

Clinical Communications

Identification of the larval serum proteins as major fruit fly (*Drosophila melanogaster*) occupational allergens

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Clinical Implications

- Respiratory symptoms observed in several laboratory workers handling *Drosophila* are due to a specific fruit fly allergy not related to tropomyosin but potentially to a hexamerin protein. Potential risk factors and candidate allergens are described.

TO THE EDITOR:

Allergy to laboratory animals is a significant occupational problem observed in about one third of an exposed population. This kind of allergy is mediated by an IgE-dependent hypersensitivity and leads to bothersome, mainly respiratory symptoms. It concerns preferentially mammals, but arthropods and more specifically insects can be involved. Workers at research institutes are particularly exposed to this kind of occupational hazard.¹

To better understand the symptoms described by several persons working with *Drosophila* in research institutes and to check if they could be due to an insect allergy, we designed and sent a detailed questionnaire to the entire *Drosophila* worker population in Montpellier (France) (n = 59). We invited workers who described respiratory symptoms (14 [47%] of 30 answers) related to *Drosophila* manipulation to an allergy testing. The study protocol was approved by our institutional ethics committee and all participants gave their informed consent before participating in the study.

We performed skin prick tests (SPT) through one intact larva and adult fly and quantified specific *Drosophila* IgE in serum (ImmunoCAP, Thermo Fisher Scientific, Uppsala, Sweden) on 11 symptomatic workers (no. 3 to 13) (Table I). The same experiments were performed with nonexposed and exposed but not symptomatic persons as controls (no. 1 and 2, respectively). The flies may be colonized by dust mites; to avoid any confusion, we also performed SPT with commercial extracts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Stallergenes-Greer, Antony, France).

Drosophila SPT were negative for the controls and 4 of the 11 symptomatic workers (no. 3 to 6) and positive for the 7 other symptomatic ones (64%) (no. 7 to 13). *Drosophila* serum-specific IgE were also detected in these patients (no. 7 to 13). No cross-reactivity with dust mites was observed.

We defined *Drosophila* allergy by the presence of respiratory symptoms related to *Drosophila* manipulation associated with positive SPT to adult and/or larva and/or positive detection of *Drosophila*-specific IgE. Of the 11 symptomatic tested workers, 7

(64%) fulfilled this definition (23% of the responders). They tended to present symptoms, especially ocular ones like conjunctivitis ($P < .1$), more frequently ($P < .05$) than the symptomatic but nonallergic workers.

We demonstrated that a *Drosophila* allergy explains most of the respiratory symptoms developed by some exposed workers. In a univariate model only, atopy ($P < .05$) and a previous allergic disease ($P < .01$) to another antigen than *Drosophila* were risk factors, as previously shown.² The small size of our population and the absence of provocative challenges outside the workplace did not allow a proper dose-response and causality assessment analysis.

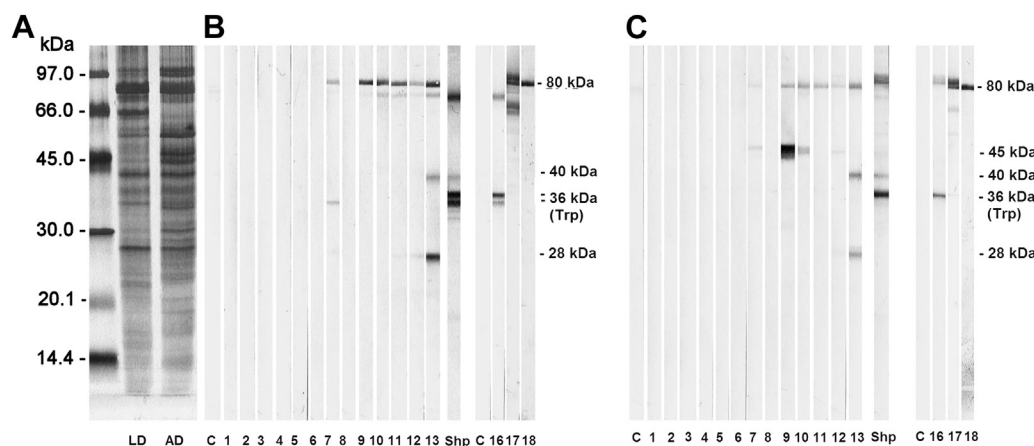
To better characterize the allergen(s) involved, western blotting analysis was performed. After SDS-PAGE and transfer onto nitrocellulose, allergens in larval (Figure 1, B) and adult (Figure 1, C) *Drosophila* were revealed with IgE obtained from patients' serum. It is well known that tropomyosin is an arthropod pan-allergen, and to check for any cross-reactivity, the nitrocellulose strips with the *Drosophila* extracts were incubated with serum from a patient sensitized to shrimp and showing IgE to Pen a 1 allergen (lane Shp), and an antitropomyosin monoclonal antibody (lane 16). Only allergic workers, except one (no. 8 with a doubtful SPT for larva only), displayed IgE reactivity to *Drosophila* proteins. In larval and adult extracts, an 80 kDa allergen was detected in 6 of 7 subjects. In addition, a 76 kDa band was detected in at least 3 workers (no. 10, 11, 13) only in larvae, and a 45 kDa band was observed in 4 workers (no. 7, 9, 10, 12) only in adult extracts. These bands were not detected with a positive Pen a 1 serum or the antitropomyosin antibody and they were substantially found in crude larva and/or adult extracts (Figure 1, A). Recently, a novel German cockroach 79 kDa allergen (Bla g 3) has been characterized, which is homologous to the 72 to 78 kDa American cockroach Per a 3 allergen.³ Interestingly, both allergens are hexamerins, a protein family largely represented in the hemolymph of insects.⁴ Since then, a hexamerin (HEX1B) was also identified as an allergen in *Gryllus bimaculatus*.⁵ Two major proteins present in *Drosophila* hemolymph corresponding to this description are the larval serum protein 1 (LSP-1) and 2 (LSP-2). Thus, we tested specific polyclonal antibodies raised against LSP-1 or LSP-2, respectively, with larval and adult fly extracts.⁶ Several isoforms were detected with the anti-LSP-1 antibody, ranging from around 60 kDa to more than 80 kDa (lane 17), and were not found in the sera of the allergic workers. By contrast, a unique 80 kDa band was detected with the anti-LSP-2 (lane 18) that perfectly matched with the 80 kDa allergen detected with the sera of the allergic workers.

One allergic worker showed additional signals around 40 and 28 kDa in larvae and adults (no. 13), and another one for a 36 kDa allergen, only in larvae (no. 7). This allergen could correspond to a tropomyosin (no. 7 vs Shp and lane 16), which is described as Dro m 7, a 33 kDa *Drosophila* allergen. A 72 kDa tropomyosin dimer is also detectable in larvae with a positive Pen a 1 serum and the antitropomyosin antibody signal (Shp and lane 16). However, we also performed ELISA analysis, and none of the tested sera showed any IgE-specific signals on purified Pen a 1 (data not shown). This observation is an additional indication

TABLE I. Summary of clinical and biological data of the 13 patients tested (1-13)

	1	2	3	4	5	6	7	8	9	10	11	12	13
Exposure to <i>Drosophila</i>	–	+	+	+	+	+	+	+	+	+	+	+	+
Symptoms to <i>Drosophila</i>	–	–	+	+	+	+	+	+	+	+	+	+	+
SPT to larva <i>Drosophila</i>	–	–	–	–	–	–	–	+/-	+	+	+	+	+
SPT to adult <i>Drosophila</i>	–	–	–	–	–	–	+	–	+	+	+	+	+
SPT to <i>Dermatophagoides pteronyssinus</i>	–	–	–	–	+	+	–	+	–	–	+	+	+
SPT to <i>Dermatophagoides farinae</i>	–	–	–	–	–	+	–	+	–	+	–	–	+
IgE to <i>Drosophila melanogaster</i>	–	–	–	–	–	–	+	+/-	+	+	+	+	+

SPT, skin prick test.

IgE to *Drosophila melanogaster*: presence or absence of specific IgE against *Drosophila* in serum.**FIGURE 1.** SDS-PAGE western blot. Silver stained proteins (A) from *Drosophila* larva (LD) and adults (AD) and allergen detection in larva (B) or adult (C) with IgE from an individual patient: nonexposed (1), nonsymptomatic (2), symptomatic nonallergic (3-6), allergic workers (7-13), a Pen a 1 positive serum (Shp), or monospecific antibodies: anti-Pen a 1 (16), anti-LSP1 (17), anti-LSP2 (18), and negative controls (C). Trp, Tropomyosin.

that a major allergen different from a tropomyosin is involved in *Drosophila* allergy.

The 28 and 40 kDa bands might correspond to other predicted *Drosophila* allergens: the 40 kDa arginine kinase (Dro m 9) and the 25 kDa superoxide dismutase (Dro m MnSOD) (www.allergome.org). Notably, the 40 kDa allergen, which was not identified by the antitropomyosin antibody, was detected with the anti-Pen a 1 serum. This indicates that arginine kinase might also be an arthropod pan-allergen.⁷

We demonstrated that respiratory symptoms developed by about a quarter of a *Drosophila* exposed worker population are related to a specific *Drosophila* allergy with atopy and previous allergic diseases as risk factors.⁸ We identified clinically relevant candidate *Drosophila* allergens. The most prominent allergen corresponds to the hexamerin LSP-2, which was never characterized as a potential *Drosophila* allergen before. Hexamerins are large extracellular proteins⁴ with high stability and remarkable resistance against proteolysis, detergents, and changes in pH and temperature.⁹ These factors may also contribute to the allergenic property of *Drosophila* LSP-2. Furthermore, this study also highlights the necessity to inform and protect the exposed workers from this occupational risk, especially atopic and otherwise allergic individuals.

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