

Asthma and anaphylaxis induced by royal jelly

F. C. K. THIEN*, R. LEUNG*, B. A. BALDO†‡, J. A. WEINER*, R. PLOMLEY* and D. CZARNY*

*Allergy and Respiratory Immunology Unit, Department of Respiratory Medicine, Alfred Hospital, Prahran,

†Kolling Institute of Medical Research, Royal North Shore Hospital of Sydney and ‡Department of Medicine, University of Sydney, Australia

Summary

Background Asthma, together with, in some cases, anaphylaxis, was observed in seven subjects following ingestion of royal jelly, a secretion of honey bees which is used as a health tonic.

Objective To determine if reactions were IgE-mediated and to identify allergenic components of royal jelly.

Methods Skin-prick tests, immunoassays for specific IgE antibodies and protein blotting studies using patients' sera and anti-IgE second antibodies were employed.

Results Immunoassays detected IgE antibodies to royal jelly proteins in sera of subjects who reacted to the substance. A total of 18 different IgE-binding components were detected on blots following electrophoretic separation of royal jelly under dissociating conditions. Examination of 63 sera from subjects allergic to bee venom showed that there is no direct relationship between IgE antibody reactivity to bee venom allergens and to royal jelly proteins although 38% of the sera reacted with a royal jelly solid phase. IgE antibody reactivity to royal jelly proteins was also detected in 52% of 75 subjects with allergies to inhalant and/or food allergens. Antibody binding of blotted royal jelly proteins was most marked in the molecular weight region 25–55 kDa and one component of MW ~ 55 kDa was detected by all of the reactive sera from royal jelly-allergic and control allergic subjects.

Conclusions Symptoms of asthma and anaphylaxis seen in subjects following ingestion of royal jelly were true IgE-mediated hypersensitivity reactions. The clinical significance of the antibodies found in the sera of control subjects is not known but they may arise in response to common inhalant allergens that show allergenic cross-reactivity with royal jelly.

Keywords: asthma, anaphylaxis, royal jelly

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Introduction

Royal jelly, a secretion of the hypopharyngeal and mandibular glands of worker honey bees (*Apis mellifera*), is a creamy yellow-white, acidic material, slightly pungent in odour and taste and made up of proteins, free amino acids, fatty acids, sugars, vitamins and some minerals [1–6]. During the early stages of development, royal jelly is the only food of female bee larvae but only

the queen bee continues to receive this diet while other larvae develop into sexually immature worker bees.

Although imprecisely defined chemically and generally unstandardized, royal jelly is widely used as a health tonic and in 'alternative' medicine but, unlike with bees, its beneficial effect for man is unproven and, in fact, some serious adverse reactions have resulted following its ingestion. Recently, Thien *et al.* [7] drew attention to five cases of asthma and anaphylaxis provoked by royal jelly and this was followed by a report of further cases of royal jelly-induced asthma and/or anaphylaxis including the death of an 11-year-old girl [8].

Correspondence: Dr B. A. Baldo, Kolling Institute of Medical Research, Royal North Shore Hospital, St Leonards, NSW 2065, Australia.

In an effort to investigate the possible allergenic involvement of constituent proteins in the reactions reported by Thien *et al.* [7], we set out to qualitatively define the protein components of royal jelly and to examine their IgE antibody reactivity in both the patients who experienced the reactions and in allergic subjects sensitive to some other allergens including bee venom.

Materials and methods

Royal jelly and royal jelly extracts

The preparation of royal jelly examined was a sample labelled Fresh Royal Jelly Natural High, Forster, NSW, Australia, batch number 10342. A water soluble extract of the preparation was made by mixing royal jelly (5 g) with distilled water (10 mL) and rotating overnight at 4°C before centrifuging at 12 000 rpm for 1 h. The supernatant was dialysed using Spectrapor Membrane Tubing (Spectrum Medical Industry Inc., Los Angeles, molecular weight cut-off 2 000 Da) at 4°C against repeated changes of distilled water and freeze-dried.

Protein content was determined with the Pierce BCA* (Bicinchoninic acid) Protein Assay Reagent [9]. The lyophilized material was stored at 4°C.

Subjects and sera

Details of seven subjects, six female and one male, who each experienced a reaction after ingestion of royal jelly, are set out in Table 1. All seven subjects were atopic and had positive skin-prick tests to grass pollens. Skin-prick testing with royal jelly was not performed on subject 4290 but the other six subjects all proved skin-test-positive to royal jelly. Sera were collected hours or a few days after reactions and stored at -20°C. Sera from a total of 75 subjects allergic to foods or, common inhalant allergens as well as some sera from patients with eczema, were obtained from the Allergy Clinic, Royal North Shore Hospital (RNSH). Serum IgE antibodies to house dust mite and grass pollens were detected in 88% and 73% of these subjects respectively. Sera from 63 bee venom-allergic subjects collected over a 4-year-period were also studied. Only 26 of these were examined for IgE antibodies to inhalant allergens; eight had

Table 1. Clinical details and serum IgE reactions of royal jelly-sensitive subjects

Subject	Age/sex	Clinical details	IgE binding to royal jelly proteins*
4289	20 F	Patient history of mild asthma. Asthma, rhinitis, angioedema following royal jelly. +ve oral challenge. Skin-prick test +ve	6.7†
4290	66 M	Patient history of mild asthma. Severe anaphylaxis and asthma following royal jelly. Oral challenge and skin-prick test not done	35.0
4291	32 F	Serum collected ~ 6 years after royal jelly reaction. Skin-prick test +ve, oral challenge +ve with bronchospasm	1.3
4292	33 F	Wheeze, rhinitis and angioedema following royal jelly. Skin-prick test +ve	1.3
4330	25 F	Patient history of infrequent asthma. Onset of asthma 1 h after first exposure to royal jelly. Skin-prick test +ve, 9 × 4 mm	12.6
4331	45 F	Patient history of mild asthma. Asthma, angioedema, hypotension after taking royal jelly. Skin-prick test +ve, 15 × 5 mm	31.8
4432	33 F	Patient history of mild asthma. Cough, chest tightness and dyspnoea after 2nd intake of royal jelly.	21.9

* Proteins bound to nitricellulose discs (see materials and methods).

† Expressed as percentage uptake of ¹²⁵I-anti-human IgE which is directly proportional to the amount of IgE bound. Uptakes with cord and 'normal' (non-allergic) sera were 0.3% and 0.6% respectively.

antibodies to house dust mite and six had grass pollen-reactive antibodies. Three of the seven royal jelly-allergic subjects were skin tested with bee venom and were negative. Skin-test reactivity of the 75 control allergic subjects and 63 bee venom-allergic subjects to royal jelly was not determined and past usage of royal jelly by these subjects was not known. Other control sera were obtained from normal non-allergic laboratory workers. Cord serum was provided by the Department of Obstetrics, RNSH and used as an IgE-'free' control. All sera were stored at -20°C .

Tests for royal jelly-reactive IgE antibodies

Sera were tested for royal jelly-reactive IgE antibodies in assays using 6 mm nitrocellulose discs [10] prepared by soaking 100 discs overnight in 2 mL of a 2 mg/mL solution of royal jelly extract in distilled water. After rinsing in a solution of Tween 20 0.1%, discs were soaked in this solution for 1 h to block unbound sites. After incubation of a disc for 4 h with serum (50 μL per tube), bound IgE antibodies were detected by the addition of

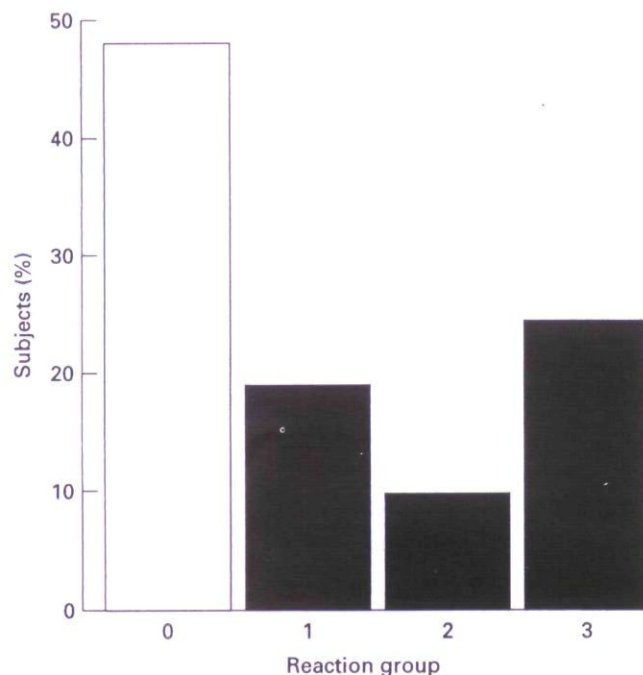


Fig. 1. Histogram showing the frequencies of negative (group 0) and weak (group 1), medium (group 2) and strong (group 3) IgE antibody reactions of subjects' sera ($n = 75$) with royal jelly proteins. Subjects were allergic patients with asthma, allergic rhinitis and/or eczema clinically sensitive to inhalant and/or food allergens but not to bee venom. IgE binding to royal jelly proteins immobilized on nitrocellulose discs was detected by the addition of ^{125}I -anti-IgE.

^{125}I -anti-human IgE obtained from Bioclone Australia Pty Ltd, Marrickville, NSW, Australia. Results were expressed as percentage uptake of ^{125}I -anti-IgE which is directly proportional to the amount of IgE antibodies bound to the disc.

Uptakes of labelled anti-IgE that were $< 3\times$ uptakes obtained with serum from a normal, non-allergic subject (0.6%) were considered negative. Positive reactions were classified as group 1 if uptakes were $3-5\times$ the normal serum value, group 2 ($5.1-10\times$ normal) or group 3 ($> 10\times$ normal value).

Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE [11] was carried out as previously described [12]. Proteins in polyacrylamide gels were stained with Coomassie Brilliant Blue. For electroblotting studies, 150 μL of a 50 mg/mL solution of royal jelly was loaded onto a 14×15 cm polyacrylamide 9–27% gradient gel before carrying out electrophoresis at 170 V for about 18 h. Molecular weight standards (Pharmacia, Uppsala, Sweden) were used to assess the approximate molecular weights of the royal jelly proteins.

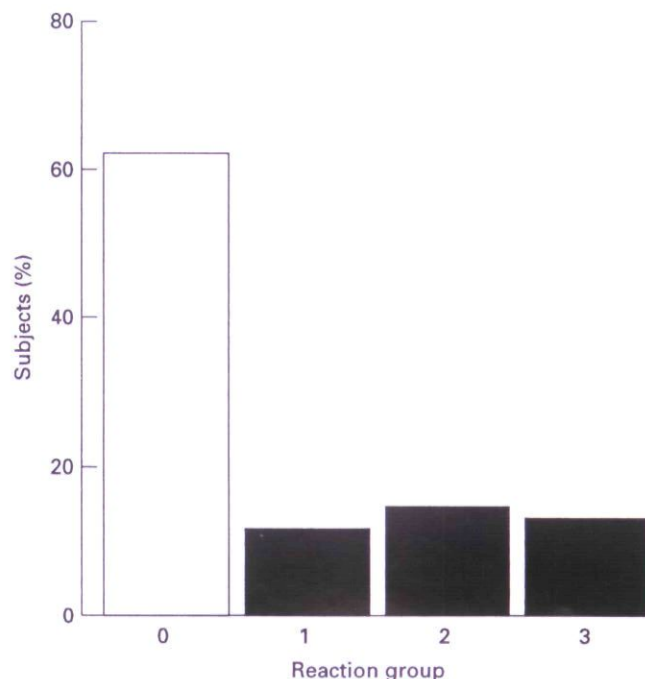


Fig. 2. Histogram showing the frequencies of reactions of sera from subjects ($n = 63$) allergic to bee venom. Negative reactions, group 0; positive reactions, groups 1–3. For other details see legend of Fig. 1.

Electrophoretic transfer of proteins to nitrocellulose membrane

Materials and methods for electrotransfer of separated proteins to nitrocellulose were as previously described [13,14]. After transfer, membranes were washed for 5 min in 10% methanol in phosphate-buffered saline for 1.5 h at room temperature to block unbound sites. Transferred proteins were detected with amido black (Sigma Chemical Co., Missouri, USA).

Probing of nitrocellulose strips and autoradiography

Materials and methods for identification of IgE-binding proteins were as previously described [15]. Detection of IgE-binding proteins was achieved by the addition of ^{125}I -anti-human IgE (Bioclone).

Results

Examinations for royal jelly-reactive IgE antibodies

Sera from seven subjects who reacted to royal jelly were

examined for royal jelly-reactive IgE antibodies in a radioallergosorbent test using nitrocellulose discs with adsorbed royal jelly proteins. In comparison to cord serum and sera from non-allergic controls, all seven sera showed elevated levels of uptake of anti-IgE by the discs but with two of the sera, numbers 4291 and 4292, the levels were not convincingly high (Table 1). In addition to the sera from non-allergic subjects, sera from 75 different subjects either with eczema and known food and/or inhalant allergen sensitivities or with known allergic rhinitis and/or asthma to foods or inhalants, were screened for IgE antibody binding to the royal jelly discs. Fifty-two per cent of the sera proved positive with approximately half of the positive sera showing strong reactions (Fig. 1). To investigate a possible association between antibody reactivity to royal jelly and sensitivity to bee venom, 63 sera from subjects with known allergy to bee venom and with bee venom-reactive IgE serum antibodies were screened for IgE antibodies to royal jelly proteins. Thirty-eight per cent of these sera proved positive (Fig. 2). Results of IgE-binding studies with some of the sera from atopics and

Table 2. Clinical details and serum IgE reactions for selected 'control' subjects used to probe royal jelly blots*

Subject No	Age/sex	Clinical details	IgE binding to royal jelly proteins†
1864	22 F	Asthma, rhinitis	13.8 ⁺
1907	34 F	Asthma, rhinitis	7.3
2054	16 F	Asthma	24.0
2310	24 F	Asthma, rhinitis	16.7
3478	37 M	Severe asthma, urticaria	19.7
3560	22 F	Asthma, rhinitis	32.5
3629	31 M	Mild asthma, rhinitis	5.4
3255	22 F	Eczema	10.1
3437	42 F	Eczema	32.6
3751	32 F	Severe eczema	25.4
3792	21 F	Eczema, urticaria, angioedema	18.7
4040	49 M	Eczema	26.6
4101	32 F	Eczema, asthma, rhinitis	30.6
4279	29 F	Eczema	15.7
4196	36 F	Eczema, asthma, rhinitis	5.9
2093	21 F	Bee venom reaction	2.7
2369	29 M	Bee venom reaction	6.6
3878	31 M	Bee venom reaction	3.3
4320	25 M	Bee venom reaction	7.1

* See Fig. 3.

† Proteins bound to nitrocellulose discs (see materials and methods).

+ Expressed as percentage uptake of ^{125}I -anti-human IgE which is directly proportional to the amount of IgE bound. Uptakes with cord and 'normal' (non-allergic) sera were 0.3% and 0.6% respectively.

bee venom-allergic subjects used in blotting studies are shown in Table 2.

Uptakes of anti-IgE by cord sera and sera from 'normal' non-allergic subjects were in the range 0.3–0.6%.

Qualitative electrophoretic definition of royal jelly proteins and identification of IgE-binding proteins

Protein staining following SDS-PAGE of reduced royal jelly proteins revealed 18–20 bands with a heavy cluster in the molecular weight region ~25–75 kDa. The dominant features of the separation profile were heavily stained bands of ~55, 47, 40 and 39 kDa (Fig. 3).

Blotting studies designed to detect IgE-reactive bands showed that sera from royal jelly-sensitive subjects 4289, 4290, 4330, 4331 and 4332 all reacted with the ~55 and 47 kDa components (group a Fig. 3 and Table 3) while

three of the five sera examined also reacted with the ~40 and 39 kDa bands. In total, the sera from the subjects allergic to royal jelly revealed 14 IgE antibody-reactive bands in the reduced royal jelly extract.

Blotting of serum samples from subjects with a clinical history of asthma and rhinitis (group b), subjects with a clinical history of eczema (group c) and subjects allergic to bee venom (group d) revealed detection profiles similar to the royal jelly serum panel except that none of the four group d sera reacted with the ~47 kDa royal jelly protein. Again, however, all sera detected the ~55 kDa band (Fig. 3; Table 3). Sera from the bee venom-allergic group, unlike the other sera examined, also reacted with the higher molecular weight (~94, 67 and 60 kDa) royal jelly proteins.

No binding of anti-IgE (and hence of IgE antibodies) was observed when cord sera or sera from 'normal'

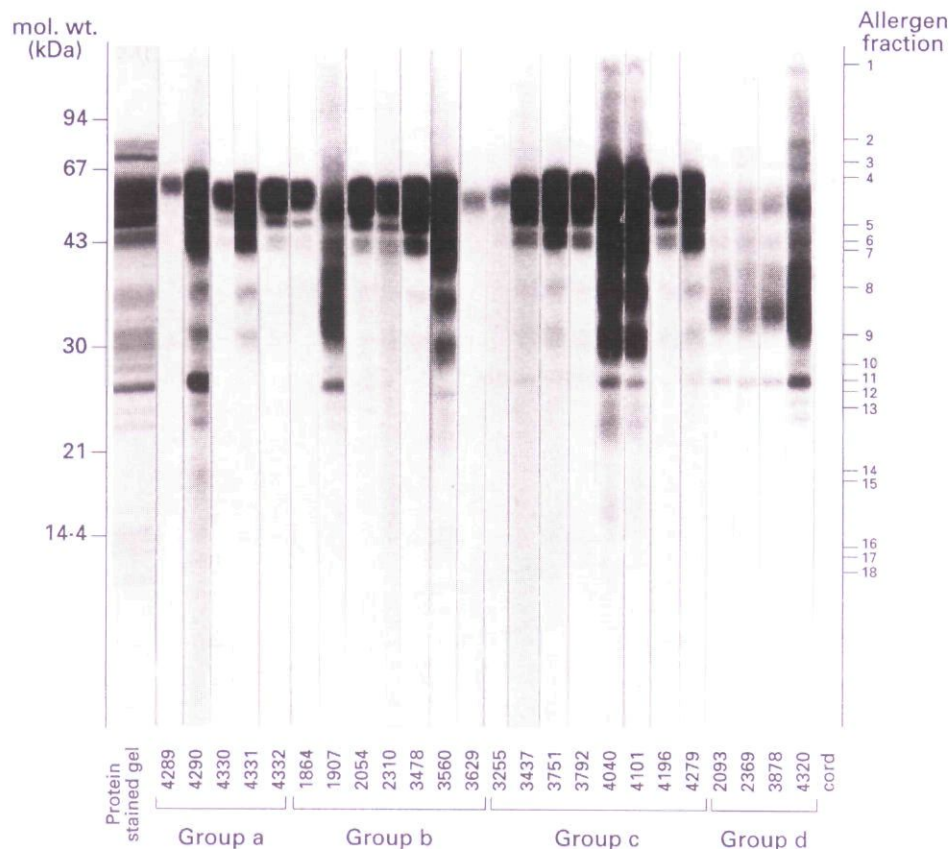


Fig. 3. Protein stained and IgE antibody-binding patterns of blotted royal jelly proteins separated by SDS-PAGE and transferred to nitrocellulose membrane. Strips in groups a–d and strip labelled cord are autoradiographs of blotted royal jelly proteins on nitrocellulose strips that were incubated with patients' sera or cord serum followed by ^{125}I -anti-human IgE. Group a: sera from subjects who experienced a reaction to royal jelly; group b: sera from subjects with a clinical history of asthma and rhinitis. Strip c: sera from subjects with a clinical history of eczema; group d: sera from subjects with a clinical history of an allergic reaction to bee venom. Autoradiographs were subjected to exposure for 7 days. For details of individual patterns of reactions of the sera see Table 3.

Table 3. Reaction patterns detected by protein blotting of sera from allergic subjects showing IgE antibody-binding components detected in royal jelly

Subject no.	Royal jelly allergen fraction and estimated molecular weight ($\times 1000$)																	
	1 > 94	2 67	3 60	4 55	5 47	6 40	7 39	8 33	9 30	10 27	11 25	12 23	13 21	14 14.7	15 14.5	16 14.4	17 14.3	18 14.0
4289				•	•													
4290				•	•	•	•	•	•		•	•	•	•	•	•	•	•
4330				•	•	•	•	•										
4331				•	•	•	•	•	•		•							
4332				•	•	•	•											
1864				•	•	•	•											
1907		•	•	•	•	•	•	•	•	•	•	•	•					
2054				•	•	•	•				•							
2310				•	•	•	•	•	•		•	•						
3478				•	•	•	•	•	•		•							
3560				•	•	•	•	•	•							•		
3629				•	•	•	•				•							
3255				•	•	•	•				•							
3437	•			•	•	•	•	•	•		•	•						
3751				•	•	•	•	•	•		•	•						
3792				•	•	•	•	•	•		•	•						
4040	•			•	•	•	•	•	•		•	•	•	•	•			
4101				•	•	•	•	•	•		•	•	•					
4196				•	•	•	•	•	•		•	•						
4279		•	•	•	•	•	•	•	•		•							
2093	•	•	•	•		•	•	•	•	•	•	•	•					
2369	•	•	•	•		•	•	•	•	•	•	•	•					
3878	•	•	•	•		•	•	•	•	•	•	•	•					
4320	•	•	•	•		•	•	•	•	•	•	•	•					

a Sera from subjects with clinical reaction to royal jelly (see Table 1).

b Sera from subjects with a clinical history of asthma and rhinitis (see Table 2).

c Sera from subjects with a clinical history of eczema (see Table 2).

d Sera from subjects with a clinical history of an allergic reaction to bee venom (see Table 2).

non-allergic subjects were employed in the protein blotting studies with royal jelly.

Discussion

Royal jelly commercially available in Australia is generally produced locally or imported from China and the different preparations taken by subjects at different times and in different locations suggests that the reactions were due to the royal jelly proteins themselves rather than to contamination or a defective batch of royal jelly.

Results of direct antibody-binding and blotting studies clearly showed that subjects who experienced an allergic reaction to royal jelly have serum IgE antibodies to a number of royal jelly protein components. As with other allergen extracts [12,14,16,17], antibody-binding patterns

were heterogeneous but two of the components of MWs ~ 55 and 47 kDa were recognized by the sera from all subjects.

Interestingly, the occurrence of IgE antibodies that react with royal jelly protein is not confined to subjects known to be allergic to the extract. Approximately half of a group of subjects allergic to inhalants and/or foods were found to have serum IgE antibodies that reacted with royal jelly and a similar spectrum of bands was detected on blots when these sera were used to probe the separated royal jelly proteins. As with the royal jelly-allergic subjects, sera from subjects with asthma and rhinitis and/or eczema all reacted with the ~ 55 and 47 kDa proteins suggesting that these two components will prove to be major allergenic proteins and useful diagnostic markers for detecting allergic sensitivity to royal jelly.

IgE-antibody binding was also detected in the sera of

24 of 63 subjects with known allergic sensitivity to bee venom indicating that there is not a direct relationship between sensitivities to royal jelly and to the venom. Overall though, the high incidence of IgE antibodies reactive with royal jelly in the 138 allergic subjects with no previously established allergy to royal jelly is intriguing and of some concern since at least some of these subjects may exhibit clinical symptoms if exposed to the substance. It must be emphasized, however, that clinical allergic sensitivities were not established or even investigated in these 'control' subjects so the significance of the royal jelly-reactive IgE antibodies in such patients is unknown and should be urgently studied. It would also be of interest to skin test a general allergic population with royal jelly extract to see if the high incidence of IgE antibodies to the proteins is reflected at the skin-test level.

The high incidence of royal jelly-reactive IgE antibodies in the allergic control subjects might be explained by contamination of royal jelly with inhalant allergens such as, for example, pollens, or crossreactions between royal jelly proteins and other commonly encountered inhaled or ingested allergen sources. The former explanation seems unlikely since a number of sera in the control group reacted strongly with blotted royal jelly proteins despite the absence of IgE antibodies reactive with grass pollens. Also, all the royal jelly-reactive sera from royal jelly-allergic and many control allergic subjects reacted directly on blots with a number of royal jelly proteins including the ~ 55 kDa protein known to be a component of royal jelly (see below). The most likely explanation therefore of the high incidence of reactivity of control allergic sera is allergenic cross reactivity and, at this stage of investigation, one might speculate that, given the high incidence of recognition of mite allergens in the allergic control group, this is the most likely source of the cross-sensitization.

The ~ 55 kDa protein detected on blots by all the royal jelly-reactive sera examined here may be the same protein recently studied and designated 'major royal jelly protein (MRJP)' by Hanes and Simuth [5]. Apart from a 5523 Da protein termed royalsin [18], the proteins of royal jelly appear to have been little studied so the relationship between MRJP and the IgE-binding ~ 55 kDa component we have detected may be of interest to both apiculturists and allergists.

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