Food allergy in breastfeeding babies. 
Hidden allergens in human milk

MF. Martín-Muñoz¹, F. Pineda², G. García Parrado², D. Guillén¹, D. Rivero¹, T. Belver¹, S. Quirce¹

1Department of Allergy, Hospital La Paz Health Research Institute (IdiPAZ), Madrid, Spain
2Diater Laboratories, R&D Department, Madrid, Spain

Institution where the work was carried out: Department of Allergy, Hospital La Paz Health Research Institute (IdiPAZ), Madrid, Spain

Key words 
breastfed infants; food allergy; hidden allergens; human milk

Summary

Background. Food allergy is a rare disorder among breastfeeding babies. Objective. Our aim was to identify responsible allergens in human milk. Methods. We studied babies developing allergic symptoms at the time they were breastfeeding. Skin prick tests (SPT) were performed with breast milk and food allergens. Specific IgE was assessed and IgE Immunoblotting experiments with breast milk were carried out to identify food allergens. Clinical evolution was evaluated after a maternal free diet. Results. Five babies had confirmed breast milk allergy. Peanut, white egg and/or cow’s milk were demonstrated as the hidden responsible allergens. No baby returned to develop symptoms once mother started a free diet. Three of these babies showed tolerance to other food allergens identified in human milk. Conclusion. A maternal free diet should be recommended only if food allergy is confirmed in breastfed babies.

Introduction

Breast milk is the optimal nutrition for infants. Whether breastfeeding protects against the development of allergies remains controversial (1,2,3). Some studies report protection with exclusive and prolonged breastfeeding (4,5,6), particularly in children prone to atopy (7,8). Other reports have suggested breast milk could be responsible for early sensitization to food (9,10,11). In a high-risk cohort, McGowan et al. (3) found an extremely high cumulative incidence of food allergy associated with breastfeeding. Food proteins ingested by women who are breastfeeding are absorbed and excreted into breast milk antigenically active. Eczema, colic, diarrhea and vomiting are frequent symptoms in exclusively breastfed infants, but rarely food allergy has been demonstrated in this group (12,13). In a multidisciplinary review of the literature concerning the impact of early feeding in infancy on later allergic manifestations, Van Odijk et al. (14) concluded that breastfeeding protects against the development of atopic disease, and this effect appears even stronger in children with atopic heredity.

We studied babies developing allergic symptoms at the time they were breastfeeding. The aim of our study was to identify breast milk allergens involved in allergic reactions after breastfeeding.

Methods

Infants with immediate erythema, hives, vomiting, diarrhea, sneezing, coughing or breathlessness, during or within 1 hour of breastfeeding, were included in this study. The study was carried out in accordance with the ethical standards established in the Declaration of Helsinki. A written informed consent document, previously approved by the Ethics Committee (Hospital La Paz) was provided by the mothers before beginning the study and collecting the breast milk samples. The mothers provided us with...
information about foods tolerated by the infant and a detailed description of the foods ingested by themselves before breastfeeding, and the symptoms developed by their baby. We studied food sensitization in the infants and looked for hidden allergens in the breast milk identifying the responsible food allergens there. Breast milk samples (≥ 30 ml) were collected from the babies’ mothers: (A) samples collected 24 hours after following a diet free of any suspected food, including those for which the infant had a positive skin prick test (SPT) or specific IgE (sIgE); and (B) samples collected at 2-hour intervals up to 8 h post-dietary challenge with each one of the suspected implicated foods (250 ml cow’s milk, 1 hen’s egg, 100 g of hake or 30 g of peanuts). A 24 h period free from ingestion of any suspected food was required before ingesting any other food and subsequently collecting the corresponding samples. Breast milk samples were stored at -20°C after collection. Milk was defatted by centrifugation (2000 g, 20 min at + 4°C) before the different assays.

Skin tests: SPT with cow’s milk, α-lactalbunin (Bos d 4), β-lactoglobulin (Bos d 5), bovine serum albumin (Bos d 6) and casein (Bos d 8); hen’s egg white; hake; lentil and peanut (DIATER Laboratories, Spain) and skin prick-by-prick tests (SPPT) with corresponding collected samples of the mother’s breast milk were performed on the infants. Histamine 1/100 and glycerinate saline were used as positive and negative controls. The averages of the diameters of the wheal were assessed after 15 minutes.

IgE: Serum total and specific IgE (sIgE) were determined by Immuno CAP® (Phadia).

An immunoblot analysis of immunoglobulin E and immunoblot inhibition experiments were performed for each patient’s serum with the suspected foods and respective breast milk samples to identify hidden food allergens. Briefly, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed under reducing and denaturing conditions. Protein extracts from suspected foods and breast milk collected at various times were loaded onto 15% gels and stained with Coomassie blue to visualize constitutive protein bands and for Western blot analysis. The separated proteins were electro-transferred onto nitrocellulose membranes and blocked with 0.5% PBS Tween. The membranes were incubated with individual samples of diluted sera and suspected foods in the case of inhibition, and then were reacted with a mouse anti-human IgE Fc-HRP (Southern Biotech, Birmingham, USA). After additional washing, the bands were made visible on the membrane using chemiluminescent development substrates.

Food tolerance was defined as the patients eating a specific food without symptoms occurring. In the case of food sensitization without previous ingestion, tolerance was assessed upon the introduction of the food by a controlled open food challenge. The clinical evolution of the infants was evaluated after establishing an infant and maternal free diet of identified allergen.

Results

Forty-seven breastfed babies (1-19 months) referred with immediate symptoms (urticaria, erythema or vomiting) at the time they were breastfeeding were evaluated to food allergy. Only five infants who had proved allergic symptoms after breastfeeding were studied.

Case 1: A 6-month-old girl with atopic dermatitis since her first month of life and exclusively breastfed suffered, from her second month, immediate erythema and pruritus on her face during breastfeeding. At 6 months of age, she developed generalized urticaria and vomiting 10 minutes after consuming her first bottle of humanized cow’s milk formula. Specific IgE to cow’s milk proteins was demonstrated in her serum and she was diagnosed with cow’s milk allergy. Her mother started a cow’s milk-free diet and the infant was fed with mixed breastfeeding with a hydrolyzed cow’s milk formula and subsequently meat, fish, egg and lentils. When the baby was 10 months old, she experienced a new episode of hives around her mouth while breastfeeding. Her mother reported that 2 hours before she had eaten peanuts.

Case 2: A 14-month-old girl breastfed from birth who was tolerating cow’s milk formula, meat, fruits and vegetables from eight and fish from ten months, developed hives affecting her face after ingesting a bottle of humanized cow’s milk continuing breastfeeding. Her mother remembered that she had eaten peanuts 1 hour before breastfeeding. She had suffered from wheezing and atopic eczema from her second month.

Case 3: An exclusively mixed breastfeeding 3.5-month-old girl, suffering mild eczema from birth, began to develop recurrent hives on her face during breastfeeding. She had been tolerating humanized cow’s milk formula since 1.5 months of her life. Her mother did not relate the appearance of symptoms with her previous ingestion of any suspicious food.

Case 4: An 8-month-old boy exclusively breastfed developed eczema on his face from 1 month of age. At 3 months of age he began to experience discomfort and occasional vomiting in the first hour after breastfeeding. He had started mixed feeding at 4 months with a humanized cow’s milk formula and subsequently he had been tolerating cereals, meat and fruit. At 5 months of age, he developed uneasiness and vomiting, pruritus, erythema and worsening of his eczema 30 minutes after breastfeeding. His mother did not associate the appearance of symptoms with prior ingestion by herself of any particular food.

Case 5: A breastfed 15-month-old girl, suffering from atopic dermatitis and wheezing from her second month, developed at 5 months of age an allergic reaction (generalized urticaria, vomiting, respiratory distress and cyanosis) 10 minutes after ingesting 20 ml of a humanized cow’s milk formula. Symptoms were controlled after applying adrenaline, corticosteroids and antihistamine treatment. Specific IgE to cow’s milk proteins was
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Figure 1 - Recognition of food allergens by patients’ sera in the breast milk samples (A collected more than 24 h after ingesting specific foods and before ingesting the specific food; sample B collected 4-8 h after ingesting the specific food).

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lane 1</td>
<td>Lane 2</td>
<td>Lane 3</td>
<td>Lane 4</td>
<td></td>
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<tr>
<td>breast milk simple A collected before peanut intake.</td>
<td>peanut</td>
<td>breast milk sample B collected after ingesting peanut.</td>
<td>breast milk B sample collected after ingesting peanut and inhibited with peanut.</td>
<td></td>
</tr>
<tr>
<td><strong>B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lane 1</td>
<td>Lane 2</td>
<td>Lane 3</td>
<td>Lane 4</td>
<td></td>
</tr>
<tr>
<td>breast milk A sample collected before ingesting peanut.</td>
<td>peanut</td>
<td>breast milk B sample collected after ingesting peanut.</td>
<td>breast milk B sample collected after ingesting peanut and inhibited with peanut.</td>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lane 1</td>
<td>Lane 2</td>
<td>Lane 3</td>
<td>Lane 4</td>
<td></td>
</tr>
<tr>
<td>breast milk A sample collected before ingesting hen’s egg.</td>
<td>white egg</td>
<td>breast milk B sample collected after ingesting white egg.</td>
<td>breast milk B sample collected after ingesting white egg and inhibited with white egg.</td>
<td></td>
</tr>
</tbody>
</table>

Patient 1. Lane 1: breast milk simple A collected before peanut intake. Lane 2: peanut. Lane 3: breast milk sample B collected after ingesting peanut. Lane 4: breast milk B sample collected after ingesting peanut and inhibited with peanut.

Patient 2. Lane 1: breast milk A sample collected before ingesting peanut. Lane 2: peanut. Lane 3: breast milk B sample collected after ingesting peanut. Lane 4: breast milk B sample collected after ingesting peanut and inhibited with peanut.

Patient 3. Lane 1: breast milk A sample collected before ingesting hen’s egg. Lane 2: white egg. Lane 3: breast milk B sample collected after ingesting white egg. Lane 4: breast milk B sample collected after ingesting peanut and inhibited with white egg.

Patient 4. Lane 1: breast milk A sample collected before ingesting hen’s egg. Lane 2: white egg. Lane 3: breast milk B sample collected after ingesting peanut. Lane 4: breast milk B sample collected after ingesting peanut and inhibited with peanut.

Patient 5. A. Lane 1: breast milk A sample collected before ingesting egg, cow’s milk or peanut. Lane 2: cow’s milk. Lane 3: breast milk B sample collected after ingesting cow’s milk. Lane 4: breast milk B sample collected after ingesting cow’s milk and inhibited with cow’s milk. B. Lane 1: breast milk A sample collected 24 h after ingesting egg, cow’s milk or peanut. Lane 2: egg white. Lane 3: breast milk B sample collected after ingesting white egg. Lane 4: breast milk B sample collected after ingesting white egg and inhibited with white egg. C. Lane 1: breast milk sample collected 24 h before ingesting egg, cow’s milk or peanut. Lane 2: peanut. Lane 3: breast milk B sample collected after ingesting peanut. Lane 4: breast milk B sample collected after ingesting peanut and inhibited with peanut.

detected in her serum and she was diagnosed with cow’s milk allergy. Thereafter, she and her mother began a diet free of cow’s milk. At the age of 10 months, she developed urticaria around her mouth while she was breastfeeding. Her mother did not recall the food she had eaten before breastfeeding. The infant was tolerating cereals, vegetables, fruits, meat and fish.

Table 1 shows detailed data concerning patients (age, sex, atopic dermatitis or asthma and results of SPPT with breast milk) and the mothers’ atopic diseases. Table 2 shows data about SPT total and specific IgE and tolerance to cow’s milk, hen’s egg, hake and peanut. No patient was sensitized to lentils. All 5 infants were sensitized to hen’s egg white, but 2 of them could tolerate it. Three were sensitized to cow’s milk but only one of them (Case 2) had tolerance. None of the infants had introduced peanuts in their diet but three of them had specific IgE to peanuts. Two infants were sensitized to hake, and both of them had eaten it without any symptoms. Figure 1 shows an image with the results of the immunoblot and immunoblot inhibition experiments.

Case 1 had a positive SPPT to breast milk sample B obtained after the mother had ingested peanuts. IgE in the patient’s serum recognized 2 proteins of approximately 14 kDa in the breast milk collected after the ingestion of peanuts, and both were inhibited by peanuts. Case 2 had positive SPPT to breast milk sample B, obtained after the mother had ingested peanuts. IgE in the patient’s serum recognized proteins of approximately 30 kDa in the collected breast milk after the ingestion of peanuts that were inhibited by peanuts. Case 3 had positive SPPT to breast milk sample B, obtained after the mother had ingested egg. IgE in the patient’s serum recognized a group of proteins of approximately 14 and 30 kDa in the collected breast milk after the ingestion of hen’s egg, and this recognition was inhibited by egg white. Case 4 had a positive SPPT to breast milk sample B, obtained after the mother had ingested hen’s egg. IgE in the patient’s serum recognized a group of proteins of approximately 14 and 30 kDa in the collected breast milk after the ingestion of hen’s egg, and this recognition was inhibited by egg white. Case 5 had a positive SPPT to breast milk sample B obtained after the mother had ingested peanuts, egg or cow’s milk. IgE in the patient’s serum recognized a group of proteins of approximately 14 and 30 kDa in the collected breast milk samples after the ingestion of peanuts, egg white or cow’s milk, and all were inhibited with their respective allergens.

None of the infants had allergic symptoms after breastfeeding once their mothers started a specific allergen-free diet. Respect the other 42 children had consulted: 14 for erythema, 20 for vomiting and 18 for discomfort after breastfeeding. Only five
hands or household objects. Lactation has been suggested as being responsible for early sensitization. About 50% of women excreted dietary antigens in breast milk, concentrations ranging 0.1 to more than 1000 ng/ml (20). Characteristics of lactating women such as atopy have not accounted for the variable secretion (16,17,21). Ovalbumin has been detected in 59% to 74% (24, 25), bovine B-lactoglobulin in 53% to 63% (3,16,17) and peanut proteins in about 48% (9) of lactating women. Also ovomucoid (10,15), alpha-S1-casein (18), gliadin (19), and other food allergens have been detected in human milk under physiological conditions. Nevertheless, breast milk rarely triggers allergic symptoms and the role of food allergens in breast milk in food allergy in infants is not clear.

Discussion

We studied infants who developed immediate allergic symptoms to human milk. We demonstrated that they had specific IgE to cow’s milk, egg or peanut, none of which had been introduced into their diet. All infants had atopic dermatitis, which is a risk factor for food allergy, and two of them suffered also from asthma. Sensitization in the studied infants could have occurred in utero or through breast milk, inhalation, contamination of

<table>
<thead>
<tr>
<th>Case</th>
<th>Total IgE Ku/L</th>
<th>Cow’s milk</th>
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<th>Hake</th>
<th>Peanut</th>
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<tr>
<td></td>
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<td>Tol</td>
<td>prick IgE</td>
<td>Tol</td>
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<tr>
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<tr>
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<td>9</td>
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<tr>
<td>4</td>
<td>5.03</td>
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<tr>
<td>5</td>
<td>92</td>
<td>13</td>
<td>25</td>
<td>6</td>
<td>12.9</td>
</tr>
</tbody>
</table>

F (female), M (male), AD (atopic dermatitis). Results of SPPT babies with different breast milk A and B samples (mm average diameter); NA (not applicable, because of patient had a diagnosis of allergy to that food or patient is tolerating it at that time).

Table 1 - Demographic and allergological data of the babies and their breastfeeding mothers.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age months</th>
<th>sex</th>
<th>baby</th>
<th>mother</th>
<th>A samples</th>
<th>B samples</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Collected 24 h after</td>
<td>Collected (1-8 h) after ingesting</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cow’s milk, egg, peanut and hake</td>
<td>cow’s milk, egg, peanut, hake</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>F</td>
<td>AD</td>
<td>AD</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>F</td>
<td>AD</td>
<td>AD</td>
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<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>F</td>
<td>AD</td>
<td>Asthma</td>
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<td>3.2</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>M</td>
<td>AD</td>
<td>AD</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>F</td>
<td>AD</td>
<td>Asthma</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2 - Results of skin prick tests, total and specific IgE; tolerance status (yes, no, or not introduced) to cow’s milk, white egg, hake and peanuts in each case.
Two of our cases, 1 and 5, developed allergic symptoms after the first bottle of a cow’s milk formula. Chandra et al. (1) found an incidence of cow’s milk allergy of 0.5% (9/1749) in the first year of life with a frequency of exclusive breastfeeding of 52% at 3 months of age. Food allergens, other than cow’s milk, could be responsible for allergic reactions in breastfed infants (15,21). Negative SPPT to breast milk samples obtained after a 24 h period of a cow’s milk, egg or peanut-free diet and positive with samples collected after the ingestion of any of these foods and the results of immunoblotting experiments confirmed in each case the presence of hidden food allergens in the breast milk, justifying the appearance of symptoms when the babies were breastfeeding. Sera of the cases 1, 2 and 5, recognized in breast milk samples B (collected after mother ingestion of peanuts) 14 and 30 kDa allergens, this recognition was inhibited by previous incubation of sera with peanut. This/these 14 kDa allergens could correspond to Ara h 2, Ara h 5, Ara h 6, Ara h 7 or Ara h 10; and detected allergens of 30 kDa to an Ara h 3 fragment. Both major peanut allergens Ara h 1 and Ara h 2 have been previously identified in human milk by Vadas et al. (9) and Bernard et al. (22) who detected peanut allergens (Ara h 6) in human milk as early as 10 min after peanut ingestion, with peak values observed within the first hour after ingestion.

Sera of cases 3, 4 and 5 recognized, in breast milk samples B collected after egg ingestion, proteins around 14 KDa, possibly between 20-30 kDa possibly Gal d 4 (lysozyme) and Gal d 1 (ovomucoid). Hirose et al. (23) detected ovomucoid in 12 out 37 (32%) human breast milk samples. Case 5 recognized cow’s milk allergens between 14 to 30 kDa which could correspond to the Bos d 4, Bos d 5 and Bos d 8 (α-lactalbumin, β-lactoglobulin and caseins of cow’s milk).

Böttcher et al. (26) and Järvinen et al. (27) observed an increased incidence of allergic disease in intentionally breastfed children. There is a prevailing opinion that breastfeeding decreases the allergy risk, and the mothers of high-risk infants might be more inclined to breastfeed than those of low-risk infants. Hong et al. (28) evaluated the effect of breastfeeding and gene-breastfeeding interactions in food sensitization in a birth cohort of 970 children, and observed that breastfeeding was associated with an increased risk of food sensitization; however, this effect was dependent on functional genetic variants in the IL-12 receptor b1, Toll-like receptor 9, and thymic stromal lymphopoietin genes. Liu et al. (29) evaluated a Boston birth cohort (n = 5,649) identifying a risk of sensitization for an IL4 gene polymorphism and 3 other genes. Food allergens detected in breast milk could promote tolerance (30). Symptoms in our patients, during / after breastfeeding, disappeared when a cow’s milk, egg or peanut maternal free diet was started. However, Case 1 had hen’s white egg and Case 2 cow’s milk, egg and fish IgE and both of them showed tolerance to maternal or direct ingestion of this potential allergens. Du Toit et al. (31) demonstrated a protective effect of peanut consumption during lactation by the proportion of UK Jewish mothers not consuming peanuts during breastfeeding, compared with Israeli Jewish mothers who ate peanuts, considering the ten times higher prevalence of peanut allergy in this population living in the UK. IgA in human milk might modulate mucosal immune processes and factors that promote gut maturation, such as intestinal microbiota, which could reduce allergy risk (32). Several breast milk peptides were found to lower regulate neonatal immune activity, suggesting they might promote neonatal immune competence. Järvinen et al. studied the role of maternal elimination diets and human milk IgA in the development of cow’s milk allergy in the infants. They concluded that maternal CM avoidance was associated with lower levels of mucosal-specific IgA levels and the development of CMA in infants (33).

A consensus states that pregnant and breastfeeding women in general should not follow food allergen free diets (34,35). However, in case of allergic symptoms in breastfed babies, an allergic study should be performed to assessed food allergy.

Conclusion

Food allergens detected in breast milk could promote tolerance. A maternal free diet should be recommended only if food allergy is confirmed in breastfed babies.

Conflict of interests and funding

Authors declare that does not exist economic or other types of conflicts of interests and that the study did not receive funding.

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