

## References

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## Type IV allergy to natural hirudin confirmed by in vitro stimulation with recombinant hirudin

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**Key words:** recombinant hirudin; raw hirudin; leech extract; allergic contact dermatitis; T-cell receptor; variable chain; lymphocyte transformation test; recombinant allergens; medicaments. © Munksgaard, 1996.

### Case Report

A 64-year-old man applied a hirudin-containing ointment for haemorrhoids. Some months later, he suffered thrombophlebitis of the forearm following i.v. infusions. After applying Exhirud® (Plantorgan, Bad Zwischenahn, Germany), containing the leech extract raw hirudin, the patient developed acute contact dermatitis within 2 days. Patch tests were performed with the European standard series, Exhirud®, raw hirudin 20% eth. and the ointment vehicle, with positive reactions to Exhirud® and raw hirudin 20% eth., but not to the ointment vehicle. 20 healthy age-matched controls were patch test negative to raw hirudin.

Anti-IgG-, IgG4-, IgM- and IgE-hirudin antibodies were not detectable (H.-J. Friesen, Behringwerke AG, Marburg, Germany, personal communication). In the lymphocyte transformation test (1), the stimulation index (ratio of recombinant hirudin (100 ng/ml) stimulated versus non-stimulated peripheral lymphocytes) was  $4.1 \pm 0.6$  (mean  $\pm$  SD). In 5 healthy age-matched controls, the stimulation index was  $<1.5$  (Fig. 1). T cell receptor (TCR) V $\beta$  analysis was performed by flow cytometry, using a broad panel of TCR V $\beta$  specific monoclonal antibodies (Immunotech, Hamburg, Germany and T cell diagnostics, Cambridge, MA, USA). This showed an increase in TCR V $\beta$ 17<sup>+</sup> lymphocytes after 10 days stimulation with recombinant hirudin (100 ng/ml) of the patient's peripheral blood lymphocytes (13.5% TCR V $\beta$ 17<sup>+</sup> lymphocytes), compared to unstimulated controls (4.2% TCR V $\beta$ 17<sup>+</sup> lymphocytes). No such shift was found after stimulation with mitogens such as PHA (1  $\mu$ g/ml) or interleukin 2 (100 U/ml), and in none of the 5 healthy controls was a shift in the TCR V $\beta$  repertoire in unstimulated compared to hirudin-stimulated cells detectable.

### Comment

Leech extracts are often applied topically in venous diseases, due to their anticoagulant, antiinflammatory and antiexudative properties (2). Allergic reactions to leech extract are rarely observed (3, 4). We have previously reported this patient with Type IV allergy to raw hirudin in the leech extract in Exhirud®. However, allergic reactions to contaminants of raw hirudin could not be totally excluded. In our present study, we therefore used the newly synthesized recombinant hirudin HBW023 (5, 6) for our in vitro studies. Using this approach, contamination with other ingredients can be ruled out, enabling us to demonstrate that the patient was sensitized to hirudin contained in the leech extract. This is, to our knowledge, the 1st case report of Type IV allergy to hirudin proved by in vitro lymphoproliferation to recombinant hirudin. The subset of hirudin-responding lymphocytes

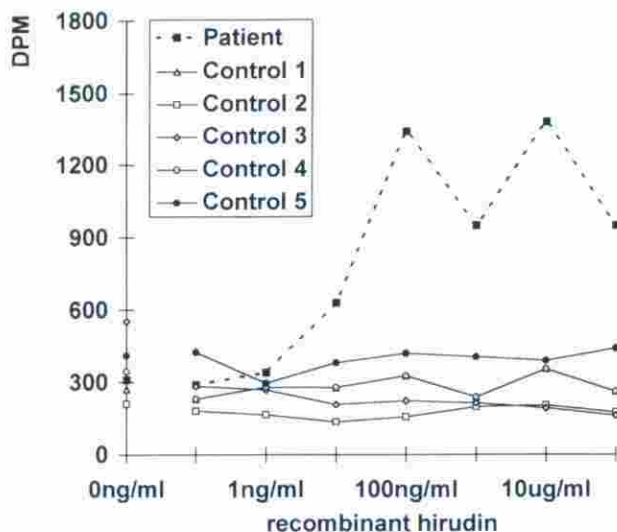


Fig. 1. Lymphocyte proliferation in response to recombinant hirudin in the patient compared to 5 healthy controls. Peripheral lymphocytes were stimulated with or without recombinant hirudin (0.1 ng/ml–100  $\mu$ g/ml) for 4 days before they were labelled with 0.5  $\mu$ Ci <sup>3</sup>H-thymidine for 18 h. DPM: decays per min.

belongs at least partially to the TCR V $\beta$ 17<sup>+</sup> lymphocytes. Additionally, we have shown that the recombinant protein technique provides a useful tool in the differential diagnosis of allergic reactions.

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## Airborne contact dermatitis from East Indian rosewood (*Dalbergia latifolia* Roxb.)

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A 54-year-old knifegrinder presented with itchy erythema of the face, eyelids, neck, forearms and dorsa of the hands, plus vesiculation in the elbow flexures. The wrist under his watch was sharply spared. The dermatitis had started abruptly while he was at work, accompanied by lacrimation, coughing and rhinorrhea. A milder episode had occurred 1 month before and cleared spontaneously. In both instances, he had been working at knife handles that he manufactured occasionally from rosewood. Since the very beginning, 1 year previously, the sawdust had caused him acute sneezing and coughing.

He was patch-tested with the GIRDCA standard series and with sawdust from his own wood sample 10% pet. Only the sawdust was positive. The sawdust was tested on 18 controls, 3 of whom gave a mild erythematous reaction at 3 days. 15 days later, 1 of these, a 51-year-old housewife, returned with intense itching at the patch test site. On retesting with the sawdust, she showed an intense erythematovesicular reaction after 2 days.

Botanical identification proved the wood to be East Indian rosewood (*Dalbergia latifolia* Roxb.). Thin-layer-chromatographic separation of an ethanol extract revealed the presence of (R)-4-methoxy dalbergione and small amounts of (S)-4,4'-dimethoxy dalbergione.

Both the knifegrinder and the positive control were then patch tested with (R)-4-methoxy dalbergione 1% and (S)-4,4'-dimethoxy dalbergione 1%. Another 3 specific known rosewood constituents, namely (R)-3,4-dimethoxy dalbergione 0.01%, (S)-4-methoxy dalbergione 1%, and (S)-4'-hydroxy-4-methoxy dalbergione 1%, were also tested. All allergens were in pure form, incorporated in pet. at subirritant concentration. The results are shown in Table 1.

### Comment

East Indian rosewood is a purple-brown timber with dark veins, a species of high commercial value. Like all *Dalbergia* species, usually known as rosewood (French: palissandre), East Indian rosewood is used for furniture veneering, wooden floors, musical instruments, and various wooden articles such as handles. All *Dalbergia* species (family: Leguminosae-Papilionaceae) contain sensitizing benzoquinones named dalbergiones (1).

In timberworkers, such as carpenters, sawyers, joiners, and cabinetmakers, sawdust may cause airborne contact dermatitis, and, more rarely, urticarial rashes and polymorphous-like eruptions. Conjunctivitis, rhinitis and asthma have also been reported (2). The mechanism of such reactions may be allergic, irritant, or both. Allergic contact dermatitis in users of finished wood objects has also been described (3-5).

In our patient, the sawdust caused both respiratory symptoms and airborne allergic dermatitis. The facts that exposure to East Indian rosewood sawdust was

Table 1. Allergic patch test reactions to dalbergiones in the patient and 1 control

| Allergens                           | Patient |    |    | Control |     |
|-------------------------------------|---------|----|----|---------|-----|
|                                     | D2      | D3 | D7 | D2      | D3  |
| sawdust 10% pet.                    | +       | ++ | -  | ++      | ++  |
| (R)-3,4-dimethoxy dalbergione 0.01% | -       | =  | ++ | ++      | +++ |
| (R)-4-methoxy dalbergione 1%        | -       | -  | ++ | ++      | +++ |
| (S)-4-methoxy dalbergione 1%        | -       | -  | +  | -       | +   |
| (S)-4'-hydroxy dalbergione 1%       | -       | -  | +  | -       | +   |
| (S)-4,4'-dimethoxy dalbergione 1%   | -       | -  | -  | -       | +   |

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