

Case Report

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Western blot analysis to confirm biscuit contamination by hidden cow's milk protein?

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Introduction

IgE-mediated food allergies can cause life-threatening anaphylaxis. Management of these allergies is based on a strict avoidance diet in relation to foods that may contain the allergen. The European regulations issued by the INCO (consumer information) provide a list of 14 allergens subject to mandatory declaration [1]. These allergens must be listed (in bold, underlined, large font, etc.) on food packaging in order to allow ready identification when consumers examine the labeling. The precautionary labeling, which is not regulated, includes statements at the end of the list of ingredients [2]. In France, the most frequent main allergens responsible for IgE-mediated allergies

in children under 2 years of age are Cow's Milk (CM) proteins, eggs, and peanuts [3], all of which are on the list of allergens subject to mandatory declaration. In this study, we report the case of a child who developed anaphylaxis after having consumed a biscuit labeled as having no cow's milk proteins.

Case report

We describe the case of an 18-months-old child (patient P), monitored for CM allergy since the age of 6 months when they experienced severe anaphylaxis that occurred within minutes after having consumed CM yogurt. When the patient was 18 months old, they experienced a second severe anaphylaxis epi-

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sode after ingestion of a biscuit that contained dark chocolate made by a family biscuit factory that had been labeled “organic and artisanal”. Before this episode, they had regularly consumed the same brand of cookies without it causing any symptoms. Indeed, CM was on the precautionary labeling, but it was not one of the ingredients listed on the packaging. Skin prick tests were performed with the remaining biscuits from the same package. The papule diameter was 6 mm for the positive control, 10 mm for the chocolate part, and negative for the negative control and the rest of the biscuit. The casein and CM IgE levels were 38.4 kU/L and 37.3 kU/L, respectively.

Results

IgE immuno-reactivities, assayed by immunoblot (see Online Supplementary Information), were compared on electrophoretically separated proteins extracted from the chocolate and the biscuit parts. Table 1 lists the available biological data for the included patients.

Patient P IgE reactivities were very strong and heterogeneous, from 94 kDa to 10 kDa, for the chocolate topping proteins but were weak for the proteins from the non-chocolate flavoured side (Figure 1A). The IgE of the three other patients (# 1, # 2, and # 3) reacted less strongly to the chocolate part (Figure 1A). The IgE reactivities of patient P to the chocolate topping -lactalbumin, P protein extract were competitively inhibited by the main cow's milk proteins -lactoglobulin, and total caseins (Figure 1B). Minor milk allergens such as lactoferrin, α -globulin, and bovine serum albumin were not inhibited (data not shown). β lactoperoxidase, Dotline experiments performed with several biscuit protein extracts (chocolate and non chocolate parts) revealed that only two batches of these biscuits (# 1 and #2) contained CM proteins, as IgE binding was observed with the serum of patient P. This binding could be fully inhibited by cow's milk at a range of concentrations (Figure 1C). Analysis of the chocolate biscuit from our patient's batch confirmed a high level of contamination of the chocolate by milk proteins on the biscuit production line. Moreover, the manufacturer was able to confirm the contamination.

Table 1: The biological data available for the patients used in this study.

N r	Patient		Specific IgE (kU/L)
	Gender	Age	
Neg Ctlr	M	3	CM: <0.1
P	F	2	CM: 37.3, Bos d 4: 6.7, Bos d 5: 10.8, Bos d 8: 38.4
1	M	5	CM: <0.1, wheat flour: 15.5, gliadin: 2.07, gluten: 23.2, Tri a 14: 6.06, Tri a 19: 0.3, rye: 10.6, barley: 7.01, oats: 1.78
2	M	2	CM: <0.1, chestnut: 2.11
3	F	5	CM: 65.7, Bos d 4: 8.85, Bos d 5: 25.9, Bos d 6: 0.23, Bos d 8: 66.5

Neg Ctlr: Negative control = non-allergic no-atopic individual;
P: studied patient;
CM: cow's milk

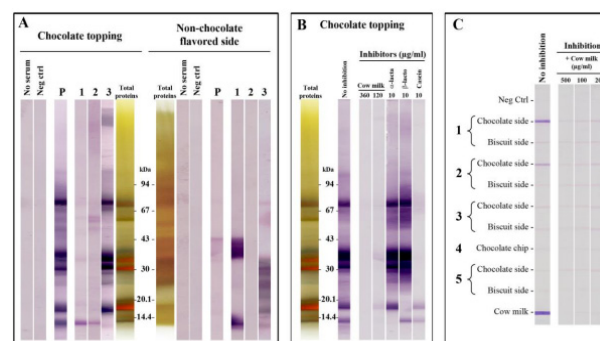


Figure 1: IgE immunoreactivity of patient P against proteins extracted from the chocolate and non-chocolate parts of various biscuits and separated by non-reducing 8-18% SDS-PAGE (A and B). A: Direct immunoblots against the contaminated biscuit # 1 (batch 1). The total proteins were revealed by silver staining. No serum: buffer only. Neg ctrl: serum from a non-184 atopic, non-allergic child. Molecular masses are indicated in kDa. B: Competitive inhibition of the binding of P IgEs against proteins from the chocolate part of the contaminated biscuit # 1 with total cow's milk proteins at two concentrations and the main individual milk allergens at 10 µg/ml: -lacta: -lactalbumin, -lacto: -lactoglobulin, Casein: total caseins. C: Dotline experiment: Proteins extracted from five different biscuits and cow's milk were dotted in a line and incubated with or without (no inhibition) cow's milk as a competitive inhibitor. Neg Ctrl: dot of buffer only.

Discussion

We reported the case of a CM-allergic child who suffered an episode of severe anaphylaxis after consumption of a biscuit that had been contaminated with hidden CM proteins during the production process in an artisanal factory. Hidden allergens are defined as allergens that have been deliberately added to food but that are not listed in the ingredients or allergens added to food by cross-contamination [4]. In our case, black chocolate was mixed with milk chocolate 4 used for another type of biscuit in the same tank. Several cases of anaphylaxis by contamination with cow's milk protein have been reported in the literature. CM proteins have been found in numerous products, such as salmon, lemon sorbet, and soy formula [5,6]. In our case, on the packaging of the biscuit, cow's milk was part of the precautionary labeling that is used by manufacturers in the agro-food sector to limit legal disputes in the event of a serious allergic accident involving one of their products. This precautionary measure is due to the difficulty with guaranteeing the absence of an allergen in the production phase, storage, transport, or distribution of food items. This precautionary labeling is not based on any regulatory legislation. A European project for the standardized detection and quantification of a number of allergens in food products has been developed by the selection of proteotypic peptides that act as markers for the presence of allergenic protein [7]. In Australia and New Zealand, what is known as Voluntary Incidental Trace Allergens Labeling (VITAL) was developed in order to define the thresholds (eliciting dose) of a number of allergen contaminants of industrial food products. To find the culprit hidden allergen, a combination of several allergy tests using either a total extract or recombinant allergens is often required, such as Western blotting, as illustrated in our case [8].

Therapeutic education sessions regarding the management of food allergies are of fundamental importance and they have been shown to have a significant positive impact on parents and children [9]. During these education sessions, patients and parents are taught how to thoroughly examine packaging labels and to be aware of the risks associated with products from artisanal manufactures.

Conclusion

Hidden food proteins represent a dangerous and sometimes even life-threatening source of allergen exposure. Allergic consumers need to be aware of the potential danger presented by handcrafted products and they should not consume such items if the precautionary labeling indicates that such entities may be present. However, they can safely consume branded products because food contamination by allergens on such regulated premises is less frequent. Improving techniques for detecting allergens in food would help to reduce the number of allergic accidents and it would enhance the quality of life of food-allergic patients.

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