

### Cross-reactivity between royal jelly and *Dermatophagoides pteronyssinus*

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#### Clinical Implications

- Immunoblot analysis confirmed the presence of cross-reactive proteins in both royal jelly and *Dermatophagoides pteronyssinus*.
- Prior sensitization to dust mites might explain allergic reactions developed after first exposure to royal jelly.

#### TO THE EDITOR:

Royal jelly (RJ) is a secretion of the hypopharyngeal and mandibular glands of worker honeybees (*Apis mellifera*). Approximately one-half of its dry weight consists of protein; other components are fatty acids, sugars, and vitamins. It is used as a health tonic, widely consumed in Asia, with believed benefits that range from promoting growth in children to improving general health status and enhancing longevity in the elderly.

Severe reactions, such as anaphylaxis and asthma have been reported especially in the adult population.<sup>1,2</sup>

We present the only reported case of allergy to RJ which suggests the presence of cross-reactive allergenic epitopes in RJ and *Dermatophagoides pteronyssinus*.

An 11-year-old girl was admitted to the emergency department, presenting with dysphonia, cough, wheezing, and eyelid angioedema. She was treated with intravenous diphenhydramine and steroids, which resulted in prompt resolution of her symptoms. Two hours before this reaction she had ingested a beverage made of crude RJ and fructose, and she had taken an ibuprofen 30 minutes before drinking the beverage to treat an upper respiratory tract infection. It was the first time she had RJ, and she had tolerated ibuprofen previously.

Over the past several years, the patient had presented with intermittent sneezing, nasal pruritus, and rhinorrhea, all of which were controlled with oral antihistamines and intranasal steroids. She had never been stung by honeybees. There was no family history of atopy.

Skin prick tests (SPTs) were performed with common inhalants found in our area (northwest region of Spain): *Dermatophagoides pteronyssinus*, *Lepidoglyphus destructor*, latex, cat and dog dander, *Alternaria alternata*, *Cladosporium herbarum*, and pollen from *Phleum pratense*, *Plantago lanceolata*, and *Parietaria judaica* (extracts provided by Leti Laboratories, Madrid, Spain). SPT was also performed with the nondiluted RJ beverage implicated in the adverse reaction, applied directly on the volar side of her forearm. Histamine (10 mg/mL) and 0.9% normal saline were used as positive and negative controls, respectively. The reaction was regarded as positive if the mean wheal diameter was at least 3 mm, in the presence of appropriate reactions to both controls. Two healthy subjects underwent prick test with the same RJ beverage, showing no skin reaction.

SPTs were positive to *D pteronyssinus*, pollen from *P pratense* and *lanceolata* and RJ, the latter of which yielded a wheal with a mean diameter of 6 mm.

Serum-specific IgE was detected by enzyme allerge sorbent test technique (Hytec; HYCOR Biomedical Ltd, Penicuik, UK) against RJ extract (3.9 kU/L), grass pollen extract (3.7 kU/L), and the grass allergens nPhl p 5 (0.8 kU/L) and nPhl p 1 (7.9 kU/L). Values of specific IgE greater than 0.35 kU/L are considered as positive. No specific IgE was detected against pollen from mugwort (*Artemisia vulgaris*), *P lanceolata*, *Salsola kali*, *Platanus acerifolia*, *Olea europaea*, and *P judaica* nor to nArt v 1 and nParj1+nParj 2.

Open challenge (OC) was initially performed with ibuprofen (total administered dose, 600 mg) without producing adverse reactions. The following week, OC was performed with the RJ beverage. After ingestion of 2 mL, the patient experienced oral pruritus and eyelid edema. OC was stopped and oral antihistamines were administered.

SDS-PAGE immunoblot analysis with RJ extract was performed according to the Laemmli method<sup>3</sup> under reducing (with 2-mercaptoethanol) and nonreducing (without 2-mercaptoethanol) conditions. After incubating polyvinylidene difluoride membrane with patient's serum, 5 bands of >97 kDa, 55 kDa, 49 kDa, 45 kDa, and 33 kDa were detected under nonreducing conditions, and 6 bands were detected under reducing conditions: >97 kDa, 55 kDa, 49–45 kDa, 39 kDa, 33 kDa, and 23 kDa. A pool of sera from non-atopic donors was used as negative control (Figure 1, A). The protein with molecular mass of 55 kDa most probably corresponds to the major royal jelly protein 1 (MRJP1) and the protein of 49 kDa to MRJP2, recently described by Rosmilah et al<sup>4</sup> with the use of proteomic technology. MRJP1 is the most abundant and best characterized glycoprotein of RJ. It represents 48% of the water-soluble proteins of RJ and appears as a single protein of 55 kDa. MRJP2 has a molecular mass of 49 kDa, but polypeptides of 40 kDa and 32 kDa have been identified as its degradation products after proteinase activity. Both MRJP1 and MRJP2 are members of the apalbumin family.<sup>4</sup>

Immunoblot inhibition with RJ extract as solid phase was performed by incubating the patient's serum with *D pteronyssinus* extract at 2 different concentrations of 0.8 mg/mL and 2 mg/mL. Total inhibition of the specific IgE binding to RJ proteins was obtained at both concentrations (Figure 1, B). Pollen extracts from grass (*Lolium perenne* and *P pratense*), *Artemisia vulgaris* (Figure 1, B) and *P lanceolata* (data not shown) at 2 mg/mL did not inhibit specific IgE binding to RJ.

Despite the widespread use of RJ, reports are few of adverse reactions to it.<sup>5</sup> Most of them occur in the adult population, although a case of fatal induced asthma by RJ in an 11-year-old girl has been described.<sup>6</sup> Allergic reactions to RJ usually develop in atopic persons,<sup>1</sup> suggesting that patients with a history of asthma or other allergic conditions may be at increased risk of allergic reactions to RJ and should avoid this product.<sup>7</sup> In several cases these reactions have been described after the first known exposure to RJ, suggesting previous sensitization to cross-reactive allergens from other sources, such as mites<sup>8</sup> or mugwort pollen.<sup>9</sup>

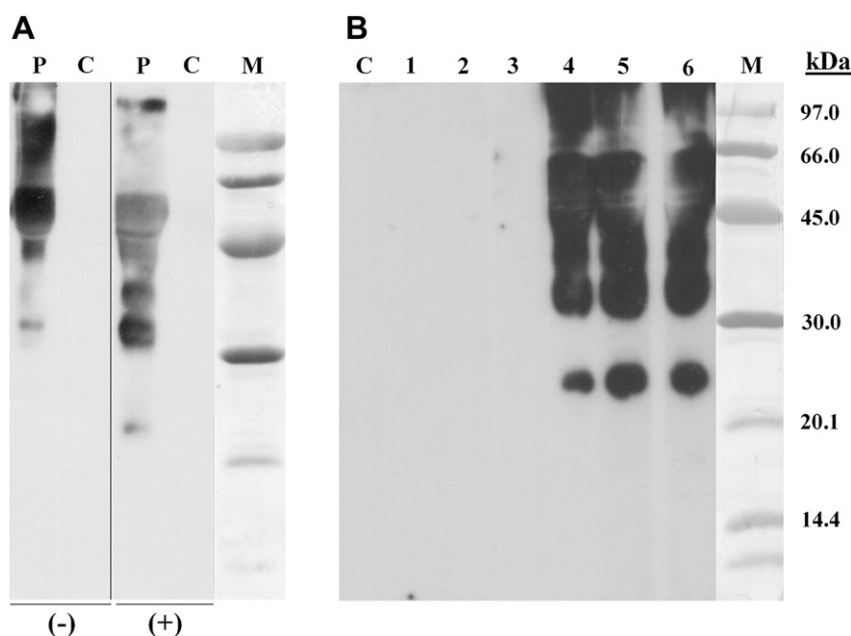


**FIGURE 1.** **A**, SDS-PAGE immunoblot analysis. *Lane P*, patient's serum; *lane C*, control sera (pool of sera from non-atopic subjects); *lane M*, molecular mass markers. (–) without 2-mercaptoethanol; (+) with 2-mercaptoethanol. **B**, SDS-PAGE immunoblot inhibition. *Lane C*, control sera (pool of sera from non-atopic subjects); *lane 1*, patient's serum after incubation with RJ extract; *lane 2*, patient's serum after incubation with *D pteronyssinus* extract (0.8 mg/mL); *lane 3*, patient's serum after incubation with *D pteronyssinus* extract (2 mg/mL); *lane 4*, patient's serum after incubation with pollen from *P pratense* (2 mg/mL); *lane 5*, patient's serum after incubation with pollen from *Artemisia vulgaris* (2 mg/mL); *lane 6*, patient's serum after incubation with lamb extract (2 mg/mL); *lane M*, molecular mass markers.

Our patient had allergic rhinitis and she was sensitized to dust mites, grass, and *P lanceolata* pollen. Immunoblot inhibition showed that proteins from *D pteronyssinus* and RJ present cross-reactive allergenic epitopes; therefore, a prior sensitization to dust mites might explain why the allergic reaction developed after her first exposure to RJ.

We also found that mugwort pollen extract did not inhibit specific IgE binding to RJ, ruling out the possibility of cross-reactivity between them.

In summary, RJ may cause allergic reactions in atopic persons, especially in those patients allergic to *D pteronyssinus*, given the presence of cross-reactive epitopes in both allergens.

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