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Persistent cow's milk anaphylaxis from early childhood monitored by IgE and BAT to cow's and human milk under therapy

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To the Editor,

Cow's milk allergy affects approximately 2 to 3% of children under 3 years. Allergic reactions often occur at an early onset within the first months of age [1]. In 80 to 85% of the described cases a natural tolerance develops until the age of 5 years. However, a proportion of 1% of adults remain cow's milk allergic, often associated with severe reactions [2].

Some breast-fed babies with cow's milk allergy still show symptoms despite a strict cow's milk-free diet of the nursing mother, and they react until the mother discontinues breast feeding. In the light of this phenomenon it is important to address the question whether or not the persistent reactions can be explained by a genuine primary sensitization to human breast milk or by a cross-reactivity between human and bovine milk proteins.

In this case presentation we refer to a 29 years old adult patient with persistent cow's milk allergy who according to his medical history reacted to breast milk as a baby even after strict cow's milk avoidance of his mother during breast feeding. This patient experienced severe anaphylactic reactions, including cardioplegia in childhood in more than one episode after accidental intake of milk, despite his life-long training of milk protein avoidance. Due to the severity of these reactions after accidental ingestion of traces of milk proteins, an OIT with cow's milk was not started unless an adjunctive treatment with omalizumab had been initiated to reduce the risk of immunotherapy [3-7]. This occurred in December 2015, whereas OIT with cow's milk was initiated nearly one year later, in October 2016.

SEVERE COW'S MILK ALLERGY DIAGNOSIS AND THERAPY: A MOLECULAR APPROACH

The study was approved by the local ethics committee (University of Lübeck, Germany, approval No. 10-126).

To get access to his severe case on the molecular level, we investigated his serum sample in terms of the IgE reactivity against cow's milk and human milk proteins in 2D immunoblots.

As depicted in Figure 1A bovine and human milk proteins showed a broad protein pattern in the molecular range between approximately 15 to 100 kDa within the pI range between 3 and 10. Among these proteins several IgE-reactive allergens could be detected by the patient's serum in both milk extracts, in particular between 25 to 45 kDa (Figure 1B). By means of N-terminal sequencing and mass spectrometry bovine α S₂-casein, β -casein und κ -casein could be identified in cow's milk. In human milk IgE-reactive proteins within the same MW range could be identified as β -casein, besides albumin and lactoferrin (Figure 1C).

IgE cross-reactivity between the milk proteins, namely the main IgE-reactive caseins, was analysed via immunoblot inhibition experiments with cow's milk, human milk and single caseins (data not shown). Here, cross-reactivities between caseins from cow's and human milk could be seen with bovine caseins inhibiting IgE against human caseins stronger than *vice versa*.

The patient received Omalizumab starting in December 2015 with 2x 150 mg Omalizumab. Afterwards the treatment proceeded with 2x150 mg Omalizumab once a month. In April 2016 a skin prick test was performed showing a decrease in reactivity. In October 2016 the patient was again skin prick tested and challenged in an oral provocation up to 1 mL cow's milk which he tolerated. He was advised to

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ingest 0.5 mL cow's milk per day since then. In November 2016 the patient started to consume 1 mL unpasteurized and homogenized milk with a fat content of 3.5%. In May 2017 the patient was again challenged and started to ingest 5 mL cow's milk which he tolerated. The treatment with Omalizumab is still ongoing.

Total serum IgE, specific IgE to milk, cheese, milk of other species and single milk allergens were analysed by CAP-FEIA (ImmunoCAP, ThermoFisher Scientific, Freiburg, Germany) before omalizumab treatment (Table 1). Six and 12 months after treatment initiation, and 2 months of OIT, respectively, the concentration of IgE specific to all analytes showed a massive increase (data not shown).

The biological relevance of IgE reactivity against cow's milk and human milk was analysed by means of the basophil activation (BAT) test before and after 6, 12 and 17 months of omalizumab treatment (and before and 2 and 7 months after OIT, respectively) (Figure 2A-B). Besides human milk we tested cow's milk and single human and bovine milk allergens. As shown in Figure 2B both milk extracts and the single allergens bovine α -casein, β -casein, κ -caseins, bovine β -lactoglobulin, bovine and human α -lactalbumin induced basophil activation. Six months after initiation of the omalizumab treatment, the allergens tested led to a reduced basophil activation and finally, after 12 months of omalizumab therapy and 2 months of oral immunotherapy, the basophil activation has further decreased. The effect of a reduced basophil activation was stronger with human milk and human α -lactalbumin compared to the bovine milk extract and single bovine allergens. Finally, after 17 months of omalizumab administration and 7 months of OIT the BAT revealed the decreased basophil reactivity.

DISCUSSION

It has been discussed for a long time that milk allergic patients may be genuinely sensitized to human milk proteins [8]. In our case study we assessed this question on a molecular level. Our patient suffered from cow's milk allergy since early infancy developing severe, almost fatal anaphylactic reactions to milk and milk products after accidental ingestion. Results of the CAP-FEIA before omalizumab therapy showed that, although the total IgE level was relatively low (85 kU/L), the concentrations of IgE specific for caseins were higher in contrast to the whey proteins α -lactalbumin and β -lactoglobulin. These findings are in line with literature reports that IgE to caseins are a predictor for more persistent cow's milk allergy than IgE to whey proteins that may be found in children who became tolerant [9]. However, when looking at the CAP FEIA data 6 and 12 months after omalizumab initiation, and 2 months of OIT, respectively, both total and specific IgE levels revealed elevated levels. The background for this phenomenon may be that omalizumab as a humanized IgG antibody binds to free serum IgE leading to increased total IgE and specific IgE [10]. Likewise, it is discussed that omalizumab cross-links IgE on the surface of IgE-positive memory B-cells, resulting in an activation and leading to an increased total IgE level [11].

Immunoblot and immunoblot inhibition studies were performed to identify the allergens in human and cow's milk and to show identical or similar epitopes on human and bovine allergens that are recognized by the patient's IgE. IgE from the patient's serum bound primarily to caseins in human milk and cow's milk with β -casein as the main isomer in human milk, and α S₂-casein in bovine milk, respectively. However, from the immunoblot inhibition studies on caseins there is evidence that the analyzed caseins from human and cow's milk are cross-reactive.

Meaning that we do not observe that the patient's serum contained IgE directed to caseins in human milk which could not be inhibited by bovine caseins. In contrast, Mäkinen-Kiljunen & Plosila [12] described an IgE-mediated allergic reaction to genuine human milk proteins; namely human α -lactalbumin.

To simulate the acute allergic reaction of the patient to cow's and human milk and the single milk allergens before and under treatment with omalizumab and, later on OIT, we performed basophil activation tests in an extensive and specific readout according to Schwager et al. [13]. Given that the patient's CD63 up regulation is hardly higher than 15 % on stimulation with cow's milk, κ -casein and β lactoglobulin and even lower against the other allergens tested, we were able to show that the highest basophil activation in our patient could be elicited by human and bovine milk allergens and single milk allergens prior to omalizumab administration and OIT, and was decreased during therapy by means of a testing setup with a very extensive gating strategy of the basophils. Actually, evidence suggests that cow's milk rather than human milk plays a bigger role in the allergic response in our patient with severe cow's milk allergy since early infancy. In our case, the positive clinical outcome of our patient within the first 17 months upon the treatment with omalizumab and after 5 months of OIT reflects the suppression of basophils.

Our observed positive clinical response to omalizumab as an adjunct to milk OIT is in line with literature reports [3-7]. Actually, milk OIT intends to either lead to desensitization (in that the patients ingest milk without any symptoms during continued OIT due to changes in effector cells, e.g. mast cells, basophils) or, in contrast, to "sustained unresponsiveness" after a period of avoidance due to the induction of allergen-specific T regulatory cell responses suppressing Th2 response [3,7]. However, milk OIT is often associated with adverse reactions which limits its

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safety and efficacy [3-7]. To reduce the risk of OIT, adjunctive treatment with omalizumab was investigated in different studies depicting an increased tolerability of milk OIT during omalizumab but not an improved longer-term efficacy (desensitization, sustained unresponsiveness) [4]. Whether or not the individual response of this patient in our case study can be explained by facilitating OIT through the administration of the adjunctive therapy with omalizumab or the effect of the OIT itself has to be assessed after omalizumab administration is suspended. Still, one has to bear in mind that even when being desensitized through milk OIT in combination with omalizumab patients are described to lose protection after 8 weeks of avoidance [4].

In summary, we do not have a clear proof whether or not primary sensitization has taken place via human milk in our patient with life-long severe cow's milk allergy although the evidence is highly suggestive. Further studies are mandatory recruiting cow's milk allergic patients with transient and persistent cow's milk allergy from early infancy and investigate for differences in epitope recognition on the molecular level.

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REFERENCES

- 1 Sampson HA. Update on food allergy. *J Allergy Clin Immunol* 2004; 113: 805-19.
- 2 Järvinen KM, Chatchatee P. Mammalian milk allergy: clinical suspicion, cross-reactivities and diagnosis. *Curr Opin Allergy Clin Immunol* 2009; 9: 251-258.
- 3 Wood RA. Food allergen immunotherapy: Current status and prospects for the future. *J Allergy Clin Immunol* 2016; 137: 973-82.
- 4 Wood RA, Kim JS, Lindblad R et al. A Randomized Double-Blind Placebo-Controlled Study of Omalizumab Combined with Oral Immunotherapy for the Treatment of Cow's Milk Allergy. *J Allergy Clin Immunol* 2016; 137: 1103–10.
- 5 Frischmeyer-Guerrero PA, Masilamani M, Gu W, B et al. Mechanistic Correlates of Clinical Responses to Omalizumab in the Setting of Oral Immunotherapy for Milk Allergy. *J Allergy Clin Immunol* 2017; 140: 1043-1053.
- 6 Martorell-Calatayud C, Michavila-Gómez A, Martorell-Aragonés A et al. Anti-IgE-assisted desensitization to egg and cow's milk in patients refractory to conventional oral immunotherapy. *Pediatr Allergy Immunol* 2016; 27: 544-6.

- 7 Fiocchi A1, Pecora V, Valluzzi RL, Fierro V, Mennini M. Use of biologics in severe food allergies. *Curr Opin Allergy Clin Immunol* 2017; 17: 232-238.
- 8 Schulmeister U, Swoboda I, Quirce S et al. Sensitization to human milk. *Clin Exp Allergy* 2008; 38: 60-68.
- 9 Järvinen KM, Celestin J. What's on the menu for prediction of natural history of cow's milk allergy beyond casein cocktails and epitope soup? *Clin Exp Allergy* 2012; 42: 1549-1551.
- 10 Takahashi M, Taniuchi S, Soejima K, Hatano Y, Yamanouchi S, Kaneko K. Successful desensitization in a boy with severe cow's milk allergy by a combination therapy using omalizumab and rush oral immunotherapy. *Allergy Asthma Clin Immunol* 2015; 11:18; DOI 10.1186/s13223-015-0084-y.
- 11 Eckl-Dorna J. Omalizumab's Impact on Total and Allergen-Specific IgE Levels: A Polyclonal Story. *Int Arch Allergy Immunol* 2016; 169: 69-70.
- 12 Mäkinen-Kiljunen, S., Plosila, M. A father's IgE mediated contact urticaria from mother's milk. *J Allergy Clin Immunol* 2004; 113: 353-354.
- 13 Schwager C, Kull S, Behrends J, Roeckendorf N, Schocker F, Frey A, Homann A, Becker WM, Jappe U. Peanut oleosins associated with severe peanut allergy—importance of lipophilic allergens for comprehensive allergy diagnostics. *J Allergy Clin Immunol* 2017; DOI: 10.1016/j.jaci.2017.02.020.

FIGURE LEGENDS

Figure 1A-C. 2D separation and 2D immunoblot of bovine and human milk. Separation was performed under reducing conditions. The bovine and human milk concentration was 600 µg. A: 2D separation of bovine (at left) and human milk (at right), Commassie stained. B: 2D immunoblot of bovine (at left) and human milk (at right), detection antibody: patient's serum 1:20 (chemiluminescent western blot detection). C: Identification of IgE-reactive bovine (at left) and human milk proteins (at right) by N-terminal sequencing and mass spectrometry, Commassie stained.

Figure 2A-B. Basophil activation test performed with blood of the cow's milk allergic patient using cow's milk and human milk and single bovine and human milk allergens. Percentages represent CD63^{pos} basophils determined by flow cytometry. Blood sample stimulation was conducted with the analytes as indicated. fMLP, anti-IgE were run as positive controls, PBS as negative control.

- A. Controls before and 6, 12 and 17 months after omalizumab treatment and before and 2 and 7 months after OIT treatment, respectively.
- B. Cow's milk, human milk and single bovine and human milk allergens before and 6, 12 and 17 months after omalizumab treatment and before and 2 and 7 months after OIT treatment, respectively.

Table 1 Synopsis of the CAP-FEIA results on specific IgE against whole cow's milk and milk from different species as well as single milk allergens (ImmunoCAP)

		Before Omalizumab therapy	
	Total IgE	85.0 kU/L	
	Target allergen	Specific IgE [kU/L]	CAP class
Bovine milk	Milk protein (f2)	34.90	4
	Milk boiled (f231)	38.00	4
	Whey (f236)	33.60	4
Cheese and milk of other species	Cheddarcheese (f81)	29.80	4
	Mould cheese (f82)	23.70	4
	Ewe's milk (f325)	30.60	4
	Ewe's whey (f326)	25.80	4
	Goat's milk (f300)	32.00	4
	Mare's milk (f286)	2.55	2
Single allergens	Bovine casein (f78); (nBos d 8)	31.30	4
	β -lactoglobulin (f77); (nBos d 5)	8.86	3
	α -lactalbumin (f76); (nBos d 4)	8.85	3

Figure 2

