

after arrest means that he had gone through 3 half lives and that the mast cell tryptase at time of arrest could have been 200 µg/l, which would be diagnostic of death due to anaphylactic shock.

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Treatment with American *Polistes* venom was ineffective in an Italian patient allergic to European *Polistes*

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Polistes is the most common genus of social wasps. The species *Polistes dominulus* (PD), *Polistes gallicus* and, to a lesser extent, *Polistes nympha* are largely distributed in Europe (1), with a greater distribution in the Mediterranean areas (Italy, Greece, Spain, France and North Africa). PD is also present around the Caspian Sea and across Russia and China (2).

Diagnostic and therapeutic extracts for European and American *Polistes* are only partially cross-reactive

Until 1996, when Anallergo (Florence, Italy) started the production of PD venom, only a mixture of the American *Polistes* (AP) species was commercially available for diagnosis and therapy. Of note, the American species are not present in Europe and recent studies (3, 4) show that American and European species of *Polistes* are phylogenetically distant. We describe the case of a patient for whom immunotherapy with AP extract was ineffective due to the poor cross-reactivity with PD venom.

The patient is a 39-year-old woman, who 7 years ago developed a severe grade 4 allergic reaction following hymenoptera sting. She had asthma, urticaria and hypotension and was therefore treated with adrenaline at the emergency department. The insect could not be directly identified, but it was supposed to be a wasp, due to the absence of the sting in the skin lesion. Intradermal tests, performed with commercial extracts (Stallergenes, Milan, Italy) gave a strongly positive reaction to AP mix (0.1 µg/ml) and a mild reaction to vespa venom (1.0 µg/ml), whereas honeybee venom was negative. Specific IgE (CAP test, Pharmacia & Upjohn Diagnostic, Uppsala, Sweden) were 2.61 kU/l for AP and negative for vespa and honeybee. Based on these results, immunotherapy

with an AP extract (Stallergenes) was started and a maintenance dose of 100 µg was reached in 7 weeks. After 4 years the patient was stung again by a wasp, perhaps a *Polistes*, with subsequent grade III reaction (urticaria, angioedema and asthma). At this point, the diagnostic workup was repeated, including also the newly available PD extract. The intradermal test was strongly positive for PD (0.1 µg/ml) and negative for bee and vespa. Specific immunoglobulin (Ig) E were 4.94 kU/l for PD, 1.93 kU/l for AP and negative for vespa and bee.

We carried out a CAP-inhibition assay, according to Straumann and slightly modified (5), in order to assess the cross-reactivity between PD and AP mix. The patient's serum was incubated overnight either with PD venom or AP mix, then IgE against PD and AP were measured. After incubation with AP extract, the AP-specific IgE were reduced, as expected, by 74% and the same happened for PD-specific IgE after incubation with PD venom (77%). On the contrary, after incubation with AP mix, the PD-specific IgE were reduced only by 39%. The protein components of PD venom (5 or 20 µg/lane) were resolved via sodium dodecyl sulphate polyacrylamide gel electrophoresis (Bio-Rad, Hercules, CA,

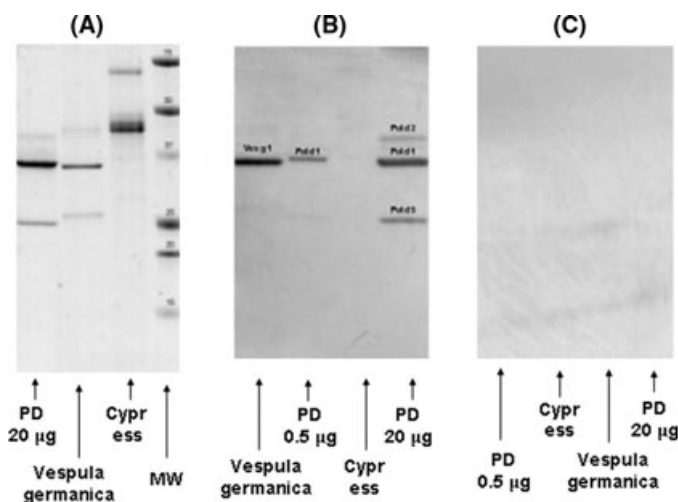


Figure 1. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting experiments. (A) SDS-PAGE: protein separation of PD venom, *Vespa germanica* venom and *Cupressus* extract, against standard molecular weights (MW); (B) immunoblotting: patient's immunoglobulin (Ig) E recognizing Pol d 1 (phospholipase PD), Pol d 2 (hyaluronidase PD), Pol d 5 (antigen 5 PD), Ves g 1 (phospholipase *V. germanica*); no IgE against *Cupressus*; Pol d 2 and Pol d 5 are recognized only at the highest concentration of venom. (C) Immunoblotting: total inhibition of patient's IgE for PD and Vg after incubation with PD venom.

USA; gel Criterion XT 12%) and stained with Coomassie blue. *Vespula germanica* venom and an extract of *Cupressus arizonica* were also run as controls. The protein bands of hyaluronidase (Pol d 2), phospholipase (Pol d 1, Ves g 1) and antigen 5 (Pol d 5) were identified at 43 kDa, 35 kDa and 25 kDa, respectively (Fig. 1A). Subsequently, the proteins were transferred onto nitrocellulose (Bio-Rad, Trans-Blot Transfer Medium 0.2 µm, Tris/Gly buffer) and incubated with the patient's serum. The protein-bound IgE were detected with peroxidase-conjugated anti-IgE (Sigma, St Louis, MO, USA) and a chemiluminescent system (Amersham, Piscataway, NJ, USA; ECL Plus). The patient's IgE recognized Pol d 1, Pol d 2, Pol d 5 and Ves g 1 (that has 75% homology with Pol d 1; Fig. 1B). Finally, the same experiment was repeated after incubating the serum with PD venom (2 mg/ml), and obtaining the complete inhibition of IgE binding.

According to these results, a new immunotherapy course with PD venom was started. After 1 year of treatment,

the patient was stung by a *Polistes* and displayed only a large local reaction. This indirectly confirms that the immunotherapy was correctly prescribed. Our clinical and immunological results suggest that, at least in the Mediterranean areas, the diagnostic workup for hymenoptera sting should always include PD, as the AP mix may lead to misdiagnosis and consequent nonprotective immunotherapy.

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