Send Orders for Reprints to reprints@benthamscience.net

Endocrine, Metabolic & Immune Disorders - Drug Targets, **2014**, *14*, **9-15**

Breast-Milk Characteristics Protecting Against Allergy

Federica Minniti[1], Pasquale Comberiati[1], Daniel Munblit[2], Giorgio L. Piacentini[1], Elisa Antoniazzi[1], Laura Zanoni[1], Attilio L. Boner[1] and Diego G. Peroni[1,*]

[1]*Department of Pediatrics, University of Verona, G.B. Rossi Polyclinic, P. le L.A. Scuro 10, 37134 Verona, Italy;* [2]*Department of Pediatrics, Imperial College London, Norfolk Place, St Mary's Campus, London W2 1PG, UK*

Abstract: Breast milk and colostrum are the first feeding sources for a child, providing nutrients, growth factors and immunological components, which are crucial for the newborn's correct development and health. Length of exclusive breastfeeding and time of solid foods introduction is a key factor that may influence allergy development. There is an emerging evidence of a relationship between breastfeeding, milk composition and lower risk of chronic diseases, such as diabetes, obesity, hypertension and allergies. This review examines current evidence regarding humoral and cellular characteristics of breast-milk, and potential role of environment, maternal diet and breastfeeding on the allergy development in children.

Keywords: Allergy, atopy, breast-milk, breastfeeding, cytokines, environment, immunoglobulin, nutrients.

INTRODUCTION

The role of breastfeeding in the prevention of allergies has been studied since 1930, but it is still controversial [1-5]. However, importance of breastfeeding in regulating newborn's immune system and gut mucosal barrier is widely accepted [6]. Newborn's gut is immature and needs the right amount of breast milk in order to develop an optimal mucosal barrier function [7, 8].

Atopic diseases are spread worldwide and their prevalence is rising [2]. It has been demonstrated that some factors in human milk are protective against atopy, while others increase the risk of allergic susceptibility [6]. There is still a lot of debate regarding optimal duration of exclusive breastfeeding and its association with the risk of atopy, as well as relationship between early infant feeding and subsequent allergy development [9]. WHO (World Health Organization) and UK Department of Health recommend exclusive breastfeeding for 6 months, whereas most European governments and allergy organizations recommend its exclusivity for 4 months [10, 11]. Maternal allergy history may be an important risk factor for asthma, allergic rhinitis and eczema development [12]. However, there is little information about the influence of the allergic status of the mother *per se* on the composition of breast milk [13, 14]. Therefore, it seems crucial to assess the different composition of milk between allergic and non-allergic mothers in order to give appropriate recommendations on the duration of breast feeding as well as to answer mothers' questions [15].

This paper reviews current data on humoral and cellular characteristics of breast milk and examines existing evidence on the potential role of environmental factors, maternal diet and breastfeeding in the pathogenesis of allergies.

HUMORAL AND CELLULAR COMPONENTS IN BREAST MILK

There is a strong evidence that breast milk and colostrum provide multiple factors, humoral and cellular immunity components required for newborn's normal growth and development [15, 16]. They also provide an appropriate microenvironment for the morphological, microbiological and immunological maturation of the gut in the first few days of life, which may play an important role in the prevention of allergies [16-18]. Breastfeeding promotes active and passive stimulation of the immune system by its biologically active molecules, with a balance between stimulatory and suppressive signals [4]. Newborn gut immunity is immature and requires the right amount of breast milk to develop an optimal mucosal barrier function [7, 8]. Sections of a fetal gut show immature enterocytes with sparse lymphoid cells, whereas sections of a breastfed child's gut show proliferating enterocytes and abundant lymphoid tissue [6]. Enterocytes incubated with colostrum have higher proliferation (measured with incorporation of titrated thymidine) than enterocytes incubated with mature milk, which may be explained by a higher levels of immune active factors than mature milk [7].

Human milk contains antibodies, predominantly secretory immunoglobulin A (s-IgA), glycoconiugates and oligosaccharides, living cells, antioxidants and fatty acids (FA), nutrients, cell surface homologues, glutamine and dietary nucleotides, lactoferrin, hormones, growth factors, anti- and pro-inflammatory cytokines and food proteins which mother have been exposed with [4, 9, 18, 19]. Allergic status of the mother, infections, mastitis, stress, delivery by cesarean section and fish oil and probiotics supplementation during pregnancy may influence human milk composition

*Address correspondence to this author at the Department of Pediatrics, G.B. Rossi Polyclinic, Piazzale L.A. Scuro 10, 37134 Verona, Italy;
Tel: +39 045 8126884; Fax: +39 045 8124790;
E-mail: diego.peroni@univr.it

2212-3873/14 $58.00+.00

[8]. Concentration of cytokines is inversely correlated with neonate maturity: colostrum contains more cytokines than human milk. Immune active molecules are not digested in the stomach and they maintain their biological activity in the digestive tract of a child [18, 19].

s-IgA (SECRETORY IMMUNOGLOBULIN A)

s-IgA are present both in breast milk as well as infant's gut and serum. However, full-term newborns are deficient of s-IgA [6] and therefore breast milk provides infant with s-IgA during the first weeks of life. It takes up to 30 days to reach protective levels of IgA. Since s-IgA directed against antigens secreted into the milk, it provides protection against environmental allergens and pathogens [20], playing an important role in passive protection provided by breastfeeding [21]. Furthermore, s-IgA presence in human milk is able to prevent an excessive uptake of foreign antigens across the mucosa, possibly lowering the risk of allergic sensitization [22]. Several studies suggested that low levels of secretory and serum IgA in early infancy may represent a consistent risk factor to develop allergy early in life [23-28]. Savilhati *et al.,* found a correlation between low total s-IgA and risk of atopy in long breast-fed children with no atopy family history, but failed to reveal a difference in breast milk s-IgA levels of mothers of children who subsequently developed cow's milk allergy and those who did not [9].

In contrast, Duchen *et al.,* found that lower levels of anti-ovalbumins-IgA and lower total levels of s-IgA in colostrum and mature milk of allergic mothers did not influence the development of allergic disease in children up to 18 months of age [1]. Pesonen and co-authors study results are in agreement with the Duchen *et al.,* data, showing that colostrum and breast milk IgA concentrations are not associated with the development of allergic symptoms [29], while higher serum IgA concentrations at the age of two months are related with respiratory allergic symptoms in the follow-up at 5 years.

BREAST MILK CYTOKINES

Numerous immune active molecules have been found in breast milk. Most of the studies carried out up to date were focused mainly on transforming growth factor-beta (TGF-β), soluble CD14 (sCD14), interleukin-10 (IL-10), interleukin-4 (IL-4) and interleukin-5 (IL-5).

TGF-β is an anti-inflammatory cytokine produced by several cell types, including T-regulatory cells. It has a suppressive role, and it plays a role in the tolerance development [16]. TGF-β1 is also responsible for the production of specific IgA, inhibition of T cell activation and induction of oral tolerance [12]. Human breast milk contains TGF-β1, β2 and β3 [30]. Data on TGF-β is far from agreement: various studies report different levels of TGF- β2 in colostrum and/or mature milk between allergic and non-allergic mothers, whereas others found no difference in levels of TGF-β1 [31]. Conflicting findings are compatible with a concept that TGF-β1 and TGF-β2 concentrations in breast milk may interfere with the development of the mucosal immune system of a breastfed child [14]. Current literature data suggests that TGF-β1 concentration is higher in colostrum compared to mature milk, with a variability between women [15, 32]. A study conducted in 2005 by Rigotti *et al.,* found that concentration of TGF-β1 was lower in milk of allergic mothers already in the first days of life. Moreover, concentration of TGF-β varied between colostrum and mature milk one month after birth, with a significantly lower concentration in mature milk of allergic mothers [14]. Few studies found a positive association between low levels of TGF-β1 in human milk and infant wheeze [20, 30]. According to Marek *et al.,* TGF-β levels are detected less often in colostrum of allergic mothers [18]. Burch *et al.,* demonstrated no difference between TGF-β1 levels in breast milk of allergic and non-allergic mothers. However, controversy between this study and study conducted by Rigotti and co-authors may be explained by a difference in the timing of milk collection (1 month after delivery vs. 2 weeks) [14]. Prokesova *et al.,* assumed that child immunity produce more TGF-β exceeding levels of this growth factor in breast milk, and though this cytokine is not essential for the inducing tolerance induction [15]. Savilahti *et al.,* found that lower concentrations of TGF-β1 were associated with IgE mediated cow's milk allergy. However, no difference in levels of TGF-β2 between allergic and non-allergic mothers was found [9]. Animal studies have demonstrated that presence of TGF- β in breast milk is able to prevent intestinal mucosal inflammation and allergy in allergic-prone rats [33].

sCD14 is a bacterial pattern recognition receptor for cell wall components such as lipopolysaccharide (LPS) in gram-negative bacteria [5, 13]. It is a soluble component of Toll like receptor 4 and participates in a signal transduction initiation, resulting in high secretion of IL-12 [34]. sCD14 plays an important role in innate immunity. Both membrane-bound CD14 and sCD14 are competing for LPS (lipopolysaccharide) and are able to bind its CD14 levels in colostrum are higher than in breast milk, decreasing over time: the newborn has low serum levels of sCD14, which has to be provided by breast milk [6]. sCD14 promotes Th-1 development thus playing an important role in prevention of allergies. It also interacts with T and B cells, increasing the production of IgG1 and reducing IgE production; it monitors the pathogen load of the gut, and it activates the immune response to pathogens and commensals. "Hygiene hypothesis" may partially explain the role of sCD14 in atopy prevention: early exposure to LPS may shift T-helper lymphocytes from Th2 to Th1 response. Low levels of this factor in breast milk are associated with atopy and eczema in children aged 6 months [35, 36]. Increased levels of sCD14 were found to be associated with a lower incidence of doctor-diagnosed asthma at the age of two years, especially in children of non-allergic mothers [5]. Snjiders *et al.,* found that breast milk collected one month postpartum from allergic mothers and from sensitized mothers had higher sCD14 levels [33, 34]. Rothenbacher *et al.,* found a synergistic action between higher levels of sCD14 in breast milk and reduced risk for asthma in early childhood, this effect was particularly evident in children of non-allergic mothers [34]. Savilahti *et al.,* followed a large group of full terms infants to four years of age and found an association between low levels of sCD14 in their mothers' colostrum and atopy development.

Children with allergic symptoms and IgE-sensitization at 4 years of age had received colostrum with a lower concentration of sCD14 than those without these symptoms [9]. IL-12 mediates same functions as sCD14. Low levels of IL-12 are associated with an atopic status [36]. Belderbos *et al.,* found that concentrations of monocytes and TLR3-mediated IL-12p70 were most sensitive to environmental modulation. Authors also found that breastfeeding is a major determinant of neonatal innate immunity, with the most significant association being that of TLR7 mediated IL-10 production (fourfold decrease). This could be explained by the direct influence of immune modulatory compounds on immuno-globulins, nucleotides, oligosaccharides and antimicrobial proteins/peptides. Another possible explanation is that breastfed neonates have a lower incidence of respiratory viral infections (RSV and rhinovirus trigger TLR7). Decrease in IL-10 production, mentioned above, may reflect a more rapid transition to Th-1-polarized innate immune system, which maybe one of the breastfeeding protective mechanisms by which against allergy. Newborn babies who were exclusively breast-fed during the first months of life have different innate immune responses in comparison to those who were not [37].

IL-10, also known as "cytokine synthesis inhibiting factor", is an anti-inflammatory and anti-allergic cytokine which can inhibit an expression of many inflammatory genes [38, 39]. A defect in synthesis of IL-10 leads to an exaggerated and prolonged inflammatory response in airways of asthmatic subjects. No difference in concentration of IL-10 between allergic and non-allergic mothers was found [14].

IL-4, IL-5 and ***IL-13*** are cytokines involved in Th-2 response: IL-4 induces switching isotype of B-cells to IgE and the development of Th2 lymphocytes by autocrine stimulation; IL-5 promotes eosinophilic proliferation and differentiation [40], whereas IL-13 increases mucus production and leads B cells to switch isotype to IgE production, but it do not activate T cells [39]. Interferon-gamma [***IFN-γ***], a type-2 interferon, promotes macrophage-mediated inflammatory reactions and inhibits eosinophil-mediated reactions [39]. Prokesova *et al.,* assessed cytokine levels in colostrum and milk up to 1 year of age. Authors found no difference in the levels of IL-5, IL-10 incolostrum of allergic and non-allergic mothers. Levels of IL-4 in colostrum of allergic mothers were higher than non-allergic. In allergic mothers breast milk IL-4 and IL-10 were found in high concentrations at 3 months postpartum with a slight decrease later, while IL-5 levels did not change during the first year of life. Authors also reported an inverse correlation between IFN-γ and allergy development [15]. Hrdy *et al.,* found that colostral cells of allergic mothers had an increased gene expression of Th-2 cytokines IL-4 and IL-13, and a decreased gene expression of IFN-γ [32]. Soto-Ramirez *et al.,* found high levels of IL-13 in milk whey of allergic mothers [41].

BREAST MILK CELLULAR COMPONENTS

Both colostrum and mature milk contain cellular and soluble components which may provide specific and non-specific protection to the newborn. Breast milk is able to orchestrate complex relation between mucosal epithelium, enteric nervous system and mucosal immune system. By means of this action, it is able to confer immediate and long-term protection against inflammatory or autoimmune diseases. Various lymphocytes subsets are responsible for this protection. Colostrum and breastmilk have a different composition, colostrum is rich of macrophages (typically 40–50%) and polymorphonucleates (40–50%), with less of 10% of lymphocytes of total colostral cells [42, 43]. Neutrophils are responsible for mother's protection, whereas monocytes regulate T and B lymphocyte functions in infants and contain IgA. Colostrum contains both T-helper/T-inducer lymphocytes CD3+/CD4+ and T-cytotoxic/suppressor lymphocytes CD3+/CD8+, with a T-cell CD4+/CD8+ ratio similar to peripheral blood. T lymphocytes are able to mediate newborn's immune response. T-CD3+ lymphocytes act as an effector cells in cell-mediated immunity and cooperate with B cells in antibody production. Peroni *et al.,* compared colostrum composition and studied mothers' peripheral blood for different subtypes of lymphocytes. They found that colostrum contain higher levels of B1cells CD19+ CD5+, effector T cells CD45RA+/CD27- and T effector memory cells CD45RA-/CD27-. Proportion of NK-T cells CD3+ and CD56+ and/or CD16+ was higher in colostrum than in peripheral blood. Memory B cells CD19+ CD27+ tended to increase in colostrum but failed to achieve statistical significance. In contrast, total B CD19+ lymphocytes, total conventional B cells CD19+CD5- and transitional B cells CD19+ CD23+, T naive lymphocytes CD45RA+/CD27+ and T memory cells CD45RA-/CD27+ are lower in colostrum than in peripheral blood cells [42]. Authors also found a difference in leukocyte formula between colostrum and peripheral blood, showing that breast milk represents an extra-lymphoid compartment where effector lymphocytes migrate and accumulate, in a specific and selective way. Thus colostrum is able to transfer an effective defense against infections to the newborn, as infant immunity is immature and slow in response [42].

ENVIRONMENT, MATERNAL DIET AND COMPOSITION OF BREAST-MILK

There is a degree of evidence that children living in a rural setting have a lower risk of developing allergic diseases [38, 44]. In 2010 Peroni *et al.,* assessed breast milk composition of mothers living in farming and urban environment. Both colostrum and one month breastmilk were analyzed, investigating levels of TGF-β1 and IL-10. TGF-β1 levels were higher both in colostrum and in mature milk of farm mothers. In the group of allergic urban mothers' levels of this growth factor declined from colostrum to mature milk. IL-10 concentrations were higher in mature milk of farm mothers compared to non-farming ones. Authors concluded that exposure to a rural environment is associated with higher levels of TGF-β1 and IL-10 in breast milk when compared to exposure to an urban environment. As these cytokines may have a protective role, higher cytokines concentration in breast milk could explain the lower prevalence of allergies in populations living on farms [38]. Tomičić *et al.,* analyzed colostrum and mature milk composition of Estonian and Swedish mothers. Authors observed a difference in microbial load between the two groups of mothers. Environmental microbes are known to be important stimuli for the immune system. The authors

postulated that immune composition of breast milk was also different between the two groups. They found lower levels of TGF-β and higher levels of s-IgA, IL10 and IFN-γ in Estonian mothers in comparison to Swedish. Despite low levels of TGF-β, low prevalence of atopy has been reported in Estonian mothers from this cohort. Authors succeeded in TGF-β levels reduction in breast milk by Lactobacillus reuteri supplementation [8].

Diet is an important aspect of lifestyle and plays a role in the allergy prevention [45]. It has been a common practice for a decent period of time, to recommend mothers so called "allergenic foods" elimination during pregnancy and lactation as a primary allergy prevention measure [4]. Food antigens such as beta-lactoglobulin, ovalbumin and gliadin are transferred through breast milk to a baby. Immaturity of the intestinal barrier of a new born may determine inflammatory responses or sensitization; it may also lead to tolerance induction [8]. In 1994 Canadian Task Force on the Periodic Health Examination stated that breastfeeding, together with maternal diet restriction during pregnancy and lactation, may reduce allergy incidence in infants with a family history of atopy, but not in the general population. Current data suggests that elimination diet results unsuccessful because of the individual variation on amount of antigens in breast milk (antigens can contribute to tolerance rather than sensitization) and of the poor compliance of mothers to the diet. Suggesting a balanced diet to atopic mothers, with moderate fat (<30% of energy intake) and antioxidants intake, even during pregnancy, seems a more useful approach [8]. Anti-oxidants role in breast milk is still obscure. There is some data that low plasmatic concentration of anti-oxidans, beta-carotene, ascorbate and alfa-tocopherol are associated with wheezing [4]. Breast milk fatty acid composition has been proved to be associated with atopy risk [4, 46]. Omega-3 fatty acids are precursors of less active inflammatory mediators than derivatives of omega-6 FA. Their level in colostrum and breast milk may vary, depending on maternal diet [47]. Dietary n-3 polyunsatured fatty acids (PUFA), are concentrated in high doses in oily-fish and may reduce a risk of atopic disease development, decreasing production of pro-inflammatory mediators [1, 48]. High levels of n-6 PUFA and trans FA, low levels of n-3 PUFA and high intakes of trans FA were suggested as risk factors for atopy [45]. Stoney *et al.*, found that high levels of omega-3 FA failed to provide protection against atopy in children and were associated with the development of atopy [47]. In contrast, Wijga and co-authors data suggests that n-3 long chain PUFA in children of allergic mothers were inversely associated with persistent symptoms. No association has been found between PUFA and sensitization. Children of non-allergic mothers, had no association between breast milk FA composition and allergic symptoms, however alfa-linoleic acid was positively associated with allergic sensitization [45]. Duchen *et al.*, found that low levels of alfa-linoleic acid and n-3 long-chain PUFA in breast milk were related to the development of atopy in children [1]. In another study, Morales *et al.*, found no associations between concentrations of arachidonic acid, docosahexaenoic acid and total n-3 in colostrum and risk of allergic manifestations [11]. Soto-Ramirez *et al.*, data suggest that high levels of total n-6 PUFA in breast milk were associated with higher risk of asthma like symptoms, while high levels of total n-3 PUFA were associated with lower risk of atopy [41]. Furuhjelm *et al.*, investigated the role of n-3 fatty acid supplementation in pregnant women in allergy development prevention. Allergic infants have higher levels of CCL-17 (a Th2 chemokine) and higher levels of CCL17/CXCL11 (Th2/Th1) ratios [49].

ATOPIC DISEASES AND BREAST MILK

Role of breastfeeding in preventing atopic diseases has been controversial for the last decades [1-5]. Existing scientific data suggest that atopic subject has Th-2 polarized response to various antigens. Th-2 lymphocytes produce such cytokines as IL-4, IL-13, IL-15. Atopic subject is predisposed to produce larger amounts of these cytokines which lead to B-cells switch towards IgE production in response to common environmental allergen exposure, thus leading to allergic response which results in clinical allergic manifestations [47]. It is known that intra-uterus environment is rich of Th-2 cytokines protecting fetus from a rejection by the maternal immune system. Mother's dietary allergens crosses placenta during pregnancy and baby gets exposed to allergens in utero which is an earliest sensitization. At birth a shift from Th-2 response to Th-1 response occurs, and influences newborn immune system development. As mentioned above, if shift does not happen and there is immaturity of Th-1 response as well as delay in IFN-delta response, then newborn is more prone to develop atopy [50]. It seems that Th-2 cytokines and chemokines (*e.g*. IL-4, IL-5, IL-13, CCL11) in breast milk, may be responsible for subsequent development of allergic diseases and asthma symptoms [41]. Data from the study conducted in 2011 in Japan suggest that breastmilk of mothers whose children developed atopic dermatitis within 6 months after birth contained more Th2 cytokines and adjuvants, measured by reversed-phase HPLC. Authors found that high concentrations of Coenzyme-A increased Th-2 differentiation in mice. Moreover, oral administration of CoA induced skin roughness, hyperplasia of the epidermis, hypergranulosis in the spinous layer and stratum thickening in mice [51].

Reduced breast-feeding length and early solid food introduction (earlier than four months of age) seem to increase the likelihood of allergy development [52, 53]. A beneficial effect of exclusive breastfeeding against asthma and allergic diseases during the first months of life has been reported [54]. Verhasselt *et al*., showed that airborne antigens can be transferred from the mother to a new born mouse through breastmilk, and so breastfeeding induce tolerance and prevention of allergies [55]. Ochiai *et al.*, found that high levels of IL-6, IL-12p40 and IL-13 in colostrum, and high levels of eotaxin and IFN-alfa2 and low levels of IL-1alfa in mature milk were risk factors for atopic dermatitis [19]. Snijders *et al.*, demonstrated that relationship between breast-feeding and infant eczema in the first two years of life was modified by maternal allergic status. Breast-feeding positive effect on recurrent wheezing may be associated with protection against respiratory infections [5].

Breastfeeding may be responsible for protection against asthma *via* induction of tolerance mechanism. Tolerance is defined as a lack of reactivity to an antigen/allergen that

leads to a permanent immunologic state in which repeated antigen exposures do not result in an allergic reaction. It is usual default response to an allergenic stimulus from the first days of life [53]. The presence of immunomodulatory factors in breastmilk may induce tolerance towards breast-milk-transferred antigen. Verhasselt *et al.*, showed that an airborne antigen can be transferred from lactating mice to their progeny through breastmilk, eventually resulting in tolerance induction and prevention of asthma [33]. Whether breastfeeding protects against the development of asthma and allergies remains a matter of controversy, since some studies do not support this beneficial effect. Kramer *et al.*, assessed effect of prolonged breastfeeding on risk of asthma and allergy development. Despite prolonged duration and exclusivity of breastfeeding, authors found no reduction in the risk of asthma, hay fever or eczema at 6.5 years of age, nor lower levels of sensitization [3].

Hygiene hypothesis cannot solely explain high rates of asthma among the low socio-economic urban groups in US. Developmental origins hypothesis for health as well as disease (DOHaD) and microflora hypothesis have been taken into account. The former, also called programming, claims that predictive adaptive responses of the foetus in utero environmental cues promote a phenotype that is optimally suited for a postnatal environment. A mismatch between this phenotype and environment results in a negative health consequences. Epigenetic mechanisms are also taken into account in this hypothesis. Fetal exposure to maternal smoking during pregnancy, separately from post-natal exposure to second-hand smoke, can increase risk of asthma development. Maternal stress or adherence to a Mediterranean diet can modify the risk of being an allergic subject. Smoking can modify fetal lung development and immune function. Role of the gut microbiota is quite challenging, commensal bacteria play crucial role in programming of a neonatal immune system. Gut microbes may induce regulatory T cells (involved in Th1/Th2 balance) and can enhance systemic innate immunity. Another part of the hypothesis is that gut microbes may produce metabolites capable of epigenetic modifications. Human-milk oligosaccharides confers “beneficial” gut microbiota to infants, increasing colonization by Bifidobacteria and reducing prevalence and abundance of Clostridium difficile compared to formula-fed infants [56]. Azad *et al.*, found decreased fecal microbiota richness and diversity in infants who are exclusively breast-fed compared to those who are partially or not breast-fed at the age of 4 months, because of the action of oligosaccharides. Breastfeeding promotes lower intestinal microbiota diversity, and low diversity is associated with an increased risk of atopic disease, yet breast-feeding is generally considered protective against atopy [57].

IMMUNE-MODULATED DISEASES AND BREAST MILK

Increased duration of breastfeeding has been associated with a reduced risk of coeliac disease. Ivarsson *et al.*, found that the risk of developing coeliac disease was reduced in breastfed children younger than two years of age when dietary gluten was first introduced. Actual breast milk protective mechanisms against the development of coeliac disease remain unclear. Continuing breastfeeding at the time of weaning may limit the amount of gluten that child receives, decreasing the chances of a child to develop gluten hypersensitivity. Moreover, breast milk can protect infant against gastrointestinal infections which are responsible for increased permeability of the intestinal mucosa, allowing the passage of gluten into the *lamina propria*. Human milk IgA antibodies may diminish an immune response to ingested gluten by mechanisms such as agglutination of an antigen to immune complexes on the mucosal surface, so that uptake is prevented [53]. A recent review confirms that breastfeeding seems to offer a protection against coeliac disease development in predisposed infants [58].

Diabetes Autoimmunity Study in the Young (DAISY) found that the safest age to introduce solid foods in children at increased genetic risk for type 1 diabetes is between 4 and 5 months of age. Breastfeeding while introducing new foods may reduce the risk of type 1 diabetes [59].

CONCLUSION

There is still lot of controversy on human milk constituents and its role in protection against allergies despite manystudies conducted. Protective factors include sCD14, TGF-beta, s-IgA, IFN-gamma, IL-10, IL-12 and n-3 PUFAs. IL-4, IL-5, IL-13, n-6 PUFA high concentrations have been shown to correlate with a higher risk of allergy development. However, it is important to promote breastfeeding as it is still a preferred method of nutrition and an established way to reduce the risk of atopic diseases. Correct timing of weaning is crucial factor in oral tolerance promotion and correct development of the child’s gut immunity. Further studies are needed in order to establish the role of a different factors presentin breastmilk, and to give mothers correct advice on breastfeeding practice. Analyzing breast milk composition maybe useful not solely in allergy prevention but many other pediatric conditions. Another potential goal should be an attempt to enrich formula milk with a variety of protective factors.

CONFLICT OF INTEREST

The Authors have no relevant conflicts of interest with the subject matter or materials discussed in this manuscript. The Authors have no financial relationships relevant to this article to disclose. An honorarium, grant or other form of payment was not given to anyone to produce this manuscript. The Authors did not receive any funding for this work from any of the following organizations: National Institutes of Health [NHI]; Wellcome Trust; Howard Hughes Medical Institute [HHMI]; or others

ACKNOWLEDGEMENTS

Dr. Piacentini, Dr. Antoniazzi and Dr. Zanoni conceived and designed the study, collected the data, analyzed the data and wrote the initial draft. Dr. Minniti and Dr. Comberiati designed the study, ensured the accuracy of the data and analysis, wrote the initial draft and critically revised the manuscript for important intellectual content. Dr. Munblit ensured the accuracy of the data and analysis,

edited the English version of the manuscript and critically revised the manuscript for important intellectual content. Dr. Boner and Dr. Peroni conceived and designed the study, collected the data, ensured the accuracy of the data analysis, and critically revised the manuscript for important intellectual content.

LIST OF ABBREVIATIONS

IL-4	=	Interleukin-4
IL-5	=	Interleukin-5
IL-10	=	Interleukin-10
IFN-γ	=	Interferon-gamma
LPS	=	Lipopolysaccharide
PUFA	=	Polyunsatured fatty acids
sCD14	=	Soluble CD14
s-IgA	=	Secretory immunoglobulin A
TGF-β	=	Transforming growth factor-beta

REFERENCES

[1] Duchén, K.; Casas, R.; Fagerås-Böttcher, M.; Yu, G. and Björkstén, B. (2000) Human milk polyunsaturated long-chain fatty acids and secretory immunoglobulin A antibodies and early childhood allergy. *Pediatr. Allergy Immunol.*, **11**, 29-39.

[2] Greer, F.R; Sicherer, S.H. and Burks, A.W. (2008) American Academy of Pediatrics Committee on Nutrition, American Academy of Pediatrics Section on Allergy and Immunology: Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. *Pediatrics*, **121**, 183-191.

[3] Kramer, M.S.; Matush, L.; Vanilovich, I.; Platt, R.; Bogdanovich, N.; Sevkovskaya, Z.; Dzikovich, I.; Shishko, G. and Mazer, B.(2007) Promotion of Breastfeeding Intervention Trial [PROBIT] Study Group: Effect of prolonged and exclusive breast feeding on risk of allergy and asthma: cluster randomised trial. *BMJ*, **335**, 815.

[4] Hoppu, U.; Kalliomäki, M.; Laiho, K. and Isolauri, E. (2001) Breast milk-immunomodulatory signals against allergic diseases. *Allergy*, **56**, 23-26.

[5] Snijders, B.E.; Thijs, C.; Dagnelie, P.C.; Stelma, F.F.; Mommers, M.; Kummeling, I.; Penders, J.; van Ree, R. and van den Brandt, P.A. (2007) Breast-feeding duration and infant atopic manifestations, by maternal allergic status, in the first 2 years of life [KOALA study]. *J. Pediatr.*, **151**, 347-351.

[6] Iyengar, S.R. and Walker, W.A. (2012) Immune factors in breast milk and the development of atopic disease. *J. Pediatr. Gastroenterol. Nutr.*, **55**, 641-647.

[7] Walker, A. (2010) Breast milk as the gold standard for protective nutrients. *J. Pediatr.*, **156**, S3-S7.

[8] Tomicić, S.; Johansson, G.; Voor, T.; Björkstén, B.; Böttcher, M.F. and Jenmalm, M.C. (2010) Breast milk cytokine and IgA composition differ in Estonian and Swedish mothers-relationship to microbial pressure and infant allergy. *Pediatr. Res.*, **68**, 330-334.

[9] Savilahti, E.; Siltanen, M.; Kajosaari, M.; Vaarala, O. and Saarinen, K.M. (2005) IgA antibodies, TGF-beta1 and -beta2, and soluble CD14 in the colostrum and development of atopy by age 4. *Pediatr. Res.*, **58**, 1300-1305.

[10] Flohr, C.; Nagel, G.; Weinmayr, G.; Kleiner, A.; Strachan, D.P. and Williams, H.C. (2011) ISAAC Phase Two Study Group: Lack of evidence for a protective effect of prolonged breastfeeding on childhood eczema: lessons from the International Study of Asthma and Allergies in Childhood [ISAAC] Phase Two. *Br. J. Dermatol.*, **165**, 1280-1289.

[11] Morales, E.; García-Esteban, R.; Guxens, M.; Guerra, S.; Mendez, M; Moltó-Puigmartí, C.; Lopez-Sabater, M.C. and Sunyer, J. (2012) Effects of prolonged breastfeeding and colostrum fatty acids on allergic manifestations and infections in infancy. *Clin. Exp. Allergy*, **42**, 918-928.

[12] Rautava, S.; Kalliomäki, M. and Isolauri, E. (2002) Probiotics during pregnancy and breast-feeding might confer immuno-modulatory protection against atopic disease in the infant. *J. Allergy Clin. Immunol.*, **109**, 119-121.

[13] Chow, L.; Munblit, D.; Dissanayeke, S.; Patel, N.; Boyle, R.J. and Warner, J.O. (2012) Soluble CD14 and hepatocyte growth factor in human colostrum of atopic and non-atopic mothers. *Clin. Exp. Allergy*, **42**, 1815-1862.

[14] Rigotti, E.; Piacentini, G.L.; Ress, M.; Pigozzi, R; Boner, A.L. and Peroni, D.G. (2006) Transforming growth factor-beta and interleukin-10 in breast milk and development of atopic diseases in infants. *Clin. Exp. Allergy*, **36**, 614-618.

[15] Prokesová, L.; Lodinová-Zádníková, R.; Zizka, J.; Kocourková, I.; Novotná, O.; Petrásková, P. and Sterzl, I. (2006) Cytokine levels in healthy and allergic mothers and their children during the first year of life. *Pediatr. Allergy Immunol.*, **17**, 175-183.

[16] Matson, A.P.; Thrall, R.S.; Rafti, E.; Lingenheld, E.G. and Puddington, L. (2010) IgG transmitted from allergic mothers decreases allergic sensitization in breastfed offspring. *Clin. Mol. Allergy*, **8,** 9.

[17] Kull, I.; Böhme, M.; Wahlgren, C.F.; Nordvall, L.; Pershagen, G. and Wickman, M. (2005) Breast-feeding reduces the risk for childhood eczema. *J. Allergy Clin. Immunol.*, **116**, 657-661.

[18] Marek, A.; Zagierski, M.; Liberek, A.; Aleksandrowicz, E.; Korzon, M.; Krzykowski, G.; Kamińska, B. and Szlagatys-Sidorkiewicz, A. (2009) TGF-beta[1], IL-10 and IL-4 in colostrum of allergic and nonallergic mothers. *Acta Biochim.*, **56**, 411-414.

[19] Ochiai, S.; Shimojo, N.; Morita, Y.; Tomiita, M.; Arima, T.; Inoue, Y.; Nakaya, M.; Uehara, N.; Sato, Y.; Mori, C.; Suzuki, Y. and Kohno, Y. (2013) Cytokine biomarker candidates in breast milk associated with the development of atopic dermatitis in 6-month-old infants. *Int. Arch. Allergy Immunol.*, **160**, 401-408.

[20] Soto-Ramírez, N.; Karmaus, W.; Zhang, H.; Liu, J.; Billings, D.; Gangur, V.; Amrol, D.; da Costa, KA.; Davis, S. and Goetzl, L. (2012) Fatty acids in breast milk associated with asthma-like symptoms and atopy in infancy: a longitudinal study. *J. Asthma*, **49**, 926-934.

[21] Dickinson, E.C.; Gorga, J.C.; Garrett, M.; Tuncer, R.; Boyle, P.; Watkins, S.C.; Alber, S.M.; Parizhskaya, M.; Trucco, M.; Rowe, M.I. and Ford, H.R. (1998) Immunoglobulin A supplementation abrogates bacterial translocation and preserves the architecture of the intestinal epithelium. *Surgery*, **124**, 284-290.

[22] Järvinen, K.M.; Laine, S.T.; Järvenpää, A.L. and Suomalainen, H.K. (2000) Does low IgA in human milk predispose the infant to development of cow's milk allergy? *Pediatric Res.*, **48**, 457-462.

[23] van Asperen, P.P.; Gleeson, M.; Kemp, A.S.; Cripps, A.W.; Geraghty, S.B.; Mellis, C.M. and Clancy, R.L. (1985) The relationship between atopy and salivary IgA deficiency in infancy. *Clin. Exp. Immunol.*, **62**, 753-757.

[24] Lúdviksson, B.R.; Eiríksson, T.H.; Árdal, B.; Sigfússon, Á. and Valdimarsson, H. (1992) Correlation between serum immuno-globulin A concentrations and allergic manifestations in infants. *J. Pediatr.*, **121**, 23-27.

[25] Lundell, A.C.; Helssmar, B.; Nordström, I.; Saalman, R.; Karlsson, H.; Lindberg, E.; Aberg, N.; Adlerberth, I.; Wold, A.E. and Rudin, A. (2009) High circulating immunoglobulin A levels in infants are associated with intestinal toxigenic *Staphylococcus Aureus* and a lower frequency of eczema. *Clin. Exp. Allergy.*, **39**, 662-670.

[26] Lúdviksson, B.R.; Arason, G.J.; Thorarensen, O.; Árdal, B. and Valdimarsson, H. (2005) Allergic diseases and asthma in relation to serum immunoglobulins and salivary immunoglobulin A in pre-school children: a follow-up community-based study. *Clin. Exp. Allergy*, **35**, 64-69.

[27] Taylor, B.; Norman, A.P.; Orgel, H.; Stokes, C.R.; Turner, M.W. and Soothill, J.F. (1973) Transient IgA deficiency and pathogenesis of infantile atopy. *Lancet*, **2**, 111-113.

[28] Mellander, L.; Carlsson, B.; Jalil, F.; Soderstrom, T. and Hanson, L.A. (1985) Secretory IgA antibody response against *Escherichia coli* antigens in infants in relation to exposure. *J. Pediatr.*, **107**, 430-433.

[29] Pesonen, M.; Kallio, M.J.; Siimes, M.A.; Savilahti, E. and Ranki, A. (2011) Serum immunoglobulin A concentration in infancy, but not human milk immunoglobulin A, is associated with subsequent

atopic manifestations in children and adolescents: a 20-year prospective follow-up study. *Clin. Exp. Allergy*, **41**, 688-696.

[30] Oddy, W.H.; Pal, S.; Kusel, M.M.; Vine, D.; de Klerk, N.H.; Hartmann, P.; Holt, P.G.; Sly, P.D.; Burton, P.R.; Stanley, F.J. and Landau, L.I. (2006) Atopy, eczema and breast milk fatty acids in a high-risk cohort of children followed from birth to 5 yr. *Pediatr. Allergy Immunol.*, **17**, 4-10.

[31] Oddy, W.H. and Rosales, F. (2010) A systematic review of the importance of milk TGF-beta on immunological outcomes in the infant and young child. *Pediatr. Allergy Immunol.*, **21**, 47-59.

[32] Hrdý, J.; Novotná, O.; Kocourková, I.; Prokešová. L. (2012) Cytokine expression in the colostral cells of healthy and allergic mothers. *Folia Microbiol.*, **57**, 215-219.

[33] Verhasselt, V. (2010) Neonatal tolerance under breastfeeding influence: The presence of allergen and transforming growth factor-b in breast milk protects the progeny from allergic asthma. *J. Pediatr.*, **156**, S16-S20.

[34] Rothenbacher, D.; Weyermann, M.; Beermann, C. and Brenner, H. (2005) Breastfeeding, soluble CD14 concentration in breast milk and risk of atopic dermatitis and asthma in early childhood: birth cohort study. *Clin. Exp. Allergy*, **35**, 1014-1021.

[35] Jones, C.A.; Holloway, J.A.; Popplewell, E.J.; Diaper, N.D.; Holloway, J.W.; Vance, G.H.; Warner, J.A. and Warner, J.O. (2009) Reduced soluble CD14 levels in amniotic fluid and breast milk are associated with the subsequent development of atopy, eczema, or both. *J. Allergy Clin. Immunol.*, **109**, 858-866.

[36] Snijders, B.E.; Damoiseaux, J.G.; Penders, J.; Kummeling, I.; Stelma, F.F.; van Ree, R.; van den Brandt, P.A. and Thijs, C. (2006) Cytokines and soluble CD14 in breast milk in relation with atopic manifestations in mother and infant [KOALA Study]. *Clin. Exp. Allergy*, **36**, 1609-1615.

[37] Belderbos, M.E.; Houben, M.L.; van Bleek, G.M.; Schuijff, L.; van Uden, N.O.P.; Bloemen-Carlier, E.M.; Kimpen, J.L.; Eijkemans, M.J.; Rovers, M. and Bont, L.J. (2012) Breastfeeding modulates neonatal innate immune responses: a prospective birth cohort study. *Pediatr. Allergy Immunol.*, **23**, 65-74.

[38] Peroni, D.G.; Pescollderungg, L.; Piacentini, G.L.; Rigotti, E.; Maselli, M.; Watschinger, K.; Piazza, M.; Pigozzi, R. and Boner, A.L. (2010) Immune regulatory cytokines in the milk of lactating women from farming and urban environments. *Pediatr. Allergy Immunol.*, **21**, 977-982.

[39] Agarwal, S.; Karmaus, W.; Davis, S. and Gangur, V. (2011) Immune markers in breast milk and fetal and maternal body fluids: a systematic review of perinatal concentrations. *J. Hum. Lact.*, **27**, 171-186.

[40] Takatsu, K. and Nakajima, H. (2008) IL-5 and eosinophilia. *Curr. Opin. Immunol.*, **20**, 288-294.

[41] Soto-Ramírez, N.; Karmaus, W.; Yousefi, M; Zhang, H.; Liu, J. and Gangur, V. (2012) Maternal immune markers in serum during gestation and in breast milk and the risk of asthma-like symptoms at ages 6 and 12 months: a longitudinal study. *Allergy Asthma Clin. Immunol.*, **8**, 11.

[42] Peroni, D.G.; Chirumbolo, S.; Veneri, D.; Piacentini, G.L.; Tenero, L; Vella, A.; Ortolani, R.; Raffaelli, R. and Boner, A.L. (2013) Colostrum-derived B and T cells as an extra-lymphoid compartment of effector cell populations in humans. *J. Matern. Fetal Neonatal. Med.*, **26**, 137-142.

[43] Wirt, D.P.; Adkins, L.T.; Palkowetz, K.H.; Schmalstieg, F.C. and Goldman, A.S. (1992) Activated and memory T lymphocytes in human milk. *Cytometry*, **13**, 282-290.

[44] Barnes, M.; Cullinan, P.; Athanasaki, P.; MacNeill, S.; Hole, A.M.; Harris, J.; Kalogeraki, S.; Chatzinikolaou, M.; Drakonakis, N.; Bibaki-Liakou, V.; Newman Taylor, A.J. and Bibakis, I. (2001) Crete: does farming explain urban and rural differences in atopy? *Clin. Exp. Allergy*, **31**, 1822-1828.

[45] Wijga, A.H.; van Houwelingen, A.C.; Kerkhof, M.; Tabak, C.; de Jongste, J.C.; Gerritsen, J.; Boshuizen, H.; Brunekreef, B. and Smit, H.A. (2006) Breast milk fatty acids and allergic disease in preschool children: the Prevention and Incidence of Asthma and Mite Allergy birth cohort study. *J. Allergy Clin. Immunol.*, **117**, 440-447.

[46] Hanson, L.A.; Korotkova, M.; Håversen, L.; Mattsby-Baltzer, I.; Hahn-Zoric, M.; Silfverdal, S.A.; Strandvik, B. and Telemo, E. (2002) Breast-feeding, a complex support system for the offspring. *Pediatr. Int.*, **44**, 347-352.

[47] Stoney, R.M.; Woods, R.K.; Hosking, C.S.; Hill, D.J.; Abramson, M.J. and Thien, F.C. (2004) Maternal breast milk long-chain n-3 fatty acids are associated with increased risk of atopy in breastfed infants. *Clin. Exp. Allergy*, **34**, 194-200.

[48] Lowe, A.J.; Thien, F.C.; Stoney ,R.M.; Bennett, C.M.; Hosking, C.S.; Hill, D.J.; Carlin, J.B.; Abramson, M.J. and Dharmage, S.C. (2008) Associations between fatty acids in colostrum and breast milk and risk of allergic disease. *Clin. Exp. Allergy*, **38**, 1745-1751.

[49] Furuhjelm, C.; Jenmalm, M.C.; Fälth-Magnusson, K. and Duchén, K. (2011) Th1 and Th2 chemokines, vaccine-induced immunity, and allergic disease in infants after maternal ω-3 fatty acid supplementation during pregnancy and lactation. *Pediatr. Res.*, **69**, 259-264.

[50] Joneja, J.M. (2012): Infant food allergy: where are we now? *J. Parenter. Enteral. Nutr.*, **36**, 49S-55S.

[51] Higashi, T.; Shimojo, N.; Suzuki, S.; Nakaya, M.; Takagi, R.; Hashimoto, K.; Nakagome, K.; Nakamura, K.; Kohno, Y. and Matsushita, S. (2011) Coenzyme A contained in mothers' milk is associated with the potential to induce atopic dermatitis. *Int. Immunol.*, **23**, 741-749.

[52] Pali-Schöll, I.; Renz, H. and Jensen-Jarolim, E. (2009) Update on allergies in pregnancy, lactation, and early childhood. *J. Allergy Clin. Immunol.*, **123**, 1012-1021.

[53] Sansotta, N.; Piacentini, G.L.; Mazzei, F.; Minniti, F.; Boner, A.L. and Peroni D.G. (2013) Timing of introduction of solid food and risk of allergic disease development: Understanding the evidence. *Allergol. Immunopathol.*, **41**, 337-345.

[54] Gdalevich, M.; Mimouni, D. and Mimouni, M. (2001) Breast-feeding and the risk of bronchial asthma in childhood: a systematic review with meta-analysis of prospective studies. *J. Pediatr.*, **139**, 261-266.

[55] Verhasselt, V.; Milcent, V.; Cazareth, J.; Kanda, A.; Fleury, S.; Dombrowicz, D.; Glaichenhaus, D. and Julia, V. (2008) Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma. *Nat. Med.*, **14**, 170-175.

[56] Azad, M.B. and Kozyrskyj, A.L. (2012) Perinatal Programming of Asthma: The Role of Gut Microbiota. *Clin. Dev. Immunol.*, **2012**, 932072.

[57] Azad, M.B. (2012) Gut microbiota diversity and atopic disease: Does breast-feeding play a role? *J. Allergy Clin. Immunol.*, **131,** 247-248

[58] Henriksson, C.; Boström, A.M. and Wiklund, I.E. (2013) What effect does breastfeeding have on coeliac disease? A systematic review update. *Evid. Based Med.*, **18**, 98-103.

[59] Frederiksen, B.; Kroehl, M.; Lamb, M.M.; Seifert, J.; Barriga, K.; Eisenbarth, G.S.; Rewers, M. and Norris, J.M. (2013) Infant exposures and development of Type 1 diabetes mellitus: The diabetes autoimmunity study in the Young (DAISY). *JAMA Pediatr.*, **167**, 808-815.

Received: 27 July, 2013 **Accepted: 17 January, 2014**