

REVIEW

Development of cow's milk allergy in breast-fed infants

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Introduction

Cow's milk allergy (CMA) is defined as an immunologically mediated adverse reaction against cow's milk antigens [1]. CMA is the first phenomenon of atopic symptomatology and the 'allergic march' because cow's milk proteins are the first foreign proteins consumed in large quantities by an infant. The overall incidence of CMA ranges from 1.9% to 7.5% in population-based studies [2–6]. Symptoms of CMA commonly appear during the first year of life and in 75–90% of patients within the first month of their life [2,7]. They occur within days or weeks after commencing feeding with a cow's milk-based formula, or already at their first exposure [2,8]. Several studies have reported appearance of CMA during exclusive breastfeeding [9–12], which occurs in 2–23% of infants with CMA [2,7,13]. The incidence of CMA during exclusive breastfeeding (with cow's milk never given to the infants) has been reported as 0.4–0.5% [2,5].

In the light of epidemiological studies, the effect of breastfeeding on the development of allergic diseases and CMA in the breast-fed infant remains controversial. The protective effect of breastfeeding in the prevention of atopic diseases has especially been defined in allergic families. However, Wright *et al.* recently reported that in mothers with low IgE, breastfeeding was associated with low IgE levels in the breast-fed, whereas in atopic mothers, breastfeeding for more than 4 months was related to development of atopy in the child [14]. This controversy may in part be due to individual variations in the levels of immunological constituents in the mother's milk. Therefore, evidence is accumulating in favour of the protective effect of breastfeeding against allergies in non-atopic families [15,16].

Clinical manifestations

There are several reports on the disappearance of symptoms in breast-fed infants already sensitized upon abstinence of the mother from cow's milk (Fig. 1) and the subsequent reappearance of symptoms upon reintroduction of milk to the mother's diet [9,11,17,18]. The age at the appearance of symptoms is usually 1 week to 2 months [9–11]. Symptoms similar to those seen in cow's milk-allergic babies fed formula can be observed in breast-fed babies [9,11,19]. However, the order of appearance of symptoms during exclusive breastfeeding is frequently distinctive: gastrointestinal symptoms, such as regurgitation, colic and crying, commonly appear first, and eczema follows shortly thereafter, at the age of 2–3 months [12].

The symptoms of CMA most commonly arise in the cutaneous, gastrointestinal and respiratory tract [20]. The majority of infants with CMA present with more than one symptom [21], and symptoms with two or more organ systems involved are seen in 58–92% of patients [2,7,13,22]. In breast-fed infants, the following reactions to cow's milk protein present in mother's milk have been reported: eczema [9–11], colic [9,11,23,24], loose stools/diarrhoea [10,11], colitis [25] with rectal bleeding [26], regurgitation and/or vomiting [9,11], constipation [9], rhinorrhoea [11,17], wheezing and coughing [10], crying, life threatening bradycardia and hypotonia [9], and anaphylactic shock [27].

Diagnostic criteria

The diagnosis of CMA is based on a definite disappearance of the symptoms of CMA after elimination of cow's milk from the diet (and breastfeeding mother's diet), reoccurrence of identical symptoms during cow's milk challenge, disappearance of symptoms after re-elimination of cow's milk, and exclusion of lactose intolerance and coincidental infections [1]. A challenge should be started after a 2-week elimination diet [28]. A double-blind, placebo-controlled food challenge is considered as the preferred diagnostic tool in confirming food allergy in children [7,29]. Some

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Fig. 1 An example of a 6-week-old infant showing facial atopic eczema during exclusive breastfeeding (a), and the disappearance of eczema during maternal cow's milk elimination diet (b).

authors have, however, suggested that open, controlled challenges are reliable in infants aged less than 1 year, when performed under professional observation in a clinical setting [7,29,30].

Challenge may also be performed through the mother's milk [9,11]. It may be performed by giving increasing

doses (100-, 200- and 400 mL doses) of cow's milk or other dairy products at 1-h intervals to the mother, with frequent breastfeedings, and continuing from the next day on with free consumption [11]. Symptoms can be caused by as little as 5 mL of cow's milk ingested by the mother [17], but commonly several hundred millilitres of cow's milk are needed [11]. The appearance of symptoms usually occurs 20 min to several hours after breastfeeding [9,11], but may also appear only after cumulating doses on the second or third day [11]. A measurable rise in total number of immunoglobulin-secreting cells of the IgA and IgG classes can simultaneously be detected in the peripheral blood of these infants by using the solid-phase enzyme-linked immunospot assay (ELISPOT) [11]. Simultaneously, an intestinal permeability test can be performed to detect alterations in intestinal permeability [9].

Laboratory tests

Neither clinical symptoms nor any laboratory test have proven to be indicative of clinical disease. In a population of infants less than 12 months of age, half of which were delayed-type reactors, serum cow's milk-specific IgE ≥ 0.35 kU/L had good sensitivity (0.72) but poor specificity (0.49) [31]. In comparison, the skin prick test (SPT) at a cut-off level of 3 mm (wheal) produces the best value for overall agreement with a sensitivity of 0.61 and a specificity of 0.76 [31]. In our previous study of young infants, mean age 5.1 months (95% CI 4.2–6.1 months), half of which were delayed reactors, SPT was positive only in 33% of infants [12]. In children with an immediate-onset reaction, SPT was positive in 47%, but only in 17% of the children with a delayed-onset reaction [12]. Interestingly, infants with a clear immediate-type of reaction to cow's milk may have no detectable cow's milk-specific IgE at the initial examination at the age of less than 6 months, but when reinvestigated during the second half of the first year, they may show a positive specific IgE response [7], suggesting that young age may influence the test results. SPT can also be performed on mother's milk by using the prick technique with a drop of mother's milk. If positive, it strongly suggests food allergy [32].

Whereas cow's milk-specific IgE and SPT assess only CMA with an IgE-mediated mechanism, there are fewer tests available for detecting delayed hypersensitivity, the mechanism of which is not fully understood. A positive patch test reaction has been associated with delayed-type reactions [33–34], and it has also been reported to correlate with the immediate-type reaction [31]. The sensitivity and specificity of skin patch test with cow's milk have been reported as 0.18–0.26 and 0.87–0.92 in infancy, respectively [31,35]. According to our findings, skin patch testing

may be performed in young infants; however, it is preferable to use it in those older than 2–3 months.

Routes of sensitization to cow's milk proteins

Cow's milk proteins are absorbed by the normal adult gut and transmitted in human milk [18,36–39]. β -lactoglobulin (BLG) has been detected in 75% of milk samples [18] in concentrations up to 16 $\mu\text{g/L}$ [11,18,36–38], and casein has been found in half of them [37,38]. The level of BLG in human milk varies inter- and intra-individually [11,40,41], decreases as a function of lactational duration, and is affected by the maternal diet and atopic constitution [42]. The sensitizing effect of food proteins present in mother's milk, however, varies between individuals [8], and there is no clear link between sensitization and presence of BLG in milk [8,43]. Significant differences in the sensitization to food proteins (e.g. cow's milk) were not found among infants in relation to maternal consumption (high vs. minimal) of foods (cow's milk) during late pregnancy and lactation [44,45]. In another study, maternal total elimination of cow's milk, egg and fish during the first 3 months of lactation did not reduce sensitization to these foods [46]. However, the development of CMA and specific IgE antibodies during the first 2 years of life were reduced when both the lactating mother avoided cow's milk during the last trimester and lactation and her infant avoided cow's milk until 1 year old [46,47]. It still remains unclear whether such small amounts of cow's milk proteins in mother's milk could sensitize an infant, although they can produce allergic symptoms in infants already sensitized.

It has been suggested that infants might also become sensitized by inhalation or by direct antigen contact, since dietary antigens are found in the home environment [48]. It is, however, difficult to assess whether this would be the most likely route of sensitization in breast-fed infants because they are simultaneously exposed to cow's milk protein through the mother's milk. Further, evidence indicates that IgE antibodies recognize ubiquitous antigens and structurally related human proteins [49,50], suggesting a possible cross-reactivity between human milk and dietary antigens, and thereby another possible means of sensitization.

Evidence in favour of sensitization *in utero* is accumulating. Feiterna-Sperling et al. [51] report a case of manifestation of CMA directly after birth, confirming an earlier finding [52]. Enhanced cord blood T cell reactivity and specific IgE antibodies to cow's milk proteins have been attributed to high-risk babies [53,54]. A more recent study provides further evidence that *in utero* exposure can result in priming to common allergens as early as 22 weeks of gestation [55]. Fetal allergen exposure has been recently

suggested to occur via the transamniotic route or transplacentally, but has not so far been conclusively reported [56].

Induction of oral tolerance and development of CMA

The systemic immune system of an infant is relatively naive at birth. IgG antibodies to cow's milk are already present at birth [57] and their subsequent increase depends on the type of feeding [58], humoral response being the strongest with neonatal exposure [57]. Production of specific IgA and IgM antibodies increases more slowly, depending on the level of exposure [59], and occurring even during exclusive breastfeeding [57]. The development of specific IgE antibodies to cow's milk is primed by early exposure, small doses and infrequent ingestion [60,61], being demonstrated in even exclusively breast-fed infants [46]. Transient low-level IgE responses to food antigens may be seen in young infants without clinical disease [62], but their levels were significantly higher in the allergic infants [62,63]. However, the development of cellular immunity to cow's milk antigens in humans is less well understood. Induction of systemic cellular responses to cow's milk proteins in infancy is followed by subsequent decline in T cell response [58]. An enhanced proliferative response of peripheral blood mononuclear cells to cow's milk proteins has been detected in patients with CMA [64,65], although contrary findings also exist [66].

Although a frequent result of the stimulation of the immune system on mucosal surfaces by food antigens is the induction of mucosal and systemic immunity, an alternative outcome is a state of unresponsiveness or tolerance. This phenomenon, called 'oral tolerance', is a state of systemic immunological unresponsiveness induced by prior oral administration of dietary antigens [67], as evidenced in experimental animals [60,68,69] and in humans [70,71]. In experimental animals, induction of tolerance is greatly affected by dose, frequency and type of antigens [72]. Evidence from human studies further shows that induction of oral tolerance is an age-dependent phenomenon [58].

CMA is a condition involving a disturbance or breakdown in the development of oral tolerance. The development of food allergy involves both a genetic predisposition and an exposure to environmental antigens [73]. At birth, the infant immune system is relatively naive and immature, as evidenced by the immaturity of the gastrointestinal barrier and immunology of the infant [73]. Immaturity of the gut involves defective antigen processing and digestion, and increased permeability of the gut mucosa [74]. Furthermore, the capacity to generate soluble mediators such as IL-4 and IFN- γ is low, at least during the first weeks of life, reflecting the functional immaturity of the immune system [75,76].

Several immune defects in children with CMA have been

described. The total number of antibody-secreting B cells, especially in the IgM class, increases during positive oral cow's milk challenge, whereas the antigen-specific response is defective [77,78]. Furthermore, the total percentage of B cells and of activated B cells is significantly higher in infants with very early symptoms of CMA than in healthy controls, suggesting a defect in the regulation of B-cell function [79]. IFN- γ production of peripheral blood mononuclear cells (PBMC)s is significantly lower in patients with CMA than in control children [80]. Further, Hill et al. [81] demonstrated that CMA patients with immediate reactions generated less IFN- γ than did patients with late reactions or healthy controls. In addition, T cell-mediated suppression has also been shown to be defective in patients with active CMA [82]. However, as these defects are not confined to patients with food allergy, they are therefore not specific for CMA. The precise immune mechanisms responsible for CMA are still unknown.

Effect of breastfeeding on the development of food allergies

In several prospective studies, prolonged and exclusive breastfeeding has been shown to prevent or delay the development of atopy and/or atopic eczema in early life [4,83–91], whereas others have not confirmed this [5,44,45,92–97]. Some studies have even reported an increased risk for development of atopic eczema and food allergies in breast-fed infants [98–101]. In a recent population-based study, the cumulative incidence of CMA was similar in infants fed cow's milk at the maternity hospital and in those exclusively breast-fed [5]. In fact, in the same study long-term breastfeeding and exclusive breastfeeding for 8 weeks of life have proven to be risk factors for IgE-positivity [5]. The problem with the majority of these studies showing controversial findings is that of the confounding factors and that the infants cannot, for ethical reasons, be randomly assigned to breastfeeding or formula-feeding [99]. A study that compares the prevalence of eczema and hay fever in two cohorts from different decades reports an increase over time [102]. With a simultaneous increase in duration of breastfeeding in the western world, this finding argues against any long-lasting protective effect of breastfeeding against allergic diseases.

The principal advantage of breastfeeding in the reduction of sensitization and of symptoms of allergy in already sensitized babies has been assumed to be the relative scarcity of food antigens in human milk. At the same time, in animal studies, small antigen amounts administered parenterally have been reported preferentially to induce IgE antibody responses [61], offering a reason to consider the low antigen content in breast milk a 'mixed blessing', since

it could result in low dose sensitization in breast-fed babies [103,104]. Other protective mechanisms include IgA antibodies against foods in human milk that may reduce the entry of such antigens through mucosal surfaces and lower the risk for sensitization [105]. Further, cell-mediated immunity is transferred from mother to infant through human milk [106], which might offer support to the infant, since allergy appears to be associated with defective suppressor function [79,82]. Antimicrobial activity of human milk may lower the incidence of infections in breast-fed babies [107], which may reduce the risk for sensitization [108]. Finally, certain hormones and growth factors in human milk may support the anatomic integrity of the mucosal barrier and IgA synthesis in the infant.

Role of immune factors in human milk

Colostrum and human milk contain numerous potentially immunologically active components such as leucocytes, cytokines and immunoglobulins that might enhance or induce the still-developing neonatal immune system [109–116]. A breast-fed infant ingests an average of 10^8 leucocytes per day with breastfeeding, which often continues for several weeks. Although concentrations of different cytokines are low in milk, it is known that cytokines exert their biological activity in the concentration range of 1 pg/mL to 10 ng/mL. Maternal cells may reside biologically intact in the gut of the breast-fed infant due to their ability to resist trypsinization [117] and to tolerate large variations in environmental pH, temperature and osmolality [118–120]. This is further due to the relatively neutral pH of the stomach of a newborn and the buffering capacity of milk [120]. In animal studies, milk leucocytes have been found in the circulation and tissues of the newborn [121–123]. Also some of the cytokines [124,125] and secretory IgA (sIgA) [126] in human milk are relatively resistant to acids and proteolysis. Further, low concentrations of proteases in the infant's stomach [127], and high levels of antiproteases in human milk [128], support the survival of these proteins intact and biologically active through the infant's gastrointestinal tract.

sIgA

Several studies have suggested that infants with CMA were receiving mother's milk with smaller quantities of total IgA antibodies [24,129] or of specific IgA antibodies to cow's milk [39] than those infants who remained healthy. Since sIgA is considered the most important protective factor at the mucosal level, its lack in milk may lead to an increased exposure of the intestinal mucosa of the breast-fed infant to potential allergens and thus enhance the risk for development of food allergies.

Leucocytes

Vassella *et al.* [130] showed that the milk of atopic mothers contains a significantly higher number of eosinophils than that of non-atopic mothers. When milk leucocyte differential counts were studied in our previous study, the predominant leucocyte in mothers of healthy infants was the macrophage, whereas in the mothers of CMA infants, the proportion of macrophages was significantly smaller, and large proportions of eosinophils and neutrophils were found more often [12]. These abnormalities in mother's milk were related to an imbalance in the CD4⁺ and CD8⁺ T cell ratio and with disturbed B-cell function in an infant. Although the causes of these phenomena are largely unknown, the chemoattractant factors, such as IL-8 or RANTES, found in higher levels in allergic mothers than in non-allergic mothers [131], may be responsible for eosinophil traffic from the maternal circulation to the breast milk. Further, HLA-DR expression on milk macrophages was less frequent in those mothers of infants with CMA [132].

Altogether, these results imply that sufficient numbers of functional milk macrophages may play a critical role in the development of oral tolerance to food antigens in the offspring, partly through the functions of the cytokines they secrete. At the same time, the presence of high numbers of eosinophils and neutrophils in mother's milk may be harmful due to their release of highly cytotoxic mediators, including eosinophil cationic protein in the infant's gut. This may lead to an immunoinflammatory process similar to that seen in asthma and to increased gut permeability, and may thus contribute to development of CMA.

Cytokines

The levels of IL-4 and IL-13, characteristic Th2-type cytokines, have been reported to show a tendency to be higher in the milk of allergic mothers than in that of non-allergic mothers [15], in contrast to the earlier finding by Rudloff *et al.* [133]. The reports on the levels of IL-8 [131] and IL-10 [133] showed similar results. Most recently, two papers have reported an association between low levels of TGF- β in mother's milk and development of IgE-mediated CMA or atopic eczema in breast-fed infants [134,135]. The levels of TGF- β were shown to correlate positively with the total number of IgA-producing cells in the peripheral blood of the infants [134] and with specific IgA antibodies to BLG and specific IgG antibodies to α -casein and whole formula, but negatively with the diameter of a SPT response to cow's milk and with lymphocyte indices to α -casein and BLG [135]. Based on their findings, the authors suggest that in an infant prone to CMA, the

TGF- β 1 content of the mother's colostrum may promote IgG-IgA antibody production and inhibit IgE- and cell-mediated reactions to cow's milk [135]. Further, the levels of TNF- α were lower in the milk of mothers having babies with CMA than in those with healthy infants [136]. As TNF- α is a cytokine involved in the maturation process of dendritic cells necessary for presentation of processed antigen to T cells, the results suggest that any lack of it in human milk may down-regulate the differentiation of dendritic cells in the breast-fed, thereby playing a role in the development of CMA.

Fatty acids

Milk from atopic mothers has aberrations in its fatty acid content. Atopic mothers' milk showed lower levels of n-6 long-chain polyunsaturated fatty acids at 1 month of lactation than non-atopic mothers [137]. Further, Duchén *et al.* [16] reported that low levels of α -linolenic acid and total n-3 long-chain polyunsaturated fatty acids in maternal milk appeared to relate to atopic sensitization of the infants.

Treatment

The treatment of CMA involves strict elimination of cow's milk protein from the infant's and the breastfeeding mother's diet [11]. Highly allergenic foods such as fish, citrus fruits, tomatoes, peanuts, chocolate and strawberries have also been advised to be avoided until the age of 12 months [138]. A protein hydrolysate or amino acid-based formula should be introduced if an additional source of milk is needed, and calcium supplementation should be given to the breastfeeding mother. Sole cow's milk-free diet is frequently inadequate, because sensitization to multiple foods, such as egg, wheat, soy and fruits during breastfeeding appears to be common [9,11,12,17,19,139,140]. Elimination of such a wide variety of foods is laborious and often impossible in mothers' diets, and may even risk the nutritional adequacy of the mother. Despite a wide elimination diet of the mother, breastfeeding in some cases may maintain allergic symptoms in sensitized infants to the extent that it may interfere with intestinal permeability [9] and normal growth [10]. Although the exact mechanism behind this is not quite known, the allergic symptoms in breast-fed infants may be due to maternal dietary antigens, which maintain the symptoms, or due to cellular and leucocyte-derived mediators in human milk, which may activate the immune system of the infant and thereby provoke allergic symptoms [11,12,24,132,136].

Conclusions

Exclusive breastfeeding does not in general seem to protect against allergic sensitization. Leucocyte, cytokine, sIgA and fatty acid composition in human milk varies between atopic and non-atopic mothers, and between mothers of infants with CMA and those with a healthy infant, irrespective of maternal atopic or non-atopic predisposition. These findings support the concept that the effect of breastfeeding on the development of allergy may depend on the composition of mothers' milk, and that variations in the levels of immunologically active constituents of human milk may partly explain the contradictory results on the protective effect of breastfeeding regarding development of allergy. Among sensitized breast-fed infants that show severe sustained symptoms of food allergy and/or impaired growth, breastfeeding should be discontinued and feeding with hydrolysate or amino acid-based formula commenced to ease the immunoinflammatory process, to ensure adequate growth and to prevent more extensive sensitization.

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