

arthritis patients. Immature B-cells are the first subset to return followed by naïve B-cells, which constituted most of the peripheral B-cells. CD27+ memory B-cell recovery was shown to be delayed and remained low for over 2 years. Furthermore, persistent in vitro failure of naïve B-cells to differentiate into mature B-cells or plasma cells in the presence of interleukin (IL)-2, IL-6, IL-10, IL-15, and B-cell activating factor in patients treated with rituximab combined chemotherapy has also been described.^{6,7} These findings further support the conclusion that rituximab combined chemotherapy may induce persistent B-cell maturation and differentiation arrest with subsequent hypogammaglobulinemia.

In addition to rituximab, several anti-CD20 monoclonal antibodies are in development for use in a variety of conditions, which will undoubtedly increase the number of patients exposed to these agents. The medical community should be made aware that the loss of memory B-cells and hypogammaglobulinemia with an increased risk of infection will likely become a more prevalent problem. Currently, B-cell subset characteristics and serum immunoglobulin levels are not universally evaluated before, during, or after treatment with rituximab and these evaluations appear to be warranted. Prolonged or perhaps permanent alteration of the B-cell compartment, which can have an indistinguishable clinical presentation and B-cell phenotype as common variable immune deficiency and certain hyper IgM syndromes (eg, activation-induced cytidine deaminase/uracil-DNA glycosylase and inducible T-cell costimulator deficiencies), may result from rituximab therapy and are not readily recognized or differentiated based on the evaluation of CD19+ B-cells alone.⁸ Delay in diagnosis and complicated evaluation of these patients can and should be avoided with appropriate baseline values. Based on our experience and review of the literature, we recommend acquiring a comprehensive baseline evaluation of the B-cell compartment as well as quantitative immunoglobulin levels before, during, and after rituximab therapy. Finally, the risk of persistent hypogammaglobulinemia and its associated comorbidities, as well as related long-term health concerns such as consequent chronic

intravenous immunoglobulin therapy should be seriously considered before recommending rituximab therapy, particularly in conditions that have other viable alternative treatments.

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A case report of bell pepper anaphylaxis: Could 1, 3- β -glucanase be the culprit allergen?

A 34-year-old woman was referred to our outpatient clinic after presenting with anaphylaxis. After eating red bell pepper she immediately presented with plantar and palmar itching, urticaria, stupor, and breathing impairment. Hypotension (80/50 mmHg) and low oxygen saturation (88%) were recorded at the emergency unit.

One month after that, she presented with a second episode of palmar and plantar itching and skin rash, immediately after eating a sauce containing crude red peppers. The symptoms resolved with self-administered adrenaline and antihistamines.

She reported a history of oral allergic syndrome to peach, walnut, and chestnut and immediate urticaria when using latex gloves, but she had never been studied by an allergist. She did not have any other medical condition; she denied respiratory symptoms and avoided red and green pepper, peach, and nuts.

We performed a skin prick test (SPT) with commercial extracts of fruits, vegetables, latex, nuts, and common aeroallergens (ALK Abelló, Madrid, Spain) and prick by prick with red and green pepper

(*Capsicum annuum*). Red or green pepper oral challenge was refused by the patient. Total and specific immunoglobulin (Ig) E was determined by the ImmunoCAP system (Thermo Scientific, Uppsala, Sweden).

A molecular diagnosis panel using microarray technology was performed using the ImmunoCAP ISAC (immuno solid-phase allergen chip) method (Thermo scientific).¹

Red and green peppers were extracted for 90 minutes at 4°C with phosphate buffer and analyzed by tricine sodium dodecyl sulphate polyacrylamide gel electrophoresis method under nonreducing conditions. Proteins were transferred to nitrocellulose membranes and incubated with patient serum about 18 hours. After that, they were incubated with anti-human IgE monoclonal antibodies and rabbit IgG anti-mouse antibodies labeled with peroxidase (Calbiochem, Nottingham, United Kingdom). Buffer incubation without serum was used as negative control. IgE binding proteins were detected by chemiluminescence (Amersham Bioscience) following the manufacturer's instructions. The same method was performed with peach.²

The SPTs with commercial extract were positive to red pepper and tomato and negative to other *Solanaceae* (eggplant and potato).

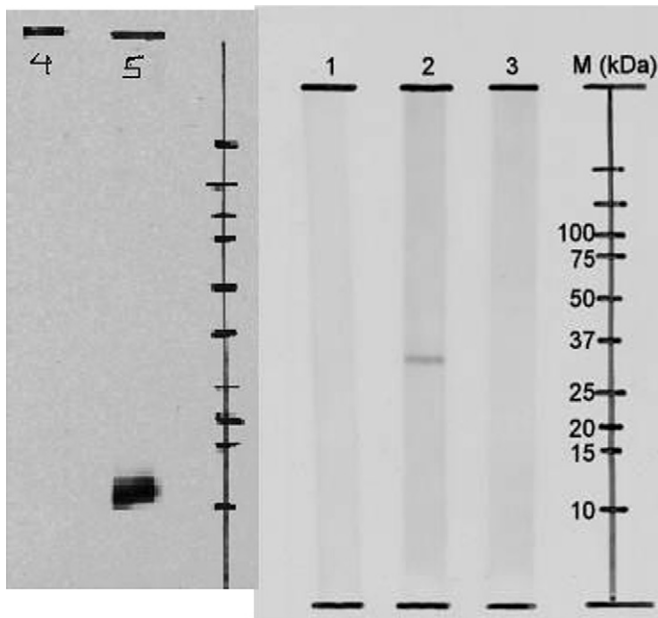


Figure 1. Bell pepper immunoblotting. Lane 1: Pepper (negative control); Lane 2: Red pepper (patient's serum); Lane 3: Green pepper (patient's serum); Lane 4: Peach (negative control); Lane 5: Peach (patient's serum).

In addition, positive SPTs were recorded for *Artemisia vulgaris*, *Parietaria Judaica*, and *Olea Europea* pollens, hazelnut, almond, chestnut, walnut, peanut, pistachio, pine nut, sunflower seed, *Rosaceae* fruits (peach and apple skin, cherry, raspberry), kiwi, and latex.

Prick by prick with bell pepper was positive for red pepper and negative for green pepper.

Total Ig E was 106 UI/mL. Serum specific IgE proved positive for bell pepper (2.7 kUI/L) peach (16.6 kUI/L), peanut (10.6 kUI/L), almond (5.53 kUI/L), walnut (12.7 kUI/L), chestnut (6.21 kUI/L), and latex (15.5 kUI/L). ISAC proved sensitization to latex hevein (Hev b 6) and lipid transfer proteins (LTP) of peach (Pru p 3), mugwort (Art v 3), and hazelnut (Cor a 9).

Immunoblotting showed Ig E binding bands of 30 to 35 kDa for red pepper and 10 kDa for peach (Fig 1).

Two major bell pepper allergens have been sequenced and characterized: Cap a 1, an osmotine-like protein of approximately 23 kDa belonging to PR 23 defense proteins group, and profilin (Cap a 2, 14 kDa).³

Bell pepper LTP has not been characterized yet, but the molecular weight of known LTPs is approximately 9 to 10 kDa.

A report has been made of severe anaphylaxis after bell pepper intake in a patient who was sensitized to birch and grass. They described an 11 kDa protein, which could be a fragment of Bet v 1 homologous.⁴

Latex hevein (Hev b 6, 20 kDa) has been described as a cause of cross-reactivity between latex and bell pepper.^{5,6}

Our patient is sensitized to a 30- to 35-kDa protein that does not match to any of these allergens. We suggest that it could be a 1,3- β -glucanase, which has been identified in latex (Hev b 2, 35 kDa). It had been previously described as a cause of cross-reactivity between latex and bell pepper.^{7,8} Other possibilities are some chitinase or peroxidase or a new, nonidentified allergen.

Interestingly an Ig E binding band was found for red pepper but not for green pepper. This could be attributable to the different expressions of proteins at different maturation stages or to culture condition of the vegetables. A paper from Jensen-Jarolim et al⁹ found different expression of profilin, osmotine, and Bet v 1 homologous in bell pepper, depending on the horticultural strain.

This is a case of bell pepper anaphylaxis in a patient with latex fruit syndrome and LTP sensitization. Surprisingly, LTP sensitization does not seem to play a role in bell pepper allergy in this patient. The most probable implied allergen is 1,3- β -glucanase, an allergen that had usually been related to oral allergic syndrome.

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