

Occupational wood-dust sensitivity from *Euonymus europaeus* (spindle tree) and investigation of cross reactivity between *E.e.* wood and *Artemisia vulgaris* pollen (mugwort)

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A 44-year-old goldsmith suffered from rhinitis and conjunctivitis after having worked with wood dust from *Euonymus europaeus* (*E.e.*) for 15 years. The material was used for drying pieces of jewellery. Very strong reactions could be seen after friction test, scratch test and nasal challenge using wood dust of *E.e.* RAST-class 3 could be measured with the serum of this patient using *E.e.* wood and *Artemisia vulgaris* (*A.v.* pollen) allergen disks. RAST-inhibition, westernblot (WB) and immunoprint (IP) indicated common allergens in extracts of *E.e.* wood and *A.v.* pollen of different degree. In addition this study indicated that subjects suffering from *A.v.* pollen allergy also show sensitization to *E.e.* wood since in 22 of 37 *A.v.* pollen allergics *A.v.* (RAST class 2-4) IgE-antibodies could be seen. The present case probably demonstrates for the first time an IgE-mediated type I allergy to *E.e.* wood.

Key words: *Euonymus europaeus*; immediate type allergy; *Artemisia vulgaris*; cross reactivity.

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CLINICAL ASPECTS

We show that patients can be sensitized with *E.e.* wood when working with this material. Further, cross reactivity can be supposed between *E.e.* wood and *Artemisia vulgaris* pollen.

The *E.e.*, a shrub or tree growing up to 8 m and belonging to the family of Celastraceae, is widespread in Europe and the Near East. Poisoning due to components of spindle trees which in the literature have been described (1, 3, 6, 7, 8, 12, 14) presumably based on the toxic effects of glycosides (digitaloids, alcaloids).

In our investigations with *in vivo* tests we examined a patient who had shown allergic symptoms of the upper respiratory tract after having worked with spindle tree wood dust. We also carried out *in vitro* tests such as immunoblotting, RAST and RAST inhibition. More-

over we investigated whether *E.e.* wood and *A.v.* pollen extracts have common allergens. Our patient showed positive skin tests to *E.e.* wood and *A.v.* pollen extracts.

MATERIAL AND METHODS

Case report

A 44-year-old goldsmith reported allergic symptoms of rhinitis, sneezing, itching eyes and irritation of the throat. The symptoms occurred exclusively during working hours while hand-

ling wood dust from *E.e.* which was used for drying pieces of jewellery after being treated with a chemical reagent. Symptoms began within a few minutes of exposure and always disappeared 1–2 h after. Occupational contact with *E.e.* wood dust, which usually took place twice weekly, lasted for 10 years and after 6 years the first allergic symptoms developed. Neither in the patient's history nor in his family were there any indications of atopic disease.

Skin prick test (SPT) was performed on the patient's forearms and on two non-atopics' forearms. A positive reaction was defined as a skin reaction showing at least a mean wheal diameter (dm) of 3 mm. SPT was done with commercial extracts from Allergopharma Joachim Ganzer KG (Reinbek, Germany). The following inhalant allergens were tested: tree pollen (birch, alder, hazel), grass pollen, weed pollen (mugwort, goosefoot) house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), cat, dog and horse dander.

Histamine solution (0.1%) and saline were used for control. The SPT extract of *E.e.* wood was made by ourselves using physiological saline and *E.e.* wood dust (1 ccm *E.e.* wood dust to 3 ml saline). Extraction time was 4 h at room temperature. The extract was filtered.

For the *scratch test* we used *E.e.* wood dust stirred in saline. The control scratch area was exposed to filter paper saturated with saline. For the *friction test* we used the original *E.e.* wood dust, rubbed on the skin under slight pressure. Sterile mull gauze was used for control rub test. Both tests were carried out on the forearms of the patient and the two non-atopics.

Nasal provocation test was performed by using an *E.e.* wood dust extract (*E.e.* wood dust extracted in physiological saline as described above) controlled by application of pure physiological saline. Both solutions were applied to the lower turbinate (0.05 ml). Nasal challenge was done under rhinomanometric control using a A 440 Rhino-manometer (Allergopharma). Control-measurements and provocations were done in two non-atopics.

RAST: Specific IgE-measurements were done by RAST (Pharmacia, Freiburg, Germany).

The serum of the patient (bleeding 21/2/1986 and 10/11/86) and sera of 37 subjects sensitized to *A.v.* pollen were measured with *E.e.* wood, *E.e.* pollen and *A.v.* pollen allergen disks (4,9). For non-specific binding the allergen disks were measured with a non-allergic control serum pool ($n = 154$). Sera of 47 persons with allergy to spices were also tested. These sera were used because there is cross-reactivity between spices and mugwort (13). They were tested in former investigations by RAST with different spice allergen disks.

RAST-inhibition (RI): *E.e.* wood dust and *A.v.* pollen extract were measured to determine allergenic activity and cross reactivity by RAST inhibition (15). Different dilutions (1–3 steps) of each allergen extract were incubated in test tubes (12 × 55 mm) with the patient's serum (1:5 diluted). The mixtures were incubated overnight at room temperature with *E.e.* wood dust and *A.v.* pollen allergen disks. Subsequently, after washing rabbit ^{125} I-antihuman IgE (Fc-specific; Pharmacia) was added as tracer for bound IgE. After a second overnight incubation, the allergen disks were washed carefully and counted in a gamma counter for bound radioactivity.

Westernblot (WB): The *E.e.* wood and the *A.v.* pollen extracts were separated by SDS-PAGE (2). The gel was calibrated with marker proteins (Pharmacia). 100 µg protein were applied to the gel (gradient 3–22%). After separation the proteins were transferred from the gel to a nitrocellulose membrane (Schleicher and Schüll, Dassel, Germany) by electroblotting. After transfer the nitrocellulose membrane was incubated overnight at room temperature with the serum of the patient (diluted 1:10). The nitrocellulose was washed again. Autoradiography (3 kBq/ml of ^{125} I-IgE, Pharmacia) was performed at -20°C using Kodak X-Omat AR films for 7, 14 and 35 days. For SDS-PAGE the proteins were stained with Coomassie brilliant blue R 250 (E. Merck, Darmstadt, Germany).

Immunoprint (IP): The proteins of *E.e.* wood and *A.v.* pollen extracts (50 µg protein) were separated according to their isoelectric points (10). After isoelectric focusing the proteins were transferred from the gel to a nitrocellulose mem-

brane by capillary blotting. Washing and incubating steps were done as described by WB. For IP the serum was 1:5 diluted. For IEF the proteins were stained with Coomassie brilliant blue R 250 (E. Merck).

RESULTS

SPT showed moderate positive reactions to weed pollen and cat dander. A differentiated SPT with various pollen species (trees, weeds) showed only weak reactions to chestnut and lime tree but a strong reaction to mugwort pollen (*A.v.*), whereas the intracutaneous test with various wood extracts only showed one positive reaction to merbau. Ten minutes after friction test with *E.e.* wood dust multiple strong itching wheals surrounded by erythema developed. The scratch test also showed an intensive allergic skin reaction of type I. During nasal challenge strong clinical type I reactions (nasal itching, sneezing, runny nose, itching eyes, tears) occurred 3 min after application of the *E.e.* wood extract. Rhinomanometric measurements in both nostrils showed a nasal blocking of 85 % compared with the initial value. There was no relevant reaction after provocation with the commercial mugwort pollen extract. Skin

tests and nasal challenge were also performed in two non-allergic persons. No reaction could be seen in these subjects.

Table 1 shows the results of RAST obtained with the sera of our patient (bleeding from 21/2/86 and 10/11/86; last occupational allergen exposure: Feb. 1986). Both bleedings showed RAST class 3 with the *E.e.* wood and *A.v.* pollen allergen disks. No significant specific IgE could be detected using *E.e.* pollen allergen disks. The negative control serum (pool serum of non-allergic subjects) showed no significant levels of specific IgE either to *E.e.* wood and pollen or to *A.v.* pollen. Thus, RAST class 3 is not due to unspecific binding but expresses specific sensitization to *E.e.* wood. Results of RAST measurements with sera of 47 patients with spice allergy are shown in Fig.1. 37 of these 47 persons showed sensitizations (RAST 2) to mugwort (*A.v.*) pollen. 22 showed specific IgE to *A.v.* pollen, *E.e.* wood and *E.e.* pollen, whereas four of them were sensitized to mugwort pollen and *E.e.* wood only. Two sera contained IgE to mugwort and *E.e.* pollen.

By RI using *E.e.* wood allergen disks the total % inhibition for *E.e.* was 96 % and for *A.v.* pollen extract 67 %. Using *A.v.* pollen allergen disks the total % binding for *A.v.* pollen was 97 % and for *E.e.* wood extract 51 %.

Table 1.

RAST-class measurements with patient serum (P) and a negative control pool serum (NP) using *E.e.* wood, *E.e.* pollen and *A.v.* pollen allergen disks. % bi = % binding, R-cl = RAST-class

Sera	Allergen disks		% bi		R-cl		
Reference system							
Reference	A	Reference	38.7		4		
"	B	"	16.0		3		
"	C	"	5.4		2		
"	D	"	2.9		1		
Test							
Allergen disks:		<i>E.e.</i> wood	<i>E.e.</i> pollen		<i>A.v.</i> pollen		
		% bi	R-cl	% bi	R-cl	% bi	R-cl
Serum P							
bleeding 21/01/86		18.2	3	1.3	0	28.4	3
bleeding 10/11/86		16.5	3	0.8	0	27.1	3
NP							
		0.8	0	0.9	0	0.9	0

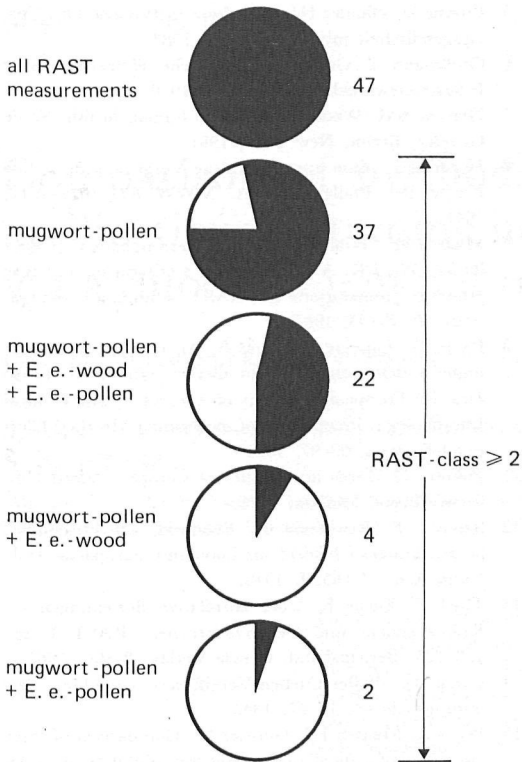


Fig. 1. RAST class measurements with sera of 47 persons with clinically actual type 1 allergies to different spices.

By SDS-PAGE and IEF the protein pattern of the *A.v.* pollen extract was more complex than that of the *E.e.* wood extract. By WB in *E.e.* wood and *A.v.* pollen extracts one common allergen band could be detected at a molecular weight of < 14 kD. By IP one common allergen band could be seen at Ip of 5.0 (Fig. 2a, b).

DISCUSSION

A 44-year-old goldsmith handling wood dust of the European spindle tree developed type I-like allergic symptoms of the upper respiratory tract. Clinical and laboratory investigations, such as SPT, friction test, scratch test, RAST and immunoblots showed that the patient had been specifically sensitized to wood components of *Euonymus europaeus*.

Unspecific effects could be excluded by performing clinical allergy tests in two healthy non-

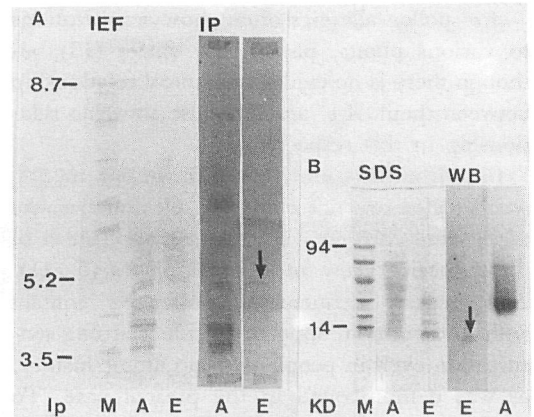


Fig. 2a, b. A: IEF (left) and immunoprecipitation (right) of *E.e.* wood and *A.v.* pollen extracts. B: SDS-PAGE (left) and WB (right) of *E.e.* wood and *A.v.* pollen using the patient serum. The arrow indicates the common allergen between *E.e.* wood and *A.v.* pollen. Time of autoradiography: 14 days. KD = kilodalton; Ip = isoelectric points; A = *A.v.* pollen, E = *E.e.* wood; M = marker protein.

atopic persons and by using control serum of non-allergics.

Avoidance of *E.e.* wood contact for 9 months did not produce any decrease in clinical reaction after allergen provocation: SPT showed the same strong reaction as seen 9 months before and RAST measurement still showed class 3 (16.5% binding).

RAST class measurements done with 37 sera of *A.v.* pollen-sensitized persons showed parallel sensitizations either to *E.e.* wood or *E.e.* pollen in 22 cases. Thus, it might be assumed that in many cases IgE-mediated sensitizations to mugwort pollen include sensitization to components of *E.e.* material (and vice versa), suggesting common allergens in the extracts of both species. A hint of the existence of common allergens between *E.e.* wood and *A.v.* pollen extract could be obtained by doing WB, IP and RAST inhibition with both extracts. For further proof of cross-reactivity immunoblot inhibition could not be done because of lack of material. However, RAST inhibition suggested cross-reactivity of a different degree. Nevertheless, an independent sensitization of each of these allergens can not be excluded, although it seems unlikely as the chance of coming in contact with *E.e.* allergens is rare.

A.v. pollen-allergics often show sensitizations to various plants, particularly spices (13), although there is no evident botanical relationship between them. *A.v.* and *E.e.* also show no relationship in this respect.

In European countries sensitizations to *E.e.* wood/pollen are not clinically relevant because these plants are not very common and most of these species grow in isolated places (5, 11). Nevertheless, frequent and intensive contact with *E.e.* material appears to induce strong sensitization even in people with no atopic history, as was demonstrated in the present case. To confirm the importance of *E.e.* wood as an occupational allergen in goldsmiths and to obtain more evidence of its allergenic potency, further investigations are necessary.

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