

suppress noxious effects of these inflammatory cytokines. IL-13 also acts directly on eosinophils and, like IL-5, prolongs eosinophil survival and enhances the pathological effects of these cells at sites of inflammation. This effect seems to be related to an increase of IL-5 and IL-13 production in our patients with DIHS/DRESS, in which marked eosinophilia is observed. The role of increased GM-CSF levels in TEN is obscure. GM-CSF might be increased to compensate for a decrease of peripheral white blood cells, which is sometimes observed in TEN.

These observations indicate the importance of controlling serum cytokine levels during the treatment of TEN, especially at the time when serum TNF- α and IL-6 levels are drastically increased together with anti-inflammatory cytokines such as IL-10 and IL-1ra. Further studies that include larger numbers of patients are needed to elucidate the roles of cytokine production in the pathogenesis of severe types of ADR.

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Major royal jelly protein 3 as a possible allergen in royal jelly-induced anaphylaxis

Dear Editor,

This is a report regarding a patient with royal jelly (RJ)-induced anaphylaxis. RJ is a secretion from the

hypopharyngeal and mandibular glands of worker honey bees. Approximately 50% of its dry weight consists of protein and other ingredients including

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free amino acids, fatty acids, sugar and vitamins.¹ Recently, RJ is widely consumed as a constituent of health tonics in Japan. There have been a few reports of acute bronchial asthma,² anaphylaxis³ and hemorrhagic colitis⁴ caused by RJ. Major RJ protein 1 (molecular weight: 55 kDa) and major RJ protein 2 (47 kDa) are reported to be the two major allergens of RJ anaphylaxis. We herein report a case of RJ-induced anaphylaxis showing immunoglobulin (Ig)E antibodies reacting to an RJ protein of 68 kDa in addition to RJ proteins of 55 and 47 kDa.

A 26-year-old woman consulted the emergency department of our hospital because of dyspnea, severe edema of the face, especially the bilateral eyelids, congestion of the conjunctivae and erythema on the face 30 min after taking an RJ tablet. She had a 20-year history of bronchial asthma, but a specific allergen including honey had not been detected until then. Prior to the onset of symptoms, she sometimes had consumed drinks containing RJ, but not RJ tablets. Laboratory examination including a complete blood cell count revealed no abnormal findings except for a slightly elevated serum IgE level (239 IU/mL). IgE antibodies to honeybees and gelatin were below 0.34 UA/mL by the radioallergosorbent test. From a diagnosis of RJ-induced anaphylaxis, she was treated with an i.m. injection of epinephrine 0.5 mg, followed by i.v. prednisolone at 40 mg/day and *d*-chlorpheniramine maleate at 5 mg/day for 2 days, resulting in recovery after the treatment.

Skin prick tests were performed after 2 weeks using crushed RJ tablets, RJ powder and crystallized cellulose which were diluted with phosphate-buffered saline (PBS) at concentrations of 1/10, 1/100 and 1/1000. Other ingredients were arabic gum, carnauba wax, maltose powder, silicon dioxide and eggshell calcium that were mixed with a little petrolatum. Skin prick tests revealed that the 1/10 diluted solution of an RJ tablet and the 1/10 and 1/100 diluted solution of RJ powder were positive (Fig. 1) and the other ingredients were negative at all concentrations. Ten healthy volunteers showed negative results to the RJ powder at all concentrations.

Immunoblot analysis was performed using the sera of our patient, two other patients with RJ-induced anaphylaxis who showed positive results to RJ powder on skin prick tests, and three normal volunteers. Pure RJ ingredients were used as a substrate and

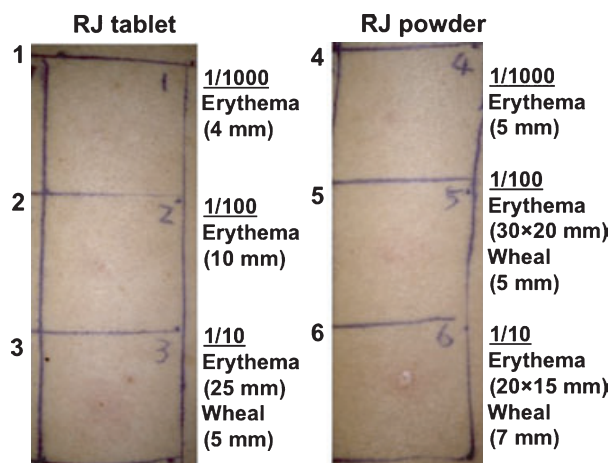


Figure 1. Results of skin prick tests. Royal jelly (RJ) tablets were applied at concentrations of 1/1000 (square 1), 1/100 (square 2) and 1/10 (square 3). RJ powder was applied at concentrations of 1/1000 (square 4), 1/100 (square 5) and 1/10 (square 6). The results revealed that the 1/10 solution of RJ tablet and 1/10 and 1/100 solutions of RJ powder were positive.

horseradish peroxidase (HRP)-labeled, rabbit-immunized, antihuman IgE antibody was used as the second antibody. Briefly, the protein extracts of pure RJ ingredients were treated with sodium dodecylsulfate (SDS) sample buffer and were boiled for 3 min, followed by SDS polyacrylamide gel electrophoresis (SDS-PAGE). Five-microgram proteins were loaded per lane. After SDS-PAGE, the separated RJ protein samples were transferred to polyvinylidene fluoride (PVDF) membranes by semi-dry blotting methods. The PVDF membranes were incubated with the patient's serum ($\times 20$ dilution in the presence of 5% skim milk in PBS containing 0.1% Tween-20 [PBST]). After incubation with the patient's serum, the membranes were washed with PBST and were again incubated with HRP-labeled antihuman IgE. Finally, signals were detected by enhanced chemiluminescence western blotting reagents (GE Healthcare, Tokyo Japan). Chemiluminescent signals obtained were recorded on X-ray film. Immunoblot analysis showed that the patient's IgE antibody reacted to bands of 47, 55 and 68 kDa and two weaker bands of 70 and 75 kDa. The band of 68 kDa was the most strongly stained. IgE antibodies in sera from two other patients with RJ-induced anaphylaxis also reacted to 55-, 47- and 68-kDa proteins. Two normal

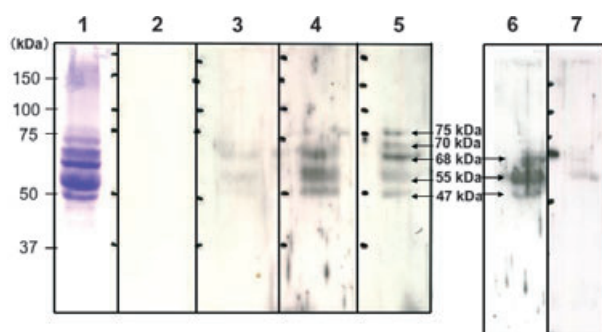


Figure 2. Immunoblot analysis using pure royal jelly (RJ) ingredients as a substrate. Lane 1 shows Coomassie brilliant blue (CBB) staining total proteins, and lane 2 shows a negative control with no serum. Lanes 3 and 7 show normal volunteers, lane 5 shows the present patient with RJ-induced anaphylaxis, and lanes 4 and 6 show two other patients with RJ-induced anaphylaxis. Lanes 4 and 6 show immunoglobulin (Ig)E antibodies reacting to 47-, 55- and 68-kDa proteins, and lane 5 shows IgE antibodies reacting to 47-, 55-, 68-, 70- and 75-kDa proteins.

volunteers' sera reacted faintly to RJ proteins of 55 and 68 kDa (Fig. 2).

Royal jelly has been reported to exhibit various physiological activities in experimental animals, including vasodilative and hypotensive activities, growth-increasing activity and anti-infection, antitumor, anti-hypercholesterolemic and anti-inflammatory activities.⁵ Since Thien *et al.*² reported RJ-induced asthma for the first time in 1993, further cases of RJ-induced asthma and anaphylaxis have been reported.³ Furthermore, they proved these symptoms to be derived from an IgE-mediated hypersensitivity reaction.⁶ In most cases of RJ-induced asthma and anaphylaxis, IgE antibodies react to two major RJ allergic proteins; major RJ protein 1 with a molecular weight of 55 kDa, and major RJ protein 2 of 47 kDa.⁷

In the present patient, IgE antibody reacted to not only the bands of 55 kDa, which probably corresponds to major RJ protein 1, and 47 kDa, which is considered to be major RJ protein 2, but also to the band of 68 kDa, which was the most strongly stained, and two weaker bands of 70 and 75 kDa. Two other RJ-induced anaphylaxis patients' sera also reacted to the 68-kDa band. This 68-kDa RJ protein probably corresponds to major RJ protein 3.⁸ This is the first clinical report of RJ-induced anaphylaxis which shows IgE antibody possibly reacting to major royal jelly protein 3, in addition to IgE anti-

bodies to major RJ proteins 1 and 2. However, the possibility that this antibody is reacting to other proteins of 68 kDa cannot be ruled out, and the weak bands of 70 and 75 kDa are still undefined. In normal volunteers' sera, bands of 55 and 68 kDa were faintly detected. There are explanations for this: a non-specific reaction and cross-reactivity between RJ allergens and environmental allergens such as other foods and pollens. In addition, some individuals may be "pre-sensitized" by a previous history of RJ ingestion,⁷ and anaphylaxis symptoms appear when some additional factors are presented. In conclusion, multiple RJ proteins including major royal jelly protein 1, 2 and 3 might be the patients' IgE-reactive allergens.

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