

Letter to the Editor

Allergic reactions to manioc (*Manihot esculenta* Crantz): Identification of novel allergens with potential involvement in latex-fruit syndrome

To the Editor:

Manioc or cassava (*Manihot esculenta* Crantz) is a tuber consumed worldwide but mainly in South America, Africa, and Asia. It is a main source of carbohydrates for more than 800 million persons in these regions. This root is usually consumed boiled or fried and can be used to prepare breads, cakes, and pastries. A major product of manioc is starch, a fine white powder extracted from pulped manioc roots, which is used as a substitute for potato starch and cornstarch and is important in manufacturing sago pearl, monosodium glutamate, fructose, glucose, and dextrose. Manioc starch is mixed with pharmaceuticals to make capsules and tablets. According to the Food and Agriculture Organization, Thailand and Nigeria are the biggest producers and export manioc starch mainly to the European and North American markets. An important reason for the vast use of manioc starch is the fact that it is considered nonallergenic and therefore largely used in baby food processing.

However, in 2004, we reported allergic reactions after manioc ingestion for the first time in Brazil.¹ Two more case reports with subjects from Mozambique and Spain were published in 2003 and 2007,^{2,3} and allergic manifestations to manioc have been suggested but not proved at the molecular level to be associated with latex allergy. Even though manioc is able to trigger anaphylactic reactions in predisposed patients, identification of the IgE-binding proteins is still missing.

In the present study we included 9 patients presenting with allergic symptoms after ingestion of boiled manioc. The study was approved by the Ethic Committee for Analysis and Research Projects, CAPPesq of HCFMUSP, and written informed consent was obtained from all patients. Clinical manifestations included skin-related symptoms, such as urticaria and angioedema, and 3 patients had anaphylactic reactions (Fig 1, A). All patients underwent prick-to-prick tests with raw and boiled manioc, presenting positive results with larger papules for raw manioc when compared with the boiled root. Notably, all investigated patients also presented with clinical relevant latex allergy. Five of 6 patients showed a positive result for basophil activation on stimulation with raw manioc extract, whereas a single patient with a negative result (<15% of activated basophils)⁴ presented with only local contact reactions and facial edema without major manifestations that might correlate to the lower response in the assay (Fig 1, A).

In ELISA experiments patients demonstrated similar IgE reactivity to both manioc extract and starch (Fig 1, B). Despite the fact that manioc starch generally contains less protein, both sources showed similar allergenic potential.

In IgE immunoblotting all patients reacted to manioc proteins migrating at approximately 40 and 30 kD, whereas 5 patients (nos. 1, 4, 6, 8, and 9) additionally recognized a 37-kD protein (Fig 1, C). To unequivocally identify those proteins, we performed 2-dimensional gel electrophoresis and mass spectrometry-based analysis by using the phytozome database (www.phytozome.net) for protein identification. Three spots were identified as

fructose-bisphosphate aldolase (GenBank: EEF49496.1) with a theoretic molecular mass of 39.9 kD, and 2 spots corresponded to glyceraldehyde-3-phosphate dehydrogenases (GAPDH; GenBank: EEF46886.1) with a mass of 36.8 kD. GAPDH was previously described as an IgE-binding protein also found in fungi and wheat (www.allergome.org).

Among patients with mold allergy in Germany, GAPDH was identified as the most important allergen of *Aspergillus versicolor*, with a sensitization prevalence of 94%.⁵ Furthermore, GAPDH was reported as a major allergen in subjects exposed to the biting midge *Forcipomyia taiwana*.⁶ Fructose-bisphosphate aldolases were identified as IgE-binding proteins in patients allergic to fungi and salmon.⁷ Both GAPDH and fructose-bisphosphate aldolase represent evolutionarily highly conserved proteins with general metabolic functions; however, their relevance as potential plant food allergens has not yet been established. In a recent study GAPDH of *Hevea brasiliensis* Muell-Arg was shown to bind IgE from sensitized patients.⁸ In our study 5 of 9 patients reacted to this protein, which might partially explain the IgE cross-reactivity of latex and manioc observed in previous studies.¹⁻³

Sera from all our patients reacted with a protein migrating at approximately 30 kD identified as Pt214, a glutamic acid-rich protein (GenBank: AAM55492.1). This protein was tentatively termed "allergenic-related protein" based solely on the primary sequence identity to Hev b 5 from latex. Hev b 5 ranks among the most important allergens of *Hevea brasiliensis*, with a high prevalence in health care workers and patients with spina bifida, and is therefore indispensable for the diagnosis of latex allergy.⁹ Although Hev b 5 and Pt214 show moderate sequence identity (41.9%), some linear IgE epitopes previously identified for the latex allergen are partially conserved in manioc Pt214.¹⁰ This protein was recently suggested not to be involved in IgE cross-reactivity because it was not recognized by sera of patients with latex allergy.¹¹ However, in contrast to our study, no data on IgE binding to Pt214 using sera of subjects with manioc allergy have been provided. Thus this new allergen might in fact be responsible for the observed link between manioc and latex allergy corroborated by the fact that all our patients with manioc allergy reacted to it. In the present study we identified a relevant IgE-binding glutamic acid-rich protein from manioc with 41.8% sequence homology to Hev b 5, an important latex allergen. Although Hev b 5 has not been reported to mediate IgE cross-reactivity, our results suggest that it is a strong candidate for involvement in a latex-manioc syndrome. In our study cohort all patients with manioc allergy demonstrated allergic reactions to latex, and clinical histories showed that allergic reactions to latex always preceded symptoms to manioc, leading to the assumption that manioc allergy might be a consequence of primary latex sensitization. In Europe and the United States the incidence of latex allergy has been reduced because of specific guidelines made in the medical field. However, there might be a second wave, especially in countries that are pursuing higher economic and technologic standards.⁹ Manioc often represents a staple food in those countries, and therefore new allergies to this plant food source might be an undesirable consequence.

Proteomic analysis with patients' sera enabled the identification of novel manioc IgE-binding proteins, demonstrating the

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Patient Nr.	Sex/age (y)	Symptoms	Prick to prick		BAT % AB	Other allergies
			Raw	Boiled		
1	F/45	SS (U)	25	Neg	ND	latex, allergic rhinitis (molds, dust mites)
2	F/41	SS (AE, U)	25	Neg	ND	latex
3	F/41	SS (AE, U)	30	9	24.7	latex
4	F/41	SS (AE)	25	7	46.9	latex, FA (kaki)
5	F/38	SS (AE, U)	25	7	30	latex, drug allergy
6	F/40	Anaphylaxis	30	7	16	latex, FA (papaya, mango, plum), allergic rhinitis (dust mites)
7	F/45	SS (AE, CU)	25	Neg	6.7	latex, CD (cobalt cloridium)
8	F/40	Anaphylaxis	30	9	ND	latex
9	F/55	Anaphylaxis	70	25	41	latex, FA (papaya, mango)

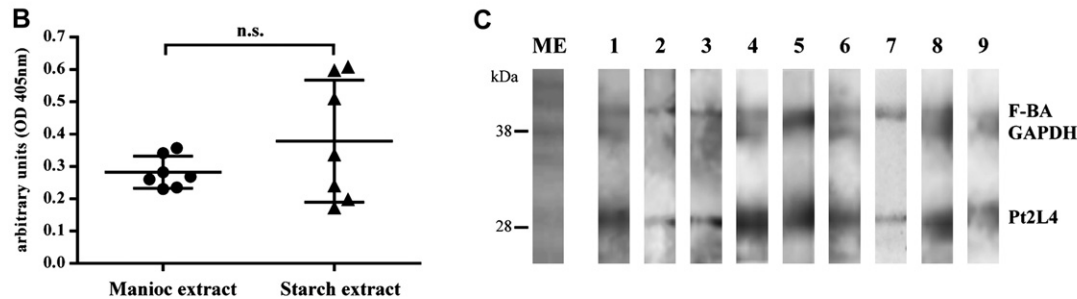


FIG 1. Allergic reactivity to manioc. **A**, Clinical features of patients with manioc allergy. %AB, Percentage of activated basophils after stimulation with raw manioc (negative for anti-FcεRI-induced CD63 expression <15%); AE, angioedema; BAT, Basophil activation test; CD, contact dermatitis; CU, contact urticaria; F, female; FA, food allergy; ND, not done; SS, Skin symptoms; U, generalized urticaria. A skin prick test wheal area (in square millimeters) of greater than 7 mm² is considered positive. **B**, Specific IgE to manioc and starch determined in ELISAs. n.s., Not significant. **C**, SDS-PAGE (left lane) and immunoblotting (right panel) with sera of patients with manioc allergy (lanes 1-9). F-BA, Fructose-bisphosphate aldolase; ME, manioc extract.

usefulness of this approach for samples containing unknown allergens. This is the first study presenting a defined cohort of subjects (n = 9) with clinically relevant allergy to manioc. Here we describe 3 new IgE-binding proteins, fructose bisphosphate aldolase, GAPDH, and a glutamic acid-rich protein similar to latex Hev b 5. Furthermore, we suggest that the glutamic acid-rich protein and, to a lesser extent, GAPDH might be involved in IgE cross-reactivity with latex. Globalization facilitates the entry of exotic foods into Europe and North America, and an increase in the prevalence of food allergy can be anticipated. Manioc has been used worldwide as source of starch and is now being gradually introduced into Europe and North America without detailed information for the consumers. Therefore, it is important for clinicians around the world to be aware of the fact that manioc is a source of allergens involved in the latex-fruit syndrome and can put persons at risk in countries that do not yet directly consume manioc.

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