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Hypersensitivity reactions to the Sabin vaccine in children with cow's milk allergy

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Clinical & Experimental Allergy

Summary

Background The Sabin vaccine is used world-wide, and most children with food allergies receive it without incident. However, in the 2009 vaccination campaign conducted in Argentina, four children experienced immediate-type hypersensitivity reactions following vaccination.

Objective We aimed to review the medical history of the affected children, study their allergic condition after the episodes and analyse the presence of allergenic vaccine components.

Methods Patients were selected based on their immediate allergic reactions following vaccination. They were assessed for allergies to cow's milk and hen's egg. The presence of cow's milk proteins in the vaccine was tested by various immunoassays involving cow's milk- or α-lactalbumin-specific polyclonal rabbit antiserum and patient sera.

Results All of the patients had a history of milk allergy, and no history or current evidence of egg hypersensitivity was found. Levels of cow's milk- and Sabin vaccine-specific IgE were increased, and the result of a skin prick test with cow's milk proteins or the Sabin vaccine was positive in each patient. In addition, an ELISA using specific rabbit antiserum detected α-lactalbumin in the Sabin vaccine. When α-lactalbumin was employed as a soluble inhibitor in a competitive ELISA, binding to vaccine-coated plates by cow's milk- or α-lactalbumin-specific rabbit antiserum or by patient serum containing IgE was inhibited.

Conclusions We have demonstrated that these patients were allergic to cow's milk, and had circulating and mast cell-bound IgE antibodies specific to cow's milk proteins. We found that the Sabin vaccine contained α-lactalbumin, which may have been responsible for the reactions elicited following vaccination with the Sabin and dual viral vaccines in combination.

Keywords cow's milk, hypersensitivity, lactalbumin, Sabin, vaccine

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Introduction

Vaccination is safe and effective world-wide. However, allergic reactions following vaccination have been described, most commonly in egg-allergic patients, due to the presence of egg proteins in some vaccines. In 2009, a vaccine programme with the oral polio vaccine (OPV) and measles-rubella vaccine (MRV) resulted in serious adverse reactions immediately following vaccination in four patients. We have examined the food allergies of these four children and whether the symptoms experienced following vaccination might be due to the presence of egg or milk proteins in the vaccines.

Food allergy is an emerging pathology, and in many regions, including Argentina, milk and hen's egg are the most common food allergens of early childhood [1, 2]. However, there is no report indicating the true incidence of food allergy in Argentina. It has been demonstrated that hen's egg allergies can lead to vaccine-triggered anaphylaxis in children [3–5], and a similar situation may exist for milk-allergic patients. Millions of doses of OPV are widely used annually throughout the world, and a significant proportion of the paediatric

population is allergic to milk. However, anaphylactic reactions are very infrequent.

In this work, we studied the current allergic status of the affected patients, and the presence of allergenic vaccine components to study the relationship between the administration of OPV and the immediate hypersensitivity reactions in four children with a previous history of allergy to cow's milk.

Methods

Patients and vaccines

Four patients were selected based on their severe immediate hypersensitivity reactions elicited within minutes of receiving a booster with MRV and OPV. The clinical characteristics of the four patients are shown in Table 1. All patients had previously experienced acute allergic reactions (skin, gut, and airway symptoms) to cow′s milk proteins (CMP), including severe reactions following accidental exposure to small amounts of cow's milk or milk-derived products. All patients had been previously diagnosed with cow's milk allergy. Following the vaccine-triggered episodes, they were tested via a skin prick test (SPT) with hen's egg, milk proteins, and OPV antigens. In addition, 10 healthy adult volunteers were given a SPT with OPV as controls. Three of four patients gave informed consent to test their serum for the presence of egg-, cow's milk-, and OPV-specific IgE antibodies. The project was reviewed and approved by the Ethics Committee of the Argentinean Association of Allergy and Clinical Immunology following the Declaration of Helsinki. Parents of the patients signed the Informed Consent forms.

The systemic clinical picture triggered by vaccines was reversed in all cases by treatment with an antihistamine (diphenhydramine) and oral steroids.

The 2009 vaccination campaign conducted in Argentina included the administration of the oral poliomyelitis vaccine (Trivalent vaccine Polioral® Sclavo, Siena, Italy), and a dual viral vaccine containing attenuated measles and rubella viruses (Serum Institute of India Ltd., Hadapsar, Pune, India). Most of the children were under 4 years of age, and they received throughout the campaign both vaccines in combination (3 324 490 doses of OPV and 2 766 691 doses of MRV).

Immunoassays

Indirect ELISA for serum IgE analysis. Blood was extracted by venipuncture, and the serum was stored at −80°C prior to analysis. The assessments of serum IgE specific for hen's egg, cow's milk, and α-lactalbumin were carried out using a fluoroimmunoassay UNICAP 100 following the manufacturer's instructions (Thermo Fisher Scientific, Uppsala, Sweden). The sera from three patients were analysed (the parents of patient 3 did not consent to the test).

In addition, an indirect ELISA for OPV- or MVR-specific IgE was performed by coating polystyrene microtitre plates (NUNC, Maxisorp, Denmark) overnight with 100 μL of undiluted whole OPV or MRV (the vaccine vials used in the 2009 vaccination campaign). After blocking with 5% horse serum in phosphate-buffered

Table 1. Characteristics of patients with history of cow′s milk allergy

Patient	Age*	Gender	Milk allergy history	Hypersensitivity symptoms after vaccination**
1	4	F	Urticaria peri-oral, rhinoconjunctivitis 5–10 min after ingestion of yogurt (6-months old). Papules and erythema minutes after an oral challenge test with 500 μL of milk. Recurrent bronchospasm episodes since 6-months old and asthma by age 6	Flushing, facial swelling, eyelid angio-oedema, oedema with hands and feet swelling, and genital itching within minutes of vaccination
2	4	F	Vomiting after ingestion of few mL of milk, followed with oral pruritus and urticaria. Rhinorrhoea and dysphonic cough Asthma with hospitalization (7-months old)	Vomiting, lips angio-oedema, flushing, loss of consciousness with loss of sphincter control within minutes of vaccination
3	16	M	Gastro-oesophageal reflux (1-month old) and urticarial peri-oral rash after ingestion of 5 mL of milk (7-months old)	Rhinorrhoea, bronchospasm and flushing 10 min after vaccination
4	3	M	Diarrhoea since 15 days of life with breastfeeding. Lip angio-oedema and flushing after ingestion of few mL of milk (4-months old)	Flushing, rhinorrhoea, abdominal cramps and vomiting 5–10 min after vaccination

*Age at vaccination.

**Symptoms were listed as appeared after vaccination.

F, female; M, male.

saline (PBS) at pH 7.4, the undiluted patient sera were added to the wells, and incubated overnight at 4°C, followed by incubation for 2 h at 37°C with alkaline phosphatase-conjugated monoclonal anti-human IgE (Sigma-Aldrich, St Louis, MO, USA) diluted 1 : 3000. Enzymatic activity was revealed by adding 0.4 M *p*-nitrophenyl phosphate in buffer at pH 9.6. The optical density (OD) was measured at 405 nm in an ELISA reader (Sirio S SAECS, Radim Company, Buenos Aires, Argentina), and the results were expressed as an OD. The cut-off value of the assays was obtained statistically (SEM+2 SD) from the OD readings of 20 sera of non-atopic and non-allergic patients that were run in parallel as negative controls (mean = 0.143, standard deviation = 0.0476, cut-off = 0.2382 for OPV-specific IgE).

Indirect ELISA using polyclonal antisera. Polystyrene microtitre plates were coated overnight with 100 μL of undiluted whole OPV, MRV, 10 μg/mL CMP, 5 μg/mL α-lactalbumin (α-La, Sigma Aldrich), 5 μg/mL β-lactoglobulin (β-Lg, Sigma Aldrich), 5 μg/mL α-casein (Sigma Aldrich), 5 μg/mL β-casein (Sigma Aldrich), 5 μg/mL κ-casein (Sigma Aldrich), 5 μg/mL lysozyme (Sigma Aldrich), or 5 μg/mL bovine seralbumin (BSA) (Sigma Aldrich) and were then blocked with 5% horse serum in PBS at pH 7.4. CMP-specific (1 : 100 000) or α-La-specific (1 : 40 000) rabbit polyclonal antiserum was then added for 60 min at 37°C, followed by the addition of rabbit IgG-conjugated horseradish peroxidase (HRP) (1 : 4000) (Santa Cruz, Santa Cruz, CA, USA) for 60 min at 37°C. The plates were developed by the addition of *o*-phenylenediamine and 30% H_2O_2 in 0.1 M citrate-phosphate buffer (pH 5.0), and the OD_{490} values were measured. The serum of a non-immunized rabbit served as the negative control.

Inhibition competitive ELISA. Polystyrene microtitre plates were coated overnight with 100 μL/well of undiluted whole Sabin vaccine, dual viral vaccine (undiluted), or CMP (10 μg/mL), and were then blocked with 5% horse serum in PBS, pH 7.4. CMP-specific rabbit antiserum (diluted 1 : 50 000 in blocking buffer) or the undiluted sera of patients were mixed (1 : 1) with different concentrations of CMP, β-Lg, casein, or α–La (0.1–10 000 μg/mL) as soluble inhibitors, and were incubated for 2 h at 37°C. A mixture (1 : 1) of the same antibodies with blocking buffer but without inhibitors was used as a negative control. Then, 100 μL of HRP-conjugated anti-rabbit IgG antibody (1 : 4000) (Santa Cruz) or alkaline phosphatase-conjugated monoclonal anti-human IgE (1 : 3000) (Sigma-Aldrich) was added for 1 h at 37°C. The plates were developed, and the absorbance values were read at 490 nm.

Skin prick test

SPT was performed using commercial extracts of α–La, β-Lg, casein, cow's milk, hen's egg, egg yolk, ovalbumin (Laboratorio Q Alergia, Buenos Aires, Argentina), or OPV. Saline and histamine (10 μg/mL) (Sigma Aldrich) were included as controls. The tests were performed on the back or the inner forearm with a metal lancet. After 15 min, the sizes of the resulting weal (papule) for each allergen were measured and recorded in millimetres (mm). Prick-to-prick tests were performed using a metal lancet with fresh cow's milk and hen's egg. Inflammation was recorded after 15 min as mm of weal.

Results

All patients experienced hypersensitivity reactions within 5–10 min following vaccination with OPV and MRV (Table 1) and were treated with antihistamines and corticoids. The clinical signs resolved completely by 6 h except for patient 2, who was hospitalized in the intensive care unit. Her symptoms resolved after 24 h.

Different allergy tests were performed following these reactions to investigate a link between vaccination and the immediate adverse events. Each of the patients had a positive SPT with OPV indicating the presence of

Table 2. Results of IgE determinations and SPT in patients with cow's milk allergy

						Cutaneous tests					
	CMP	Egg	α-La	OPV	MRV	SPT			Prick-to-Prick		SPT[¶]
Patient	specific IgE (kU/L)*	specific IgE (kU/L)*	specific IgE (kU/L)*	specific IgE (OD)[†]	specific IgE (OD)[‡]	α-La (mm)	β-Lg (mm)	Casein (mm)	CMP (mm)	Egg (mm)	with OPV (mm)
1	8.6	0.35	2.2	0.711	0.149	15	15	4	nd	nd	6
2	1.9	0.35	1.9	0.246	0.188	12.5	11	10	15	Negative	4
3	nd	nd	nd	nd	nd	17	15	8	25	nd	5
4	30.3	0.35	1.8	1.297	0.117	12	17	4	nd	Negative	5

*UNICAP cut-off value: 0.35 kU/L.

[†]ELISA cut-off value: 0.238.

[‡]ELISA cut-off: 0.203.

[¶]SPT, skin-prick test; mm, millimeters of weal diameter. Positive result: > 3 mm OD, optical density of ELISA; CMP, cow's milk proteins; MRV, measles-rubella vaccine; OPV, oral polio vaccine; nd, not done.

OPV-specific IgE bound to skin mast cells (Table 2). Furthermore, serological tests were performed in all patients except for patient 3, for whom parental permission was not obtained. Although all sera had an increased OPV-specific IgE titre, the binding of IgE to whole MRV was not observed (Table 2). Collectively, these *in vivo* and *in vitro* findings suggest the presence of allergenic components in OPV that were recognized by soluble and cell membrane-bound IgE.

To identify a correlation between OPV-specific IgE and the allergic histories of the patients, a clinical history was taken, and cutaneous tests and serological determinations against cow's milk and hen's egg allergens were performed. The main clinical manifestations of cow's milk allergy that were observed in the patients are summarized in Table 1. It can be observed that different hypersensitivity symptoms were induced within minutes of ingesting a few millilitres of milk or milk-derived products or accidental exposure to dairy products. Skin, gut, and airway allergic symptoms were observed. To assess concomitant allergy, we assayed for the presence of specific IgE antibodies in serum samples and in skin mast cells (Table 2). CMP- and α-La-specific IgE were detected in all sera, whereas egg-specific IgE was not detected. These results led us to rule out the possibility of an allergic reaction to hen's egg and confirm the sensitization to CMP, which is consistent with a history of milk allergy.

To understand the relationship between the reactive IgE profiles of the patients and the composition of the vaccine, we investigated the presence of different cow's milk allergens in OPV and MRV. According to the information obtained from the website of the manufacturer, the Polioral vaccine does not contain milk components as excipients. However, the vaccine was labelled as containing less than 0.25 mg α-La/doses. To test whether milk proteins were present in OPV and MRV, the vaccines were analysed by indirect and competitive ELISA using cow's milk- or α-La-specific rabbit polyclonal antiserum. Fig. 1a shows the results of the indirect ELISA using OPV or MRV antigens and the CMP- or α-La-specific antisera. Although the CMP-specific antiserum was previously characterized [6–8], we assessed the binding specificity of both antisera using different commercial milk proteins as coating antigens; lysozyme and BSA were included as negative controls. In addition, serum of a non-immunized rabbit was used as a control for the immunoassay (data not shown). As shown in Fig. 1a, both antisera yielded positive results with OPV (OD = 1.25 vs. OD = 0.05 for anti-CMP antiserum vs. control serum respectively; OD = 0.71 vs. OD = 0.03 for anti-α-La antiserum vs. control serum respectively). When MRV was used as the coating antigen, OD readings of 0.285 and 0.105 were obtained with the anti-CMP and anti-α-La antisera respectively. These results indicate that α-La is present only in OPV.

To confirm the presence of α-La in OPV, we performed an inhibitory ELISA by incubating the CMP-specific antibodies with the CMP-, OPV- or MRV-coated solid phase and then adding a competitive soluble inhibitor (CMP, α-La, β-Lg, or casein) at different concentrations (Fig. 1b). We found that α-La inhibited the binding of rabbit antibodies to OPV antigen in a dose-dependent manner, while inhibition of antibody binding to the MRV antigen was not observed. A control curve showing the inhibition of the CMP-specific antiserum with the identity antigen, CMP, is also depicted. In addition, β-Lg and caseins employed as control competitive soluble proteins did not show an inhibitory effect (data not shown), thus disproving the presence of these proteins in the vaccines.

Finally, we performed a similar competitive assay to address whether serum IgE could be used as an immunological tool to confirm the presence of α-La in OPV. As

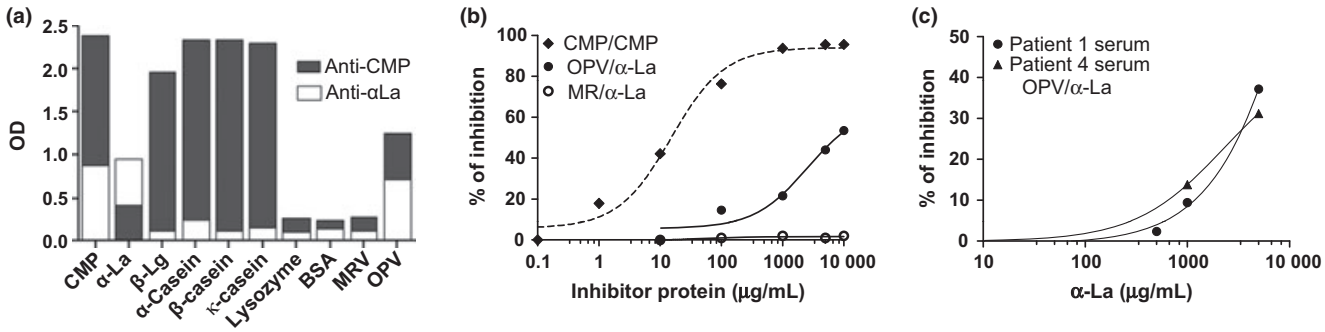

Fig. 1. Immunoassays using α-La- or CMP-specific polyclonal antiserum or patient sera. (a) Indirect ELISAs were performed with 1 : 40 000 α-La- (white column) or 1 : 100 000 cow's milk-specific antiserum (black column) with different target antigens coated to the solid phase. Horseradish peroxidase-conjugated rabbit IgG (1 : 4 000) was used as a secondary antibody. (b and c) Competitive ELISAs were performed with 1 : 50 000 CMP-specific antiserum (b) or undiluted patient sera with IgE antibodies (c). Plates were coated with whole Sabin vaccine, dual viral vaccine, or CMP, and different concentrations of CMP or α–La (0.1–10 000 μg/mL) were used as soluble inhibitors mixed 1 : 1 with sera or antisera. Coated antigen and soluble inhibitors are indicated as *coated/soluble antigens*.

observed in Fig. 1c, the same inhibitory dose-dependent curve could be achieved with the sera of patients 1 and 4. Overall, these results enable us to confirm that OPV contains α-La and that CMP are not present in MRV.

Discussion

In this study, we demonstrated that patients who showed hypersensitivity reactions minutes after vaccination with OPV and MRV were allergic to cow's milk, and had IgE antibodies specific to cow's milk proteins and OPV. In addition, we identified the presence of α-La in OPV, raising the concern that this whey protein might have triggered the allergic reactions following combined vaccination with OPV and MRV.

A number of studies have shown that anaphylaxis can be induced by vaccination and that it is among the most serious vaccine-associated adverse reactions [9–11]. However, the risk of anaphylaxis following vaccination is very low (1-10 cases per million doses) [9, 12]. Although we do not know which of the vaccines used in the 2009 campaign caused the severe allergic episodes, we suspected that OPV was the main allergen contributor. Nevertheless, we cannot discount that the MRV given concomitantly might contain an undetermined allergen. It has been reported that these vaccines may trigger anaphylactic episodes when they are administered in combination with other vaccines, such as those for tetanus-diphtheria-pertussis or hepatitis B [9].

Rates of anaphylaxis vary depending on the vaccine, and adverse reactions have been caused by both viral and bacterial vaccines. We estimated the risk of severe allergic reactions following vaccination during the 2009 campaign at 1.2 cases/million doses of OPV. Bohlke et al. have reported vaccine-specific risks of 9.2 cases/million doses of OPV and 14.4 cases/million doses of measles-mumps-rubella vaccine, although they reported that only two of the five cases of anaphylaxis had a history of atopy [2].

We reason that there is no cause to suspect the presence of allergenic proteins from egg or CMP as regular components of the polio vaccine because the strains of poliovirus were initially expanded in primary African green monkey kidney cells and ultimately expanded in the Vero cell line. However, to prevent degradation of live vaccine virus, skim milk or milk derivatives may be added to the aqueous phase. Although α-La is widely used as a gelling, emulsifying, or stabilizing agent in different dairy and non-dairy food products, it is not regularly used as a vaccine stabilizer.

Yavuz et al. [4] reported anaphylactic reactions following immunization with the measles-mumps-rubella vaccine in patients with allergies to cow's milk and eggs. Although they could not detect egg-specific IgE, they assumed that egg-derived proteins present in the vaccine might have been responsible for the systemic symptoms. They did not consider that there might be residual milk proteins or intentional milk proteins used as excipients. Kattan et al. [13] reported eight children with anaphylactic reactions following the administration of toxoid-based vaccines (tetanus, diphtheria, and pertussis). In this study, they suggested that the residual bovine casein present in the vaccine might be responsible for the induction of the anaphylactic reactions in sensitive patients.

In our study, we found no evidence of milk proteins in the dual viral vaccine, but we could detect α-La in the Sabin vaccine. In addition, the cutaneous and serological tests that were performed with milk components revealed that the patients were sensitized to milk proteins. According to *in vivo* and *in vitro* results of tests performed with milk components (Table 2), it can be concluded that these patients were mostly sensitized to whey proteins. As most milk-allergic patients are sensitized to bovine caseins [14], which are the major milk allergens, many milk-allergic subjects might not react to vaccination with OPV. Prior to vaccination, these patients had a history of milk allergies, with immediate systemic reactions following the ingestion or accidental exposure to small volumes of milk or dairy products at various ages. On the other hand, sensitization to egg proteins could not be demonstrated in these children. For these reasons, we assume that the OPV, rather than the dual viral vaccine, might be responsible for the severe IgE-mediated anaphylactic reactions within minutes of vaccination in the patients who were sensitized to milk proteins.

While allergic reactions to vaccines are rare, we have demonstrated a possible causal relationship between OPV (given concomitantly with MRV) and allergic reactions in children with a history of CMP allergy. The presence of α-La in OPV and specific IgE antibodies in the serum samples, along with the immediate elicitation of symptoms following vaccination, leads us to suggest that this whey component may be responsible for the IgE-mediated hypersensitivity reactions. We believe that children should receive OPV because there is no evidence-based recommendation for avoidance [15]. However, providers should be prepared to respond with emergency medications if an anaphylactic reaction occurs. We additionally suggest performing a SPT with OPV in milk-allergic patients prior to being immunized and considering the use of the Salk vaccine in the event of a positive reaction.

Conflict of interests

Guillermo Docena has received funds for research from the government (grant PICT 2202). Claudio Parisi, Paola Smaldini, Maria Gervasoni and Jorge Maspero have no conflict of interests to declare.

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