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Severe anaphylaxis to sheep's milk cheese in a child desensitized to cow's milk through specific oral tolerance induction

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KEY WORDS

Cow's milk allergy, food anaphylaxis, immunoblotting, oral food challenge, sheep's milk allergy, specific oral tolerance induction.

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SUMMARY

Specific oral tolerance induction to food (SOTI) is a new promising treatment for persistent IgE-mediated food allergy. Our paper reports a case of a 5-year-old girl with cow's milk allergy, who developed severe anaphylaxis after the ingestion of a croissant containing sheep's milk ricotta cheese, even though she had been previously desensitized to cow's milk through SOTI. The sheep's milk specific allergen causing the severe allergic reaction (a derivative of alpha-casein of 54,1kDa) was identified by SDS-PAGE and immunoblotting.

We conclude that SOTI is a species-specific procedure and the induced tolerance to cow's milk doesn't necessarily provide protection against milk of other mammals. Therefore, children desensitized to cow's milk through SOTI should strictly avoid the intake of milk of other mammals, until tolerance to those kinds of milk is documented by an oral food challenge.

ABBREVIATIONS

CM: Cow's milk
CMA: Cow's milk allergy
DH: Day hospital
OFC: Oral food challenge
SM: Sheep's milk
SOTI: Specific oral tolerance induction

Introduction

Children affected by cow's milk allergy (CMA) may present allergic symptoms after the ingestion of sheep's milk (SM) or food containing it, due to the possible cross-reactivity

between cow's and sheep's milk proteins (1-2). In contrast, allergy to SM with tolerance to cow's milk (CM) is relatively rare (3-5). To date, a milk-free diet is the treatment of choice for CMA. However, strict avoidance is difficult in everyday life and the risk of developing allergic reactions after accidental exposure compromises the quality of life of the entire family (6). Specific oral tolerance induction (SOTI) to food is a new promising treatment for persistent IgE-mediated food allergy. Nevertheless, this procedure can be conducted only in highly controlled clinical settings due to the risk of fatal anaphylaxis (7).

Our paper reports a case of severe anaphylaxis to sheep's milk ricotta cheese in a 5-year-old girl with CMA, who had been previously desensitized to CM through SOTI.

Case Report

At 4 months of age, a baby girl presented an episode of generalized urticaria and localized angioedema to the face and eyelids, few minutes after the first ingestion of CM. A similar episode occurred 3 months later; therefore CM and dairy products were eliminated from her diet. Since the age of 2 years, she started suffering from moderate-persistent asthma. At 3 years of age, a complete allergologic work-up was performed for the first time, resulting in strongly positive skin prick tests (SPT) (Lofarma, Milan, Italy) and specific IgE antibodies (UniCAP, Pharmacia, Uppsala, Sweden) to all CM proteins and fresh CM (Tab.1). At 4 years of age, an open oral food challenge (OFC) with fresh pasteurized CM was performed in day-hospital setting (DH), and resulted in vomiting, generalized urticaria and asthma (Grade 4 according to Sampson's criteria)(8), confirming the diagnosis of CMA. Five days later, a SOTI treatment to CM was started:

increasing doses of CM were periodically given in DH setting, starting from 1 ml, that was the dose of two fold less than the one has caused the reaction during OFC, while the patient continued with a maintaining dose at home. When an intercurrent illness (common cold, viral diarrhea, fever, etc.) intervened during SOTI, the CM dose was not increased and the previous dose was repeated. During the SOTI course, a progressive decreasing of specific IgE antibodies and SPTs to all CM proteins was observed (Tab.1). After eight months of treatment, the child was able to tolerate 150 ml of CM in a single dose, in DH setting; therefore CM and dairy products were re-introduced in her diet. Fifteen days later, after eating a piece of croissant containing sheep's milk ricotta cheese, the child experienced a severe anaphylactic reaction with generalized urticaria, acute asthma, hypotension, loss of bowel control and loss of consciousness (Grade 5)(8), that was subsided by intramuscular epinephrine administered by her mother. Ten days later an OFC to CM was

Table 1 - SPTs and IgEs results to different cow's and sheep's milk proteins. Date 1 (Outcomes during the first allergologic work-up); Date 2 (Outcomes during OFC to CM before starting SOTI*); Date 3 (Outcomes at the end of SOTI); Date 4 (Outcomes during OFC to CM after anaphylaxis to SM ricotta cheese); Date 5 (Outcomes 10 months after anaphylaxis to SM ricotta cheese); Date 6 (Outcomes during the last allergologic work-up); * SOTI to CM : from 05/19/2008 to 01/20/2009.

		Date 1	Date 2	Date 3	Date 4	Date 5	Date 6
		07/07/2007	05/14/2008	01/20/2009	02/13/2009	12/02/2009	03/16/2011
SPTs (mm)	cow's milk (CM)	19.0	8.5	4.0	11.5	5.0	9.0
	α -lactalbumin	8.0	5.0	4.5	6.0	1.0	0.0
	β -lactoglobulin	8.0	5.0	0.0	4.0	1.0	0.0
	casein	7.5	5.5	0.0	4.0	1.0	2.0
	sheep's milk (SM)	/	/	/	19.0	12.0	/
	sheep's whey	/	/	/	12.0	5.5	/
	sheep's milk ricotta cheese	/	/	/	4.5	/	/
Specific IgE (kU/L)	cow's milk (CM)	>100	>100	40.1	31.2	19.5	6.3
	α -lactalbumin	21.8	6.1	2.2	2.1	0.6	0.2
	β -lactoglobulin	3.8	4.5	0.5	0.5	0.2	0.2
	casein	>100	>100	45.7	35.7	21.8	6.8
	sheep's milk (SM)	>100	/	55.5	58.6	35.8	14.5
	sheep's whey	95.8	/	18.3	15.5	10.8	4.5
Total IgE (kU/L)		1230.0	/	662.0	747.0	1074.0	632.0

performed in DH setting, but it resulted negative, whereas SPTs with fresh CM, α -lactalbumin, β -lactoglobulin, casein, fresh SM, sheep's whey, and specific IgE antibodies to CM and SM proteins were strongly positive (Tab.1). To date, the girl takes CM and dairy products without problems, but she follows an elimination diet for milk of other mammals.

Materials and Methods

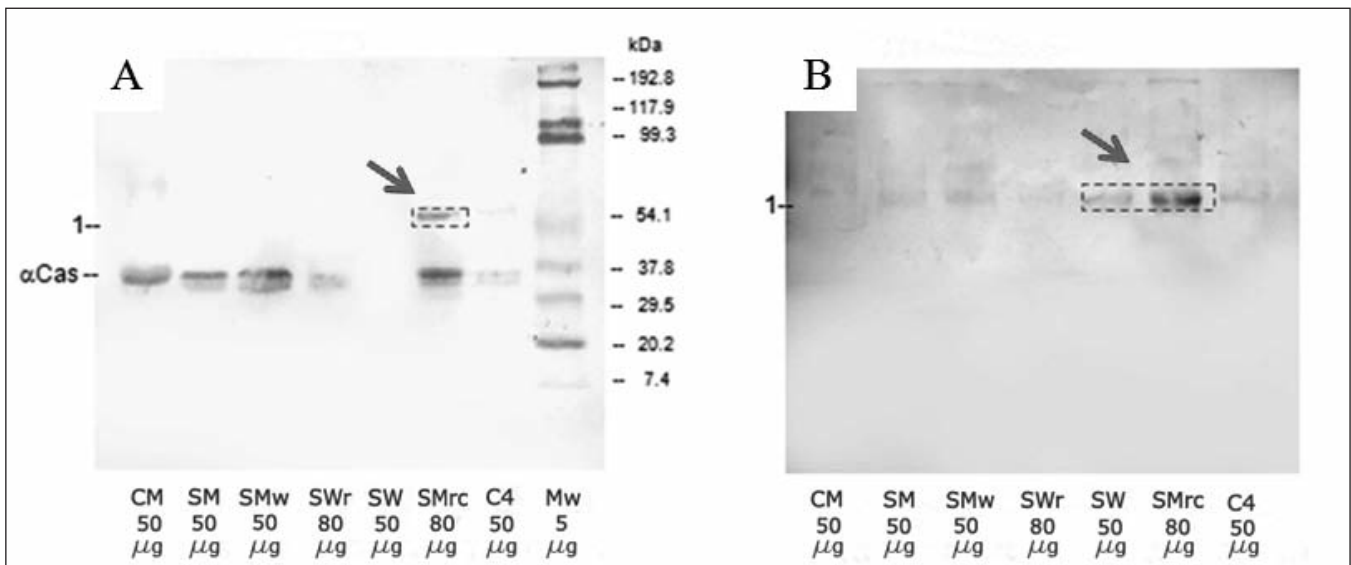
The spectrum of IgE binding to milk allergens was assayed by immunoblotting. Milk/cheese proteins were separated by SDS-PAGE in a gradient gel (9-19% acrylamide) and transferred from the gel onto a polyvinylidene difluoride membrane by electrophoretic elution as previously described (9). The membranes were immersed in 10 mL of 0.25% gelatine solution containing 0.3 mL of allergic patients' serum. Antigen-IgE complex was detected using goat anti-human IgE antibodies labelled with alkaline phosphatase (SIGMA Aldrich, Milan, Italy). Milk samples used were diluted 1:10 (v:v) with sample buffer (0.25 M Tris-HCl buffer pH 6.8, containing 7.5% glycerol, 2% SDS and 5% beta-mercaptoethanol). Sheep milk derivatives were freeze-dried and so-

lubilized in Sample buffer at the final concentration of 10 mg of dried product/mL

Results

Immunoblottings performed by using CM, SM, sheep's whey and SM ricotta cheese, showed the presence of specific IgE antibodies with high affinity for cow's and sheep's casein, and for a protein with a molecular weight of 54.1 kDa, that was present mainly in SM ricotta cheese and in sheep's whey, whereas it was absent in CM (Fig.1). The pre-absorption of serum with CM and SM reduced the intensity of IgE reactivity only to casein, but not to the band of 54.1 kDa in SM cheese lane on immunoblot (data not shown), confirming that this protein is present in significant quantity mainly after SM cheese production. The immunoreactive protein was identified as a derivative of sheep α -casein (a dimer or a glycosylated product) by using specific antibodies for different milk fractions. Only anti α -casein antibodies were capable to recognize with very high affinity the 54.1 kDa band on immunoblot (data not shown), and the identification was then confirmed by a partial sequencing of the isolated band. Since the sheep's ricotta cheese was contained in

Figure 1 - SDS-PAGE Immunoblottings. Amount of serum sample used is μ g (microgram). A) Immunoblotting with serum sample taken during the first allergologic work-up (Date1). B) Immunoblotting with serum sample taken during OFC to CM after anaphylaxis to SM ricotta cheese (Date 4). The following samples were used: cow's milk (CM), sheep's milk (SM), raw whole SM (SMw), sheep's whey (SW), sheep's whey obtained from ricotta cheese processing (SWr), sheep's milk ricotta cheese (SMrc), and one pieces of croissant with sheep's milk ricotta cheese (C4). \Rightarrow : indicates derivative of α -casein (54,1 kDa)



the croissant that triggered the anaphylactic reactions, we assume that this derivative of α -casein was the main allergen responsible for anaphylaxis in this case.

Discussion

SOTI to CM, achieved through an incremental exposure to the relevant allergen, may represent an alternative treatment for patients with persistent IgE-mediated CMA (10).

Preliminary studies on SOTI to CM have shown promising results: Meglio et al. reported that 15 over 21 (71.4%) children with CMA could tolerate the maximum amount of 200 ml/day of whole CM after a 6-month treatment (11), whereas Longo et al. demonstrated that 27 over 30 (90%) children with severe CMA achieved at least partial tolerance to CM after a 1-year treatment, with a substantial improvement in the quality of life, due to the decreased risk of developing fatal allergic reactions after accidental introduction of low quantities of CM (12). However, despite these encouraging results, SOTI cannot be recommended in routine practice yet, due to the risk of fatal anaphylaxis during the treatment (7). Thus, this procedure should be conducted only in selected clinical settings, and further randomized clinical trials are needed to clarify the long term efficacy, safety and cost-effectiveness of SOTI (13).

Furthermore, the acquired tolerance to CM after SOTI may not be effective against allergy to the milk of other ruminants. Alonso-Lebrero et al. reported a case of two children with CMA, who successfully completed a course of SOTI to CM, but developed moderate allergic reactions after eating goat's and sheep's milk cheese (14), whereas Rodríguez del Río et al. found that in a population of 58 CM allergic patients who could tolerate CM after SOTI, 15 of them (25.9%) were allergic to either goat's or sheep's milk or to both, with cross-reactivity between CM casein and goat's and sheep's milk casein showed by ELISA inhibition assays (15).

In this paper we report a case of a severe anaphylaxis to sheep's milk cheese in a child with CMA, who had been previously desensitized to CM through SOTI. This case confirms that SOTI is a species-specific procedure and, although it may represent a promising alternative to the elimination diet in the treatment of persistent CMA, the induced tolerance to CM doesn't guarantee tolerance to the milk of other ruminants. Therefore, children desensitized to CM through SOTI should strictly avoid the in-

take of milk of other mammals, especially sheep's and goat's milk, until tolerance to those kinds of milk is documented by an OFC.

Another point of clinical interest is the detection of the provoking allergen. Though it's commonly believed that the significant homology between cow's and sheep's milk proteins results in clinical cross-reactivity, in our case immunoblotting showed an IgE-binding band of 54.1 kDa that was present mainly in SM cheese, but was absent in CM (Fig.1), as also confirmed by a pre-absorption of serum with CM and SM, that didn't reduce the intensity of IgE reactivity to the band of 54.1 kDa in SM cheese lane on immunoblot. This band corresponds to a derivative of sheep alpha-casein, and we assume it was the triggering allergen. With reference to Fig.1B differences between ELISA and immunoblotting are due to the fact that when values of specific IgE are so different there is the technical need to block the reaction in immunoblotting in order to avoid overstraining, and consequently lower and less significant binding can be underestimated. However we believe that, identifying the triggering allergen before SOTI is not feasible in clinical routine, due to the possible immunological cross-reactivity among milk proteins of different animal species (1, 2, 15). OFC remains the gold standard to document the absence of sensitization against a specific type of milk.

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