Hev b 5: Latex Allergen Implicated in Clinically Relevant Cross-Reactivity With Manioc

Â Gaspar,[1] M Raulf-Heimsoth,[2] HP Rihs,[2] G Pires,[1] M Morais-Almeida[1]

[1] *Immunoallergy Department, CUF Descobertas Hospital, Lisbon, Portugal*

[2] *Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University, Bochum, Germany*

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Manioc or cassava (*Manihot esculenta*) is a very important food in South America, Africa, and Asia. Native to Brazil, which is still one of the largest producers of this root vegetable, it was carried to Africa and Asia by Portuguese traders during the 16th century. The tuber, also known as cassava root, can be eaten boiled, fried, toasted or in the form of flour for bread, pastry, and cakes. It is the main source of carbohydrates for large populations in the tropics. Mainly eaten as a substitute for potato, manioc is slowly entering the European and North American diet, although it has been present for many years in the form of tapioca, which is dry starch obtained from cassava root.

In 2003 we published the first report of an allergic reaction to manioc [1]. In that paper, we proved by immunoglobulin (Ig) E immunoblotting inhibition analysis the existence of cross-reactivity between manioc and latex, leading to the inclusion of manioc on the growing list of foods involved in the latex-fruit syndrome. Afterwards, we described another latex-allergic patient with anaphylaxis to manioc [2], and 2 further cases were reported in Brazil [3]. Another case of anaphylaxis to manioc was reported in a Spanish woman in 2007 [4], and recently, 9 cases were reported in Brazil; they all had skin-related symptoms, such as urticaria and angioedema, and 3 patients had anaphylaxis to manioc [5].

The aim of the present study was to identify the latex allergen implicated in the latex-manioc cross-reactivity syndrome.

We included 2 patients with manioc-induced anaphylaxis, both of whom also prick tests (SPTs) with latex extract (ALK Abelló) and prick-to-prick tests with fresh manioc. Serum specific IgE to latex and manioc was measured by ImmunoCAP (Phadia), and the latex allergen sensitization pattern was studied using a panel of individual recombinant (rHev b 1, 3, 5, 6.01, 7, 8, 9, 10, 11 and 12) and native (nHev b 2 and nHev b 13) latex allergens, which were each coupled to ImmunoCAPs [6]. All the recombinant latex allergens were produced in *Escherichia coli* as a fusion protein with maltose-binding protein (MBP). MBP coupled on ImmunoCAPs served as a control. Sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblots were performed with manioc and latex extracts (AlaBLOT Specific IgE Procedure, DPC). Inhibition studies were performed by immunoblotting (AlaBLOT Inhibition Assay Procedure) and by ImmunoCAP inhibition.

Patient 1 was a 59-year-old Portuguese woman, born in Mozambique, with a previous history of asthma, severe latex allergy related to multiple surgeries, and latex-fruit syndrome. She had had an anaphylactic reaction with generalized urticaria, bronchospasm, and laryngeal edema 30 minutes after eating boiled manioc, and a similar reaction 5 minutes after eating raw manioc (tapioca flour). Previously, she had eaten manioc with no adverse reactions.

SPTs were positive to latex extract (9 × 7 mm) and fresh manioc (9 × 8 mm), and specific IgE was positive to latex (14.6

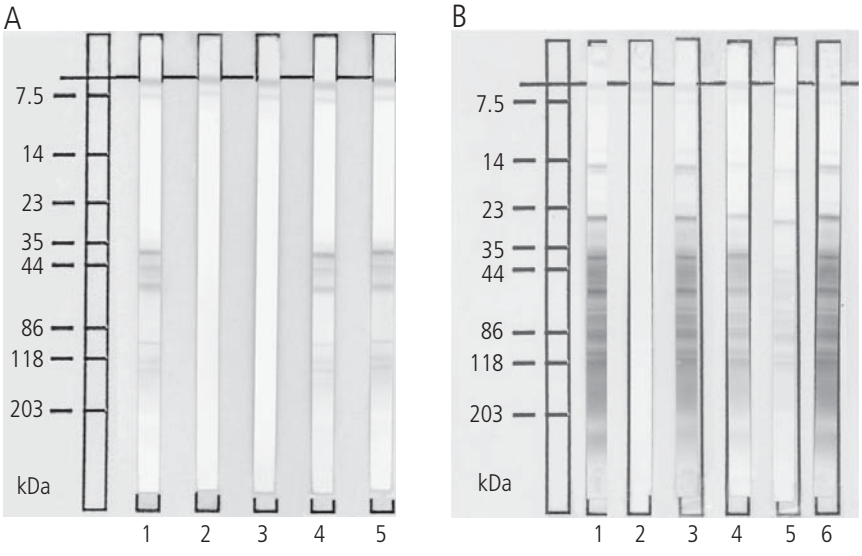

Figure. A. Manioc immunoblotting (AlaBLOT) immunoglobulin (Ig) E inhibition assay in patient 1. Lane 1, manioc immunoblotting (noninhibited); Lane 2, inhibited with manioc extract (10 µL of manioc extract, concentration of 10 mg protein/mL) as positive control; Lane 3, inhibited (100%) with latex allergen recombinant (r) Hev b 5 (concentration of 1.5 µg/µL); Lane 4, inhibited (5.7%) with latex allergen rHev b 7 (concentration of 3.2 µg/µL); Lane 5, inhibited with maltose-binding protein as negative control.
B. Manioc immunoblotting (AlaBLOT) IgE inhibition assay in patient 2. Lane 1, manioc immunoblotting (noninhibited); Lane 2, inhibited with manioc extract (as positive control); Lane 3, inhibited with latex allergen rHev b 7 (38.8%); Lane 4, manioc immunoblotting diluted 1:10 (noninhibited); Lane 5, inhibited with latex allergen rHev b 5 (64%); Lane 6, inhibited with maltose-binding protein as negative control.

kU/L) and manioc (5.1 kU/L). Specific IgE to individual latex allergens was positive only for rHev b 5 (19 kU/L) and nHev b 13 (0.81 kU/L). Immunoblotting of the manioc extract showed IgE-binding to 3 protein bands around 35, 42-44, and 50 kDa, which were 100% inhibited with latex allergen rHev b 5 (Figure 1A). In the ImmunoCAP inhibition study, we also observed 100% inhibition of manioc-specific IgE with rHev b 5 (concentration 1 μg/μL), and minor inhibition (24%) with nHev b 13 (concentration 0.75 μg/μL). In the cross-inhibition experiments, manioc extract (concentration 1 μg/μL) inhibited only 44% of the latex-specific IgE and 39% of the rHev b 5-specific IgE.

Patient 2 was a 46-year-old Portuguese woman, born in Guinea-Bissau, with a previous history of severe latex allergy related to occupational exposure (health care worker) and multiple surgeries, and latex-fruit syndrome. She had had an anaphylactic reaction with generalized pruritus, lip and hand angioedema, bronchospasm, and laryngeal edema 10 minutes after eating boiled manioc, and a similar reaction 5 minutes after eating raw manioc (tapioca flour). Previously, she had eaten manioc with no adverse reactions.

SPTs were positive to latex extract (5 × 5 mm) and fresh manioc (12 × 6 mm), and specific IgE was positive to latex (>100 kU/L) and manioc (40.1 kU/L). Specific IgE to latex allergens was positive to rHev b 1 (91.3 kU/L), nHev b 2 (1.5 kU/L), rHev b 3 (2.8 kU/L), rHev b 5 (>100 kU/L), rHev b 6.01 (>100 kU/L), rHev b 7 (0.5 kU/L), rHev b 8 (0.5 kU/L), rHev b 9 (0.6 kU/L), rHev b 10 (0.4 kU/L), rHev b 11 (31.9 kU/L), rHev b 12 (0.5 kU/L), and nHev b 13 (18.9 kU/L). Immunoblotting of the manioc extract showed IgE-binding to several protein bands, ranging from 16 to greater than 100 kDa, which were inhibited (64%) with latex allergen rHev b 5 (Figure 1B). In the ImmunoCAP inhibition study, we observed 100% inhibition of manioc-specific IgE with rHev b 5 and minor inhibition (ranging from 34%-49%) with nHev b 2, rHev b 7, rHev b 11, and nHev b 13 (concentration 0.16-1 μg/μL). In the cross-inhibition experiments, manioc extract inhibited only 15% of latex-specific IgE and only 26% of specific IgE to rHev b 5.

Our results strongly suggest that manioc allergy was a consequence of primary latex sensitization. The latex allergen responsible for this cross-reactivity was Hev b 5. This acidic structural protein is a strong antigen and one of the most important latex allergens, with a high prevalence in health care workers; furthermore, it has previously been associated with a homologous protein in kiwi fruit [7,8].

In accordance with recent findings suggesting that Hev b 5 may be a strong candidate for involvement in latex-manioc syndrome [5], and in contrast to a previous publication [9], we confirm for the first time, using IgE-binding inhibition studies, that Hev b 5 is the major latex allergen implicated in clinically relevant cross-reactivity with manioc. The immunoblotting inhibition results, where IgE-binding to several protein bands in the manioc extract was inhibited with rHev b 5, could be explained by the existence of multiple isoforms of Hev b 5 [8]. Another explanation could be the presence of multiple IgE-binding epitopes, since 11 epitopes have been identified to date, making Hev b 5 a multivalent allergen [10].

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Ângela Gaspar
Centro de Imunoalergologia
Hospital CUF Descobertas
1998-018 Lisboa, Portugal
E-mail: angela.gaspar@sapo.pt