

Immunoglobulin g as milk allergen

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Allergy to cow's milk proteins is one of the most frequent allergies in childhood and caseins are the main allergens involved [1]. The goat and sheep's milk casein allergy, without cross-reactivity with cow's milk casein, was described in 1995. Since then, several cases of food allergy to goat and sheep milk have been described, with tolerance to cow's milk [2]. Recently a case of allergy to goat and sheep milk with tolerance to cow's milk through alpha-S2 casein has been described [3].

We present the case of a 12-year-old patient under study in our department for five anaphylaxis. The patient had mild-intermittent allergic rhinitis and persistent moderate extrinsic bronchial asthma due to allergy to mites and cat epithelia. In August of 2017, after ingesting chicken batter and chocolate ice cream, he presented sneezing, runny nose and dyspnea. In September of 2017 he presented two reactions, one after eating salad with lettuce, tomato, carrot, mozzarella, cucumber and tuna, presenting rhinorrhea and generalized urticaria, and the other unrelated to food intake he presented episode of urticaria, rhinitis and dyspnea. In January of 2018 after eating spaghetti with carbonara sauce and a chocolate yogurt he presented dyspnea and hives. The last one in February of 2019 after eating a beef steak and loin stuffed with battered cheese, he presented abdominal pain, hives and dyspnea. The episode was not related with exercise or drugs. The patient follows a free diet between episodes and tolerates cow's milk.

The allergology work-up consisted of skin tests with the foods involved and were positive with cow casein, goat and sheep milk. They were negative to cow's milk, cow alpha lactoalbumin, cow beta-lactoglobulin, egg, tomato, lettuce, carrot, peach, beef meat, chicken meat, tuna, shrimp, latex, *Anisakis*, profilin/Pho d 2 and peach nsLTP/Pru p 3.

Prick by prick skin tests also was performed with raw and cooked turkey meat, raw and cooked beef and tartrazine that were negative and with loin stuffed with industrial battered cheese, without a label, negative on the outside and positive on the inside of cheese. Complete blood count, biochemistry and tryptase were normal. Total immunoglobulin E (IgE) serum was 229.9 IU/ml by ImmunoCAP (Thermo Fisher). Serum specific IgE levels by ImmunoCAP (Thermo Fisher, kUA/L) were as follow: sheep's milk 5.47, goat's milk 9.52, cow's milk 0.25, cow alpha-lactalbumin <0.10, cow beta -lactoglobulin <0.10, cow casein 0.17, cow seroalbumin (nBos d 6) <0.10, tartrazine <0.35, alpha 1.3 gal <0.1, r Tri a 19 <0.1 and rPru p 3 <0.1.

Protein extracts from cow, goat and sheep milk were prepared by homogenization in phosphate-buffered saline (15% W/V) (50 mM phosphate buffer, 100 mM NaCl, pH 7.5), dialyzation against distilled water and liophilization. In order to study the molecular mass of the possible allergens, the three types of milk extracts were studied with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) – Immunoblotting as described by Laemmli [4] (with 2-mercaptoethanol). The assay revealed in the three extracts, an intense IgE binding band of approximately 50 kDa and a much lighter zone around 33 – 30 kDa. As the molecular mass of 50-kDa band match with the heavy chain of the IgG, SDS-PAGE immunoblotting was carried out with purified goat and sheep IgGs and the patient's serum, and a band of 50 kDa was detected in both extracts (Figure 1.I). The presence of serum cross reactive IgE was analyzed by SDS-PAGE Immunoblotting-inhibition, using in solid phase the sheep's milk extract and as inhibitors the goat's and cow's milk extracts. The goat's milk extract produced a total IgE binding inhibition whereas a partial one was produced by the cow's milk extract (Figure 1.II). All these results together with the higher serum IgE value detected against goat milk extract than to sheep milk and the non total IgE binding inhibition obtained with sheep's milk extract (homologous inhibition) as inhibitor, lead us to suspect that goat milk could be the primary sensitizer of the patient's milk allergy.

According to these results, we assume the patient was first sensitized to goat's milk and presented allergic symptoms due to consumption of this milk, and later, due to cross-reactivity, he would react with sheep's milk proteins also with allergic symptoms. Specific IgE have been detected in the patient's serum that recognize IgGs and caseins from goat, sheep and cow milk that can justify an allergic reaction due to exposure, contact or ingestion of these foods. However in this case, sensitization against cow IgG must be produced by a cross-reactivity event that does not give rise to allergic symptoms. The band detected around 33 kDa in cow milk is a very faint one and this low intensity corresponds with the low specific IgE level detected in ImmunoCAP assay against cow casein (0.17 kUA/L). The patient was diagnosed with anaphylaxis due to a food allergy to goat and sheep's milk with a correct tolerance to cow's milk. Allergy to sheep and goat's milk is rare, but its frequency is increasing and it must be taken into account in the context of idiopathic anaphylaxis. In the cases published so far in cow and sheep's milk allergy, the proteins involved are caseins [5,6]. IgE sensitization mediated to IgG of goat and sheep's milk have not been found in the literature review. Further clinical studies should be considered to confirm their importance and evolution.

Conflicts of Interest

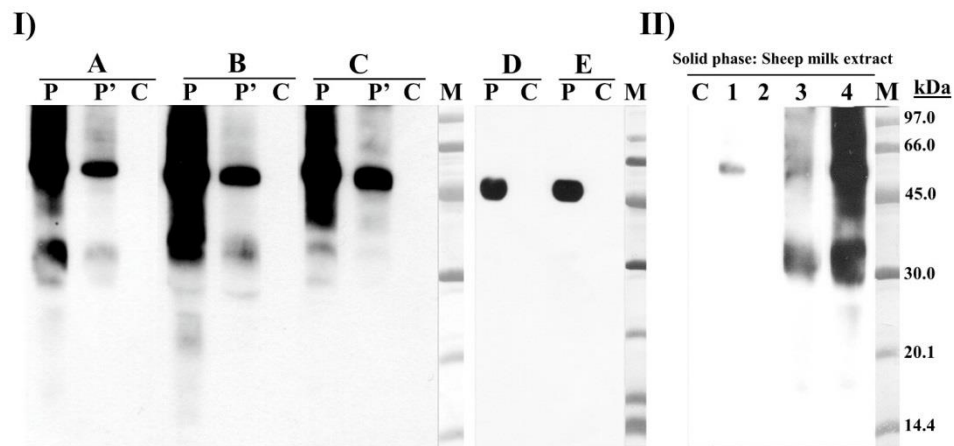
The authors declare that they have no conflicts of interest.

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Figure 1.

I) SDS-PAGE Immunoblotting. A) Sheep milk extract, B) Goat milk extract, C) Cow milk extract, D) Goat IgG, E) Sheep IgG. Lane P, P': patient serum (two dilutions). Lane C: control serum (pool of sera from non-atopic subject). Lane M: Molecular mass standard. **II) SDS-PAGE Immunoblotting-inhibition.** Solid phase. Sheep milk extract. Lane C: Control serum (pool of sera from non atopic subjects) Lane 1 – 4: Patient serum pre-incubated with sheep milk extract (lane 1), with goat milk extract (lane 2), with cow milk extract (lane 3), with sunflower pollen extract (line 4).