig 1). The sera from the 5 carpenters reacted to allergens with apparent molecular weights of 63.8, 57.5 kd, and particularly with an intense band at 28 kd. An additional very faint band at 16.8 kd was also visualized. Other bands with the ability to bind IgE with apparent molecular weights of 50.5, 43.2, and 36.7 and 30.3 kd could be seen in patients 1, 2, and 3. With the serum pool of subjects allergic to grass pollen, no IgE-binding bands were observed (lane 6). Moreover, no bands were seen with serum from 5 obeche-exposed, symptom-free, control subjects (not shown).

## DISCUSSION

The immunologic studies performed strongly suggest that the pathogenic mechanism responsible for the devel-

opment of respiratory symptoms in workers who inhale obeche wood dust is IgE dependent. There appears to be several major allergens in obeche wood, with an allergen of 28 kd being predominant both in the intensity of its reaction and in the frequency of its recognition by sera from human allergic subjects. The negative results obtained in both unexposed subjects and obeche-exposed, symptom-free subjects support the specificity of these findings.

## REFERENCES

1. Hinojosa M, Moneo I, Dominguez J, et al. Asthma caused by African maple (*Triplochiton scleroxylon*) wood dust. *J Allergy Clin Immunol* 1984;74:782-6.
2. Reijula K, Kujala V, Latvala J. Sauna builder's asthma caused by obeche (*Triplochiton scleroxylon*) dust. *Thorax* 1994;49:622-3.
3. Hinojosa M, Subiza J, Moneo I, et al. Contact urticaria caused by obeche wood (*Triplochiton scleroxylon*). Report of eight patients. *Ann Allergy* 1990;64:476-9.
4. Fernández-Caldas E, Quirce S, Marañón F, et al. Allergenic cross-reactivity between third stage larvae of *Hysterothylacium aduncum* and *Anisakis simplex*. *J Allergy Clin Immunol* 1998;101:554-5.
5. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.