Immunological Engineering
Department of Engineering
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EUROIMMUN

Component resolution for improved diagnostics of Hymenoptera venom allergy using a set of recombinant Vespula and Polistes venom allergens

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Background: Species-specific and CCD-free venom allergens have redefined diagnostic approaches in Hymenoptera venom allergy (HVA), in particular in patients with double sensitization to both honey bee (HBV) and yellow jacket venom (YJV). Differentiation of patients with allergy against different cross-reactive vespid venoms however remains highly difficult. Here we established a prototype assay including a panel of recombinant allergens from *Vespula vulgaris* and *Polistes dominula* venom (PV) for molecular dissection of IgE reactivities in patients with HVA displaying double sensitization to YJV and PV.

Methods: Expression of cloned Polistes and Vespula venom allergens Ves v 1, Ves v 5, Pol d 1, Pol d 4 and Pol d 5 in CCD-free form was performed in *E. coli* (Ves v 1 and Pol d 1) as well as Sf9 insect cells. Recombinant venom allergens, venom extracts (i3, i77) and a CCD-marker were immobilized on membrane chips assembled as a multi-parameter test for sIgE testing (EUROLine) **(Fig. 1)**.
Diagnosis of HVA was based on a history of anaphylactic sting reaction, skin test and sIgE measurements. Sera from hymenoptera venom allergic individuals from Polistes endemic or non-endemic areas were analyzed for IgE reactivity and band intensity was assessed with the EUROLineScan software. Relative antibody affinity for Ves v 5 and Pol d 5 was measured by treatment of test-strips with a chaotropic agent after serum incubation to remove lower affinity antibodies and comparison of band intensity of the treated and non-treated strips.

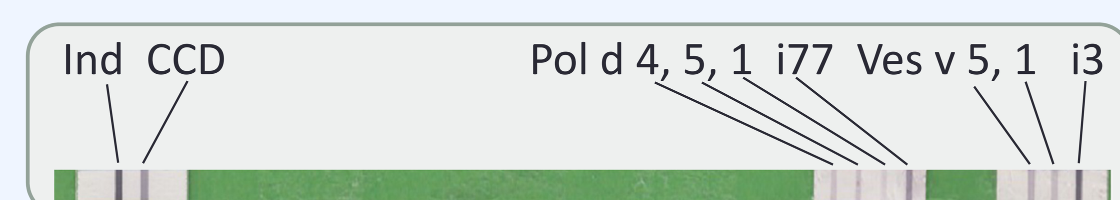
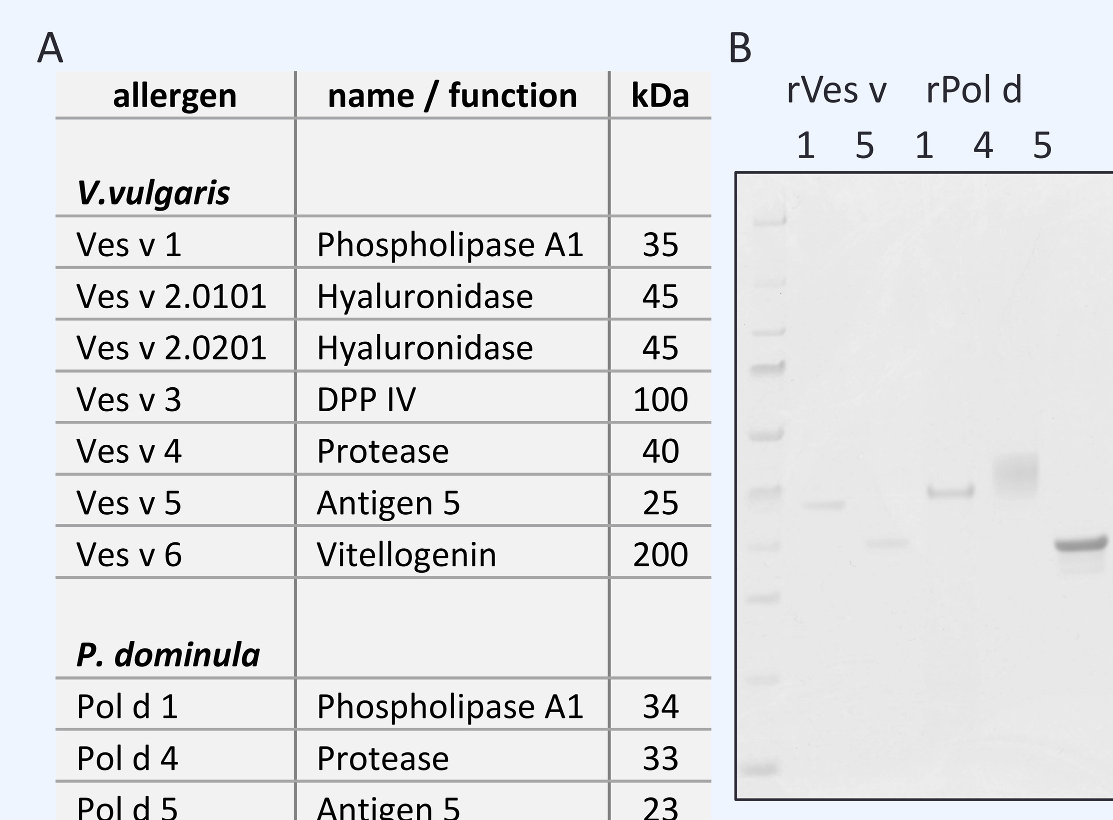


Fig. 1: Prototype EUROLine assay

Results: The known PV allergens Pol d 1 (phospholipase A1), Pol d 4 (protease) and Pol d 5 (antigen 5) as well as the YJV allergens Ves v 1 (phospholipase A1), and Ves v 5 (antigen 5) **(Fig. 2A)** could be expressed in either insect cells or *E. coli.* Notably, the venom phospholipases Ves v 1 and Pol d 1 were obtained most efficiently from E. coli after solubilizing and refolding from inclusion bodies. Molecular identity was verified by SDS-PAGE **(Fig. 2B)** and MALDI analysis (data not shown).



A

allergen	name / function	kDa
V.vulgaris		
Ves v 1	Phospholipase A1	35
Ves v 2.0101	Hyaluronidase	45
Ves v 2.0201	Hyaluronidase	45
Ves v 3	DPP IV	100
Ves v 4	Protease	40
Ves v 5	Antigen 5	25
Ves v 6	Vitellogenin	200
P. dominula		
Pol d 1	Phospholipase A1	34
Pol d 4	Protease	33
Pol d 5	Antigen 5	23

Fig. 2: *P. dominula* and *V. vulgaris* venom allergens (A) and recombinant venom allergens (B)

56 sera from a Polistes endemic area (Italy, Polistes group) and 50 sera from a non-endemic area (Eastern Germany, Vespula group) all with a confirmed history of hymenoptera venom allergy were screened for presence of sIgE **(Table 1)**.

allergen	PV group	YJV group	PV	PV	YJV
Ves v 1	46,4	84	0	1	4
Ves v 5	51,8	86	3	0	5
Pol d 1	46,4	52	2	5	1
Pol d 4	32,1	30	2	0	0
Pol d 5	76,8	82	5	0	2
negative	8,9	0			

Table 1: IgE reactivity (%) to the individual recombinant venom allergens in the different groups (left) and sIgE (EAST-class) of individual patients (right)

In 43 of 56 sera (76,8%) sIgE reactivity to Pol d 5 was detected. Of these sera, 36 (83,7%) also had sIgE to the YJV homologue Ves v 5 underlining the broad cross reactivity of these components **(Fig. 3)**. For Pol d 1, sIgE reactivity was detected for 26/56 sera (46%). Of those, 5 sera (19%) exclusively displayed sIgE reactivity for Pol d 1. Therefore the phospholipases appear less cross reactive, compared to the antigens 5, which was even more obvious in the Vespula group, were 38, 1% of Ves v 1 positive sera did not cross-react with Pol d 1. This is in line with results of studies using natural purified venom allergens [1,2].

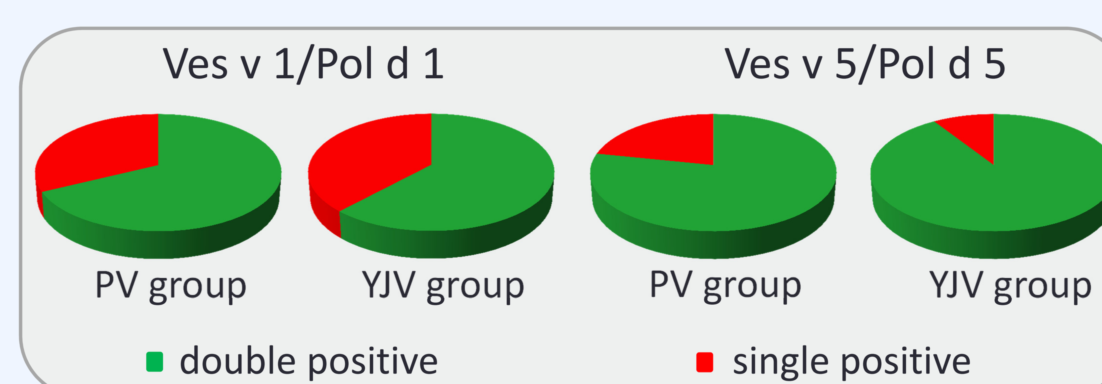


Fig. 3: Cross-reactivity between allergens

Low sIgE levels to Pol d 4 were found in 18/56 (32,1%) sera, primarily those with high sIgE reactivity for Pol d 5. Notably, 30% of the sera in the Vespula group exhibited low levels of sIgE to Pol d 4. This might suggest a minor cross-reactivity to the putative venom protease Ves v 4.
The CCD-marker showed positive reactions in 16/56 sera (29%) which is considerably lower than in the Vespula group (62%) and in line with the absence of CCDs in Polistes venom [3].
Although the cross-reactivities between the antigens 5 and the phospholipases do not always allow for definite conclusion, the levels of sIgE to the individual components provide valuable information that can be used for a plausible identification of the culprit venom.

For sera in which sIgE levels do not provide clear evidence of primary sensitization, additional criteria are needed. To allow reliable analyses of those patients with nearly equal sIgE levels to Ves v 5 and Pol d 5 we aimed for evaluation of IgE affinity as measure of primary sensitization. The relative band intensity of the treated and non-treated strips was used as a measure for relative antibody affinity.
We obtained three different patient individual patterns, which reflect the possible sensitizations. A pronounced decline of binding to Pol d 5 **(Fig. 4A)** was obtained throughout the Vespula group and suggests a primary sensitization by Vespula spp.. No decrease in residual binding for neither antigens 5 suggests double sensitization **(Fig. 4B)** and a pronounced decline of the Ves v 5 binding **(Fig. 4C)** points to primary sensitization to Polistes spp.. The two latter patterns were only observed for the Polistes group. Notably, the less cross-reactive phospholipases showed no comparable differences in antibody affinity.

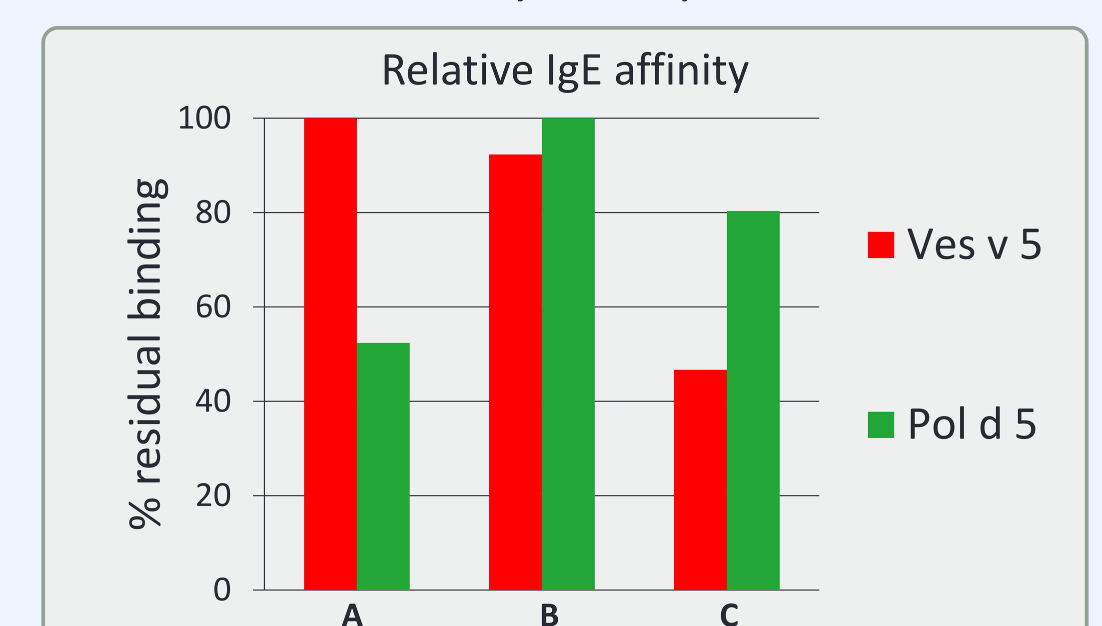


Fig. 4: Patterns of relative IgE affinity to Ves v 5/Pol d 5

Conclusion: Component-resolved diagnostics in vespid venom allergy using a set of recombinant allergens allows for detection of specific IgE reactivity to all components used. Despite cross-reactivity between YJV and PV components exclusive detection of sIgE to PV allergens without sensitization to the YJV homologues suggests an essential role of additional PV allergens to overcome the limitations of molecular allergy diagnostics in HVA. Antibody affinity might provide an additional measure for differentiation and should be further investigated.

References:

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[3] Blank et al., J Allergy Clin Immunol. 2013 131(4):1239-42.

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