

Positive skin and oral challenge responses to potato and occurrence of immunoglobulin E antibodies to patatin (Sol t 1) in infants with atopic dermatitis

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The clinical significance and molecular specificity of hypersensitivity reactions to raw and cooked potatoes remain ambiguous. We therefore investigated the clinical hypersensitivity to raw and cooked potato in infants suspected to have potato allergy and compared the findings with the occurrence of immunoglobulin E (IgE) antibodies to patatin (Sol t 1), characterized as the primary allergen of potato. Twelve infants (10 to 24 months of age) suffering from atopic dermatitis (AD) and suspected to have adverse reactions to potato, were examined. As a skin exposure test we used rubbing with both raw and cooked potato, and used open oral challenge with cooked potato for 7 days. A special eczema scoring system (SCORAD) was used to assess the severity of symptoms and signs of AD. Skin-prick tests (SPTs) were performed with raw potato and natural Sol t 1, and serological studies included measurement of total serum IgE and IgE antibodies to Sol t 1, and potato radioallergosorbent testing (RAST). The skin-rubbing test with raw potato was positive in seven (58%) and the oral challenge positive in eight (67%) infants. One infant presented with an immediate reaction and seven with a delayed reaction, i.e. exacerbation of AD, after oral challenge responses to cooked potato. Nine (75%) infants had IgE antibodies to Sol t 1 in enzyme-linked immunosorbent assay (ELISA), and SPT to natural Sol t 1 was positive in six (50%) potato-allergic infants. In conclusion, we observed positive challenge responses to both raw and cooked potato in food-allergic atopic infants. The presence of IgE antibodies and concomitant positive SPTs to the heat-stable potato allergen, Sol t 1, suggest that cooked potato can be an allergenic food for infants suffering from AD.

**Heli Majamaa^{1*}, Ulla Seppälä^{2,3*},
Timo Palosuo³, Kristiina Turjanmaa¹,
Nisse Kalkkinen² and Timo Reunala¹**

¹Department of Dermatology, Tampere University Hospital and University of Tampere, Finland, ²Institute of Biotechnology, University of Helsinki, Finland, ³National Public Health Institute, Helsinki, Finland

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Heli Majamaa, MD, PhD, Department of Dermatology, Tampere University Hospital, PO Box 2000, FIN-33521 Tampere, Finland
Tel.: (+358-3)-247-5167
Fax: (+358-3)-247-5654
E-mail: heli.majamaa@tays.fi

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Clinical and laboratory studies demonstrate increasingly that food allergy plays a pathogenic role in a subset of patients with atopic dermatitis (AD) (1). Yet, a direct relationship between immediate skin test reactivity to suspected allergens and the course of AD has been difficult

to establish (2). In this regard, identification of potential food allergens that trigger the immunoglobulin E (IgE) response in early infancy is essential in the management of AD (1).

Allergy to cow's milk, eggs, and cereals is common among atopic infants and small children (3–5). In contrast to these foods, allergy to potato has been considered uncommon and the relationship between hypersensitivity reactions to raw and cooked potato has remained an enigma (6,7).

*Equal contribution by the first two authors.

Castells et al. described an infant who at the age of 5 months developed anaphylactic symptoms after ingestion of cooked potatoes (8). Hannuksela examined infants and children suspected of food-related AD and reported that cooked potato was able to cause atopic eczema in infants under 1 year of age (9). Delgado et al. described a 4-year-old boy who exhibited contact urticaria from raw potato, but was able to eat cooked potatoes (10).

In our hospital, children who are suspected of multiple food allergies are commonly skin-prick tested (SPT) with various food allergens, including raw potato. Recently, we identified patatin of potato tuber (Sol t 1) as an IgE-binding allergen among food-allergic atopic children with positive SPT reactions to raw potato (11). To obtain more detailed information on the clinical reactions to potato (8–11), we performed skin and oral challenges in 12 infants who had a positive SPT to raw potato and were suspected of being allergic to potato.

Subjects and methods

Patients

Twelve atopic infants (six girls, six boys; mean age 13 months, age-range 10–24 months), who were suspected of allergy to potato based on a clinical history, were included in the study. All infants had AD and five exhibited gastrointestinal symptoms such as loose stools and diarrhea. When tested by the prick–prick method, all infants had a positive SPT response to raw potato (7). In addition, all infants presented multiple food allergies: 12 had positive SPTs to eggs, 10 to cow's milk (confirmed by open and double-blind placebo-controlled food challenges [DBPCFCs]) and cereals (ALK A/S, Hørsholm, Denmark), one to birch pollen, but none to natural rubber latex (Stallergènes, Antony Cedex, France). All infants were put on a potato-free diet and were invited to participate in a potato challenge study. The study was approved by the Ethical Committee of Tampere University Hospital and informed consent was obtained from the parents prior to the start of the study.

Potato challenges and blood sample collection

At examination, potato had been eliminated from the diet for a mean of 8.5 months (range 2–18 months). Open potato challenge was started by rubbing both raw and cooked potato (*Solanum tuberosum* cv Bintje) for ≈ 30 s on the volar aspect of the forearm, and the exposed skin was observed for 20 min for any reaction. Thereafter, oral potato challenge was performed by giving the

infant, at 30-min intervals, increasing doses of cooked potato until one medium-size potato had been eaten. If no reaction occurred within 4 h, the challenge was continued at home where the patient ate one to two cooked potatoes each day. The parents recorded any clinical symptoms, especially skin eruptions, pruritus, and gastrointestinal symptoms. If any abnormal reaction developed, the parents were advised to bring the child back to the hospital. If no reaction occurred, the final examination was performed on day 7. Seven of the infants exhibiting positive oral challenge responses to cooked potato were re-examined after 6 months on a potato-free diet.

The skin-rubbing test was considered positive if at least two to three wheals were seen on the exposed skin. Immediate oral challenge response was regarded as positive if symptoms such as generalized urticaria or acute gastrointestinal symptoms appeared within the first 2 h. The severity of AD was scored (SCORAD) (12) at the start and at the end of oral challenge. A delayed oral challenge response was considered to be positive if the score increased by at least 50%. All the challenges were performed and evaluated by the same physician (H.M.).

Blood samples were collected from all 12 infants for IgE immunoblotting and enzyme-linked immunosorbent assay (ELISA) studies before and after the 7-day potato challenge, and from the seven infants who exhibited positive oral challenge responses to cooked potato after they had continued on a potato-free diet for 6 months. The total serum IgE concentration (Phadebas RAST; Pharmacia, Uppsala, Sweden) and potato-specific IgE (CAP RAST; Pharmacia) were measured. Control sera were obtained from 10 children with AD (mean age 1.3 years, range 5 months to 2 years) and a negative SPT to raw potato but positive SPTs to various other allergens such as cow's milk, egg, and/or cereals. In these control children the total IgE ranged from 5 to 41 kU/l (including four children with a total IgE of <5 kU/l).

SPT with raw potato and purified Sol t 1

SPT was performed before undertaking the skin and oral potato challenges on the volar aspect of the forearm with raw potato (cv Bintje) and natural Sol t 1 (11). The latter allergen was prepared as described previously (11) and was tested at a concentration of 250 μ g/ml. Histamine dihydrochloride 10 mg/ml (ALK) served as a positive control and saline was used as a negative control. The SPT was read after 15 min and recorded as positive if the mean diameter of the

Table 1. *In vivo* and *in vitro* results for open potato challenge study

Patients	Gender (F/M)	Age (months)	Elimination (months)	Raw potato SPT*	Skin challenge†	Sol t 1 SPT	Total IgE (kU/l)	Potato RAST	Sol t 1 Western	Sol t 1 IgE-ELISA‡	Oral potato challenge§		SCORAD¶
											Immediate	Delayed	
1	F	21	3	8/5	+	+	641	6.5	+	0.73	++	NC	4/4
2	M	24	12	9/8	+	+	2324	4.1	+	0.86	–	+	17/31
3	F	19	14	10/5	+	+	119	3.6	+	0.67	–	+	1/14
4	F	10	2	4/5	–	+	312	1.8	+	0.33	–	+	6/19
5	M	18	14	9/5	+	+	231	6.4	+	1.23	–	–	16/5
6	M	18	18	6/5	+	+	68	2.6	+	0.63	–	–	19/19
7	M	13	8	4/5	+	–	1529	2.2	–	0.31	–	+	12/40
8	F	16	12	7/5	+	–	471	3.5	–	0.71	–	–	20/20
9	M	13	8	3/5	–	–	148	1.0	+	0.12	–	+	4/16
10	F	12	5	10/5	–	–	<5	<0.4	–	0.05	–	+	5/12
11	M	16	3	3/5	–	–	54	<0.4	–	0.00	–	++	17/36
12	F	12	3	5/5	–	–	198	<0.4	–	0.02	–	–	28/28

*Mean diameter of potato wheal ÷ mean diameter of histamine control (in mm).

†With raw potato.

‡Cut-off level for positivity: mean + 3SD 0.0679.

§With cooked potato.

¶Score before/after 7-day challenge.

ELISA, enzyme-linked immunosorbent assay; IgE, immunoglobulin E; NC, not continued; RAST, radionallergosorbent assay; SPT, skin-prick test.

wheal was at least 3 mm and the negative control caused no reaction.

Immunoblotting and ELISA with Sol t 1

IgE immunoblotting and ELISA were performed as described previously (11). ELISA inhibition was performed using sera of four challenge-proven potato-allergic infants (nos 1–4, Table 1). In the ELISA inhibition, increasing amounts of natural Sol t 1 (0.001–200 µg/ml) were used as inhibitors when measuring the binding of the patient's IgE against total potato protein extract in the solid phase (11).

Results

Skin and oral challenges with potato

Seven of the 12 infants had positive skin challenge reactions to raw potato and one infant also had a positive skin challenge to cooked potato (no. 1, Table 1). A total of eight (67%), comprising one immediate and seven delayed oral potato challenges, were positive. Of these eight infants, four (33%) had positive skin challenge reactions to raw potato. The infant with an immediate reaction developed angioedema, urticaria, and vomiting after eating no more than one spoonful of cooked potato. After that the challenge was stopped. This infant also had a positive skin reaction to cooked potato. A positive delayed challenge response, i.e. exacerbation of AD (>50% increase in the SCORAD), was recorded in seven infants (Table 1). Of the eight infants with challenge-proven potato allergy, seven had elevated total

IgE levels (from 50 kU/l to 2,324 kU/l) and six a positive (>0.4 kU/l) potato RAST (Table 1).

SPT

SPT with Sol t 1 was positive in six infants (Table 1). Five of these infants had a positive skin reaction to raw potato and four had positive oral challenge responses to cooked potato.

IgE reactivity to Sol t 1 in immunoblotting and ELISA

Seven infants (58%) showed, in immunoblotting, IgE binding to Sol t 1 (Fig. 1). Nine infants (75%) had IgE antibodies to Sol t 1 in ELISA analyses (Fig. 2). Seven out of the nine infants with IgE antibodies to Sol t 1 had positive skin responses to potato, and five had positive oral challenge responses to potato (Table 1). In ELISA analyses, four out of five infants (nos 2, 3, 4, 9, Table 1, Fig. 2) with challenge-proven potato allergy

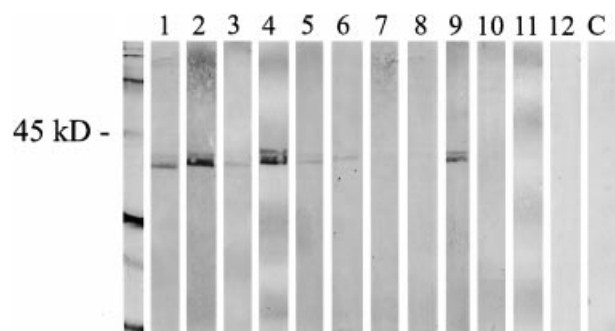


Fig. 1. Immunoblot analysis of immunoglobulin E (IgE) binding to purified patatin (Sol t 1) in the sera of 12 infants; for numbering of infants, see Table 1. Seven out of 12 infants (nos 1–6 and patient no. 9) showed IgE binding to Sol t 1 (C refers to the conjugate control).

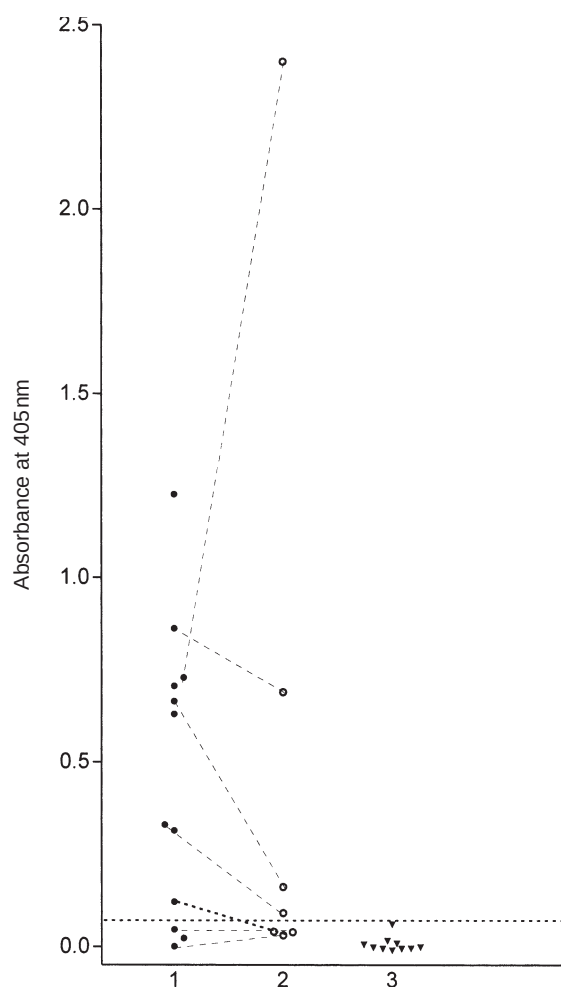


Fig. 2. 1: Occurrence of immunoglobulin E (IgE) antibodies to purified patatin (Sol t 1) in 12 infants after a 7-day potato challenge. 2: IgE binding to Sol t 1 in seven infants continuing elimination of potato in their diet for 6 months. 3: Ten atopic controls. The cut-off level for a positive response (mean OD+3 SD of 10 controls) is indicated by the dotted horizontal line.

showed a moderate decrease in their IgE antibody levels to Sol t 1 after maintaining a potato-free diet for 6 months, whereas one child (no. 1) showed a striking increase in the IgE antibody level. The two potato challenge-positive, but Sol t 1 IgE-negative, infants (nos 10 and 11) remained negative for Sol t 1 during the 6-month follow-up.

In ELISA inhibition testing, using sera of four challenge-proven potato-allergic patients (nos 1–4, Table 1), low concentrations (0.1–10 µg/ml) of Sol t 1 inhibited $\geq 50\%$ of the binding of IgE individual sera against total potato protein extract on the solid phase (Fig. 3).

Discussion

Allergy to basic foods, such as cow's milk and eggs, frequently affects atopic infants and young children (1–5,9). In our hospital, we perform

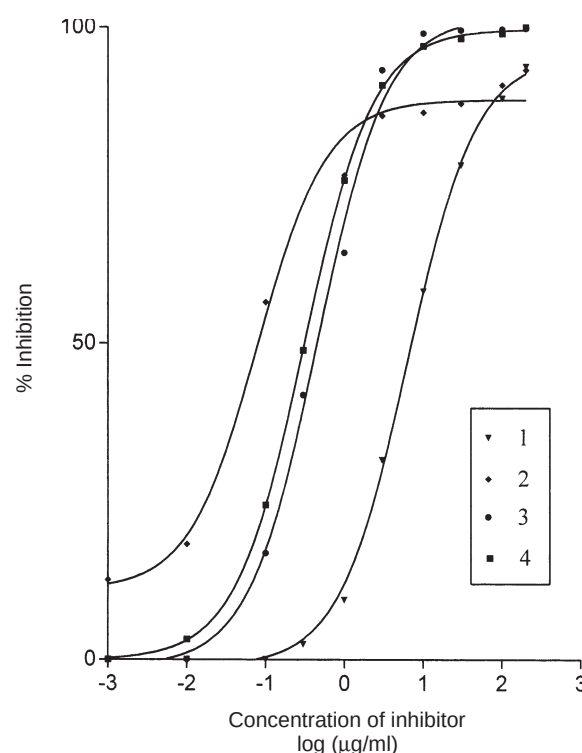


Fig. 3. Inhibition of immunoglobulin E (IgE) binding to solid-phase total protein extract of potato by increasing amounts of Sol t 1. The inhibition experiment was performed using sera of four challenge-proven potato-allergic infants (nos 1–4, Table 1).

SPTs annually in ≈ 800 infants suspected of food allergy. Of these, 15% show positive reactions to eggs, 9% positive reactions to cow's milk, and 5% positive reactions to potato. Although it has been reported previously that cooked potato can cause atopic eczema in infants under 1 year of age (9), the clinical importance of potato as an allergenic food in infants with AD has not been systematically assessed. In the present study, we performed skin and oral challenges with potato in 12 infants with positive SPTs and clinical suspicion of potato allergy. Seven of the 12 (58%) infants reacted to raw potato in skin-rubbing tests and eight (67%) showed a positive oral challenge, comprising one immediate and seven delayed-type responses to cooked potato (Table 1).

In recent years, oral food challenges have become important in the diagnosis of food allergy in children (13–15). In the present study, the oral challenge was performed openly as in this age-group open and double-blind challenges have shown comparable results (16).

Elimination of potato from the diet of these infants resulted in an improvement of the atopic eczema (17) and therefore the skin appeared to be asymptomatic when the study began. After 1 week of repeated oral challenges with cooked

potato, seven infants demonstrated eczematous skin lesions, which were graded using SCORAD (12) (Table 1). In agreement with the results of the present study, delayed eczematous skin lesions have been observed in patients with AD after 1 week of repeated oral challenges with common food allergens (4–5,14–16). Supporting our results, De Swert et al. recently reported that cooked potato elicited immediate, late, and delayed onset of food-induced symptoms in young atopic children (18). Together, these findings suggest that the onset of allergic reactions to cooked potato differs among individuals suffering from food-related AD (16,18–21).

It should be noted, however, that not all of the present infants had positive skin and/or oral challenge responses although they all showed positive SPT to raw potato. One explanation could be the relatively long potato-free period (mean 8.5 months), suggesting that some infants had already grown out of clinical allergy to cooked potato (Table 1). This suggestion is in agreement with the findings that children with positive SPTs and RASTs remain negative in oral cow's milk challenge, and *vice versa* (4–5,16). Furthermore, positive SPTs and IgE antibodies to specific foods are frequently observed in patients who have already achieved clinical tolerance to a specific food (2,9,22).

At the beginning of the study, specific IgE antibodies to heat-stable Sol t 1 were found by ELISA in nine (75%), and a positive SPT in six, of the present 12 infants (23). At a follow-up time-point of 6 months after starting a potato-free diet, five infants showed reduced levels of IgE to Sol t 1 (Fig. 2). Interestingly, the infant with an immediate-type response to cooked potato showed increasing levels of specific IgE antibodies to Sol t 1 (no. 1, Table 1, Fig. 2). Considering that cooked potato was eliminated from the diet of this child and the skin appeared to be asymptomatic, the specific IgE binding to Sol t 1 suggests that other plant food allergens might cross-react with Sol t 1 (22). Therefore, further studies are needed to identify other botanically related plant foods containing patatin-like food allergens.

The importance of Sol t 1 as a major potato allergen was further supported by the results of the ELISA inhibition with four challenge-proven potato-allergic infant sera. In each serum sample, the binding of IgE against total protein extract of potato was significantly inhibited by Sol t 1 (Fig. 3). This finding is in agreement with the immunologic inhibition results of previous studies denoting Sol t 1 as a major food allergen of potato tuber (11,24,25).

On the other hand, the detection of infants with positive SPTs and challenge responses to raw and cooked potato, but negative IgE binding and negative SPTs to Sol t 1 (Table 1), suggests that potato may contain other, as yet unidentified, allergens. In this regard, our results suggest that rather than the heat-stable Sol t 1, these additional allergens are probably responsible for the clinical hypersensitivity to raw potato among older children and adults (6,7,10).

In conclusion, the present study shows that cooked potato can be a relevant allergenic food in a selected group of infants with AD and suggests that the heat-stable Sol t 1 is a clinically important food allergen for young atopic infants with a positive SPT to raw potato.

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