

REVIEW

Factors affecting breast milk composition and potential consequences for development of the allergic phenotype

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Summary

There is conflicting evidence on the protective role of breastfeeding in relation to allergic sensitization and disease. The factors in breast milk which influence these processes are still unclear and under investigation. We know that colostrum and breast milk contain a variety of molecules which can influence immune responses in the gut-associated lymphoid tissue of a neonate. This review summarizes the evidence that variations in colostrum and breast milk composition can influence allergic outcomes in the infant, and the evidence that maternal and environmental factors can modify milk composition. Taken together, the data presented support the possibility that maternal dietary interventions may be an effective way to promote infant health through modification of breast milk composition.

Introduction

The prevalence of atopic conditions is increasing worldwide. This is particularly evident in urbanized and 'Westernized' areas of the world [1]. The reasons for this rise are poorly understood. It is evident that genetic predisposition for atopic disorders is important [2], but only environmental changes can explain the recent rapid increases. It is possible that substances which promote allergy appeared in the modern environment or that factors that provided protection from allergic disease have been lost. Migration studies suggest that residence in an environment with low allergic disease prevalence during the first few years of life is associated with long-term protection from allergic conditions. This emphasizes the importance of early life exposures for allergic disease development [3, 4].

In this article, we will discuss the role of human milk composition as a potential source for these early life environmental influences. If nutrition is an important influence on allergy development, then breast milk (BM) composition may be vital. BM is the main source of nutrition during a critical period of immune development and contains many immunoactive constituents which are likely to influence infant immune

responsiveness [5]. Treatment of pregnant or lactating mothers with interventions to optimize their BM composition may be a safe and effective modality for allergy prevention, if we are able to identify the key factors involved [6].

In this review, we aim to answer three key questions in relation to the role of BM composition and allergy development:

- What is the evidence that breastfeeding and its duration plays a major role in influencing allergic disease development?
- What are the potential effects of specific immune variations in BM composition, on the immune phenotype of the developing infant?
- Which specific maternal exposures alter BM composition sufficiently to lead to variations in health outcomes in the developing infant?

Early infant milk feeding and its influence on allergy development

BM is the optimal source of nutrition for a newborn and an important factor helping the newborn adapt to the new extra-uterine environment. Human milk provides the developing infant with a range of bioactive

factors influencing immune system maturation, physical and cognitive development and the infant intestinal microbiome [7]. At the time of birth, the infant intestinal immune system is relatively mature compared with systemic immune activity, and therefore able to actively respond to signals from antigens and other immune constituents in BM [8].

We know that the ontogeny of the infant's immune system is influenced by maternal immunity via BM [9], in addition to other routes [10]. Breastfeeding during introduction of gluten into the infant diet may reduce the risk of coeliac disease, suggesting important interactions between BM components, dietary antigens, and the gut-associated lymphoid tissue (GALT) [11]. Non-human milk feeds during infancy are known to increase the risk of infectious diarrhoea particularly in developing countries, in part due to the lack of exposure to pathogen-specific sIgA in human milk [12–14]. As cow's milk-derived formulae also increase the risk of respiratory infections [15], this suggests that human milk has a broader effect on infant immune development than that achieved by sIgA supplementation to the gastrointestinal tract. In the early 20th century, Grulee and Sanford were among the first to show that artificial milk use was associated with a higher incidence of eczema in comparison with exclusive or partial breastfeeding [16]. More recent data suggest that this may in part be due to the absence of prebiotics in the artificial milk used at that time [17].

The World Health Organisation (WHO) recommends exclusive breastfeeding for at least 6 months in all infants [18]. However, this is only achieved for 35% of infants worldwide and 19% in Europe [19]. The effect of the WHO recommendations on risk of allergy development has been investigated in several observational studies as a link between milk feeding and eczema was originally made by Grulee and Sanford [16]. It is important to highlight that in many studies, authors used various definitions of exclusive breastfeeding, and these are not always consistent with the WHO definition. According to WHO, 'Exclusive breastfeeding means that the infant receives only breast milk. No other liquids or solids are given – not even water – with the exception of oral rehydration solution, or drops/syrups of vitamins, minerals or medicines' [20].

Evidence that mode of infant milk feeding changes allergy risk

A systematic review of observational studies has shown a protective effect of exclusive breastfeeding for at least 3 months on the development of asthma, at least for children with a positive family history of asthma or atopy [OR = 0.52, 95% CI 0.35 – 0.79] [21] and a protective effect against atopic dermatitis in children with

a family history of atopy [OR = 0.58, 95% CI 0.41 – 0.92] [22]. A comprehensive but non-systematic literature review by van Odjik *et al.* [23.] supported the conclusion that breastfeeding protects against the development of allergic disease, especially among children with an atopic heredity. They found that exclusive breastfeeding reduced the risk of asthma and any breastfeeding decreased the risk of recurrent wheezing and development of atopic dermatitis. Furthermore, the protective effects increased with the duration of breastfeeding, up to at least 4 months and persisted through the first decade of life. Two high-risk population large cohort studies published subsequent to these systematic reviews, showed breastfeeding protective effects for eczema [OR = 0.64, 95% CI 0.45 – 0.90], for wheeze in general [RR = 0.67, 95% CI 0.48 – 0.96, $P = 0.021$] and for wheeze in the first 3 years of life [OR = 0.80, 95% CI 0.70 – 0.90], but one study, in contrast, found that exclusive breastfeeding increased the risk of eczema [RR = 2.09, 95% CI 1.15 – 3.80, $P = 0.016$], and Elliott *et al.* [24–26] highlighted that the protective effect on wheeze did not persist at ages 7–8 years [OR = 0.98, 95% CI 0.79 – 1.22].

None of these studies were controlled trials, and therefore, they have a significant risk of confounding. For example, mothers may feed infants with a strong family history of atopic disease differently to mothers of infants who have no family history of allergy. The large randomized control trial of a breastfeeding support programme from Kramer *et al.* [27] did not show a protective effect of prolonged and exclusive breastfeeding against asthma and showed an increased risk of inhalant sensitization at age 7, but did find reduced risk of early eczema [OR = 0.54, 95% CI 0.31 – 0.95]. A Cochrane review by Kramer and Kakuma in 2012 did not find any evidence that exclusive breastfeeding for 6 months reduced the risk of any allergic disease, compared with lesser durations of exclusive breastfeeding [14]. However, the number of studies contributing data to several of the meta-analyses was small, and in some analyses, there was high heterogeneity between study findings.

Data on the relationship between breastfeeding and food allergy are not sufficiently robust at the moment to make firm conclusions. The review by Kneepkens and Brand [28] does not provide us with any additional data to support or reject the protective effect of breastfeeding against allergic diseases. However, there is a suggestion that the effect of breastfeeding on food sensitization is influenced by genetic predisposition. Hong *et al.* [29] found that the effect of breastfeeding on food sensitization was modified by single nucleotide polymorphisms (SNPs) in the interleukin (IL)12RB1, TLR9, and TSLP genes in infants both individually and jointly. This suggests that genetic variability must be considered

alongside other factors when analysing BM composition in relation to effects on atopy development. It is also likely that conflicting data in relation to the protective effects of breastfeeding are due to differences in immune-modulatory constituents between populations. Thus, Tomicic et al. [30] studying BM from Estonian and Swedish mothers found differences in immunological constituents, and Amoudruz et al. [31] showed that levels of IL-6, IL-8, and TGF- β 1 were higher in the milk of mothers who migrated to Sweden from developing countries in comparison with local mothers. Variability in nutritional constituents may also be important, and Plagemann et al. [32] found that BM from diabetic mothers can lead to a higher risk of obesity in comparison with donor milk. Asthma and obesity commonly coexist [33, 34].

One of the reasons why breastfeeding may have beneficial effects compared to mixed or exclusive feeding with bovine milk, may be species differences in protein composition. Milk proteins are usually classified into four main groups: caseins, peptones (low molecular weight peptides), whey, and milk fat globule membrane proteins. D'Alessandro and co-authors listed 285 human milk proteins, of which only 106 were 'protein core' based on homology in human and bovine milk [35]. This highlights the significant difference between human and bovine milk in protein composition.

Randomized controlled trials have shown that variations in formula milk constitution may alter allergy risk, and this suggests that the same may be true for variations in breast milk composition. Data from studies performed on large cohorts have not consistently shown a preventative effect of hydrolysed milk formulas on allergy and eczema development [36, 37], but

oligosaccharide-enriched formula was found to have an eczema preventive effect in children with low atopy risk in three studies [17, 38, 39].

The majority of published studies investigating the association between breastfeeding and atopic disease suffer from significant methodological shortcomings – for example, there is a lack of data on the precise duration of breastfeeding and degree of exclusivity [14]. There are problems with accurate measurement and control for potential confounding factors, including family allergic history. Finally, there is a lack of objective or unified classification of some allergic outcomes [40]. Nevertheless, the evidence taken together supports the concept that variations in the timing and nature of infant milk feeds can impact on immune development and risk of allergic disease. Individual studies focused on breastfeeding and allergy development are nicely presented in the paper by Matheson et al. [41]. We summarize recent meta-analysis results in Table 1.

Immune components of BM which may influence infant immunity

Human infants are born with a physiological relative immune deficiency and are dependent on the maternally transplacentally delivered IgG antibody for systemic humoral immune protection. Their T cells have a predominantly naïve phenotype, and the capacity of newborn circulating mononuclear cells to generate pro-inflammatory cytokines is low [42]. This immune deficiency is partly compensated by immunoactive factors in human milk including IgA, antimicrobial peptides, and cytokines, in addition to growth factors and essential nutrients which promote development of the infant

Table 1. Mode of Infant feeding and immunological outcomes

Author and year	Breastfeeding assessment	Outcome	Effect size
Gdalevich 2001 [21, 22]	Breastfeeding for at least 3 months in children with a family history of atopy	Asthma Eczema	OR 0.52; 95% CI 0.35–0.79 OR 0.58; 95% CI 0.41–0.92
Mimouni Bloch 2002 [139]	Breastfeeding Breastfeeding in children with a family history of atopy	Allergic rhinitis Allergic rhinitis	OR 0.74; 95% CI 0.54–1.01 OR 0.87; 95% CI 0.48–1.58
Ip 2007 [140]	Breastfeeding for at least 3 months in children with a family history of atopy	Asthma	OR 0.73; 95%CI 0.59–0.92
Yang 2009 [141]	Exclusive breastfeeding Breastfeeding vs. formula feeding Breastfeeding in atopic heredity populations	Eczema Eczema Eczema	OR 0.89; 95% CI 0.76–1.04 OR 0.70; 95% CI 0.50–0.99 OR 0.78; 95% CI 0.58–1.05
Kramer 2012 [14]	Exclusive breastfeeding for 6–7 months vs. 3–4 months, developed countries	Eczema in first year of life Eczema in first 5–7 years Wheezing in first 1 year of life Asthma at 5–7 years Allergic rhinitis at 5–7 years	RR 0.65; 95% CI 0.27–1.59 RR 0.86; 95% CI 0.47–1.58 RR 0.79; 95%CI 0.49–1.28 RR 1.02; 95%CI 0.72–1.44 RR 0.80 95% CI 0.39–1.65
Dogaru 2014 [142]	Breastfeeding	Asthma	OR 0.78, 95% CI 0.74–0.84

GALT [43, 44]. Cytokines from colostrum and BM are not thought to be destroyed in the stomach; some are protected by being bound to other molecules such as soluble components of their receptors [45]. A variety of protease inhibitors (e.g. inhibitor of cysteine proteinases, serine protease inhibitor (SERPIN)-A1, A3, B1, C1, G1, and serine peptidase inhibitor (SPINT1)) which are present in human milk have been proposed to limit the activity of pancreatic enzymes [46, 47], and in the critical first few weeks of life, the gastric pH is high which considerably reduces peptic digestion.

In the past 10 years, several cytokines and other immune-reactive substances have been identified in human milk and colostrum [2]. The principle BM immune modulators are listed in Table 2. The actual functional and physiological effects of each of these factors regarding their influence on the infant's evolving immune responses have not been clarified [44, 48]. However, we know that certain immune active molecules (e.g. IgA, soluble cluster of differentiation 14 (sCD14), transforming growth factor- β (TGF- β), hepatocyte growth factor (HGF)) are present in very high concentrations in colostrum [8, 49] and levels of some, such as IFN- γ , are higher in colostrum in comparison with maternal serum [50] which suggests they are actively secreted in BM and may therefore be presumed have a role in infant immune defence or development. It is known that TGF- β is able to promote IgA production

[51–53] and is actively involved in induction of oral tolerance [54]. CD14 which exists in two forms: membrane bound and soluble, is expressed mainly by monocytes or macrophages and plays an important role in innate immunity as a component of the complex that recognizes endotoxin, together with Toll-like receptor (TLR) 4 [55, 56]. The levels of CD14 on monocytes and macrophages and sCD14 in serum are very low in the neonate, and the high levels in BM may compensate for this relative deficiency. sCD14 is increased in clinical conditions where local or systemic activation of macrophages or monocytes is involved [57].

Other potentially important BM constituents

Microbiome

Few studies have been focused on the detection of BM bacterial constituents which may influence development of the infant microbiome, and consequently their immune development. According to the recent review by Fernandez *et al.*, [58] BM, the bacterial profile, has predominantly staphylococci, streptococci, lactic acid bacteria, propionibacteria, and closely related Gram-positive bacteria.

Below, we summarize the known or proposed effects of some of these immune modulators on infant immune function.

Table 2. Major Immunomodulatory components detected in colostrum and BM

Cellular components	Humoral components	Cell signalling molecules	Other components
Macrophages [36]	<i>Antibodies</i>	<i>Cytokines</i>	<i>Hormones</i>
Neutrophils [36]	IgA [69, 72, 73, 75–77, 127, 146]	IL-1 [35, 120] IL-2 [35, 79, 124, 147] IL-3 [35] IL-4 [30, 35, 50, 68, 80–82, 124, 125, 147]	Leptin [120] β -endorphin [120]
Dendritic cells [36]	IgG [69, 146]	IL-5 [35, 50, 68, 80] IL-6 [31, 35, 50, 68, 80, 122, 124, 131]	<i>Lipid and carbohydrate components</i>
B2M, HLA-DRB5, C4A, CFB, ANPEP, CTSS, ERAP1 [35]	IgM [69, 76, 146]	IL-7 [35] IL-8 [31, 35, 68, 122, 147] IL-10 [30, 35, 50, 68, 73, 75, 80–83, 122, 124, 125, 131, 137, 147–149]	n-3 and n-6 polyunsaturated fatty acids [48, 52, 83, 113, 124, 129, 155, 156]
MFGM [61, 63]	<i>Antigens</i>	IL-12 [35, 83] IL-13 [30, 35, 50, 68, 80, 122, 131] IL-15 [35, 92] IL-16 [35, 68]	Prostaglandin E2 [125]
Exosomes [67]1	β -lactoglobulin [73]	TNF- α [75, 122, 124, 125, 131, 148, 150] IFN- γ [30, 35, 50, 68, 76, 80, 82, 124, 131, 147]	Oligosaccharides [104, 108, 109]
Bacteria and their components [143–145]	Ovalbumin [73]	<i>Chemokines</i> Eotaxin [68] RANTES [68, 147] MCP-1 [151] MIP-1 α [82] <i>Growth factors</i> TGF- β 1 [30, 31, 35, 50, 68, 72, 75–77, 80, 81, 83, 122, 127, 131, 137, 148, 149, 152, 153] TGF- β 2 [30, 31, 35, 50, 52, 68, 72, 73, 75, 77, 80, 122, 125, 127, 152] TGF- β 3 [35] EGF [122] HGF [49, 154]	<i>Protease inhibitors</i> CST3 [46, 47] SERPIN-A1, A3, B1, C1, G1 [46, 47] SPINT1 [46, 47] <i>Other</i> Coenzyme A [157] β -casomorphin [158] sCD14 [8, 31, 72, 75, 77, 83, 131, 148, 153]

Lipids

A triglyceride core surrounded by a thin membrane (about 10 – 20 nm in cross section) forms fat globules which can be found in milk and is known as the *Milk fat globule membrane* (MFGM). This membrane works as an emulsifier and protects the globules from coalescence and enzymatic degradation. An enzyme xanthine oxidase, present in the MFGM, has antimicrobial properties in the gut [59]. This protein is expressed in gut mucosa cells, and its antimicrobial function is associated with production of reactive oxygen species, superoxide, and hydrogen peroxide in the gut. Xanthine oxidase catalyses the reduction of inorganic nitrite to nitric oxide, and in the presence of oxygen conversion to peroxynitrite, which has bactericidal properties. Sphingomyelin (SM) is one of the components of MFGM lipid fraction. There is some evidence that SM intake plays an important role in neonatal gut maturation during the suckling period in rats and contributes to the process of myelination of the developing rat central nervous system [60], but relevance to humans remains to be established.

Milk phospholipids in the MFGM can have protective properties in the gut [61]. Phosphatidylcholine may protect the gut mucosa from toxic attack and reduce the rates of necrotizing enterocolitis in preterm infants [62, 63]. To our knowledge, there have been no studies on the levels of BM MFGM lipids or proteins in relation to infant immune function and/or outcomes.

Exosomes are small, 30–100 nm membrane vesicles, which are released extracellularly after fusion of multivesicular endosomes with the cell membrane of a wide range of mammalian cells. The mechanism of action of exosomes within the immune system is not fully understood. They can be secreted by various cell types (dendritic cells, mast cells, epithelial cells, B cells, T cells). Those secreted by mast cells can induce dendritic cells maturation [64]. There is also a suggestion that mast cell exosomes can transport functional mRNA to recipient cells [65]. Exosomes from B cells can present Bet v 1 peptides and stimulate Bet v 1-specific T-cell lines to proliferate and to produce the Th2-like cytokines IL-5 and IL-13 [66]. Exosomes have been identified in colostrum and mature BM expressing MHC class II, CD86, and the tetraspanin proteins CD63 and CD81. These milk exosome complexes inhibit anti-CD3-induced cytokine production from PBMC and increase the number of Foxp3⁺CD4⁺CD25⁺ T regulatory cells. These findings suggest that exosomes in human BM could have a significant influence on immune ontogeny and modify risk of atopic and other immune-mediated diseases [67].

Immunoglobulins are found in both colostrum and BM in significant concentrations [68–70]. IgA is the major immunoglobulin class found in human colostrum

and BM (88 – 90% of total immunoglobulin), whereas IgG is the primary immunoglobulin class found in bovine colostrum and milk. The content of IgG in human colostrum is of little consequence as it is actively transported across the placenta to the foetus in the last trimester of pregnancy [71]. Savilahti and co-authors found associations between low levels of IgA antibodies to cow's milk in colostrum and atopy and allergy development [72], and Kuitunen et al. [73] showed that high OVA IgA antibodies in colostrum were associated with atopy development by 2 years of age. Recent study by Orivuori and co-authors suggests that high levels of sIgA may be protective against eczema development up to the age of 2 years, although this protection does not last up to the age of 6 years [74]. This study used an interesting and novel approach to immune modulators quantification in relation to the duration of breastfeeding, which merits a validation in other settings. In contrast, other studies failed to reveal any influence of sIgA on immunological outcomes in children [68, 75–77]. Maternal intestinal antigens shape their GALT during infancy, and that GALT migrates to the mammary gland to produce BM Igs. These BM Igs then act by binding microbial and non-microbial antigens in the infant intestine and preventing them from breaching infant intestinal epithelium and causing inflammation [71]. Therefore, maternal intestinal exposures may be priming both their and their own infants' immune development.

Orivuori et al. [74] postulated that total amount of immune modulators received by the baby directly related to the length of breastfeeding. They calculated the dose of IgA received by multiplying the levels of each constituent with the duration of any breast feeding for each child. This approach seems reasonable and aims to evaluate the total amount of immune mediators consumed by the baby, but it is still not accurate enough as length of breastfeeding is not equivalent to the volume ingested. To get accurate measurement, breast milk flow speed should be assessed, which is hard to address.

Cytokines

A large number of *interleukins* are found in colostrum and BM. Their levels are usually much lower in comparison with growth factors. This makes it doubtful whether any other than IL-10 and growth factors are likely to have any biological activity. IL-2 has been detected in colostrum and BM. Ustundag and co-authors found that IL-2 level in colostrum from mothers of preterm infants was higher compared with the mothers of term babies [78]. However, this effect was diminished in later stages of lactation, and the effect on the infant's immune development was not established.

Bryan and co-authors [79] found detectable levels of IL-2 in BM of 42 of 52 BM samples and a significant correlation between milk aqueous IL-2 and maternal serum IL-2, but there was considerable diversity in the timing when BM was collected (15 – 357 days post-partum).

Studies on IL-4 in colostrum and BM of allergic and non-allergic mothers have produced conflicting results. Bottcher et al. [80] found higher concentrations of IL-4 in colostrum from allergic compared with non-allergic mothers. In contrast, median IL-4 level did not differ significantly between the two groups in a Polish study; nevertheless, IL-4 was more often detected in the colostrum of allergic compared with non-allergic mothers [81]. In both studies, IL-4 was less commonly detected in colostrum and BM, than several other cytokines [80, 81]. Similarly, Rudloff and co-authors detected IL-4 in only 20% of the colostrum samples with no significant difference between allergic and non-allergic mothers [82]. In the milk of allergic mothers, IL-4 levels rise from delivery and peak at 3 months postdelivery [50].

There are few studies analysing IL-5 level in colostrum or BM in general. Prokesova et al. [50] failed to find any difference in IL-5 concentration between mothers with atopic dermatitis and controls. Two studies by Bottcher and co-authors [68, 80] detected IL-5 in less than 10% of the colostrum and 1 month mature milk samples. There was no significant difference between concentrations of IL-5 in colostrum of allergic and non-allergic mothers, but a significant correlation between levels of IL-5 and IL-10 [80]. This is consistent with our understanding of cytokine interactions in that IL-10 suppresses IL-12 production and therefore IFN- γ which will remove one of the regulatory influences on Th2 activity.

A few studies have detected levels of IL-6 in colostrum or BM but with no significant difference in colostrum of allergic and non-allergic mothers [50, 68], although there was a correlation with IgA in colostrum as well as with IL-10 and TGF- β which are involved in IgA synthesis [80].

A very high concentration of IL-10 has been demonstrated in samples of human milk collected during the first 80 hours of lactation [45]. IL-10 is present not only in the aqueous phase of the milk, but also in the lipid layer. Its bioactive properties were confirmed by experiments showing that human milk samples inhibited blood lymphocyte proliferation and that this property was greatly reduced by pre-incubation with anti-IL-10 antibody [2]. Some studies have either detected no IL-10 [83], or only rarely in colostrum (found in 12% of those tested) and mature milk (6%) [80], while others have found IL-10 in most if not all samples [45, 81]. This variation may be due to the timing of colostrum collection. In the Garofallo's study, high concentrations of

IL-10 were detected in samples collected during the first 24 hours and concentrations were lower in milk collected later [45]. The colostrum samples in the study performed by Bottcher et al. [80] were collected 3–4 days post-partum.

A variety of cells are found in human milk during the first few months of lactation [84], and monocytes, dendritic cells and/or macrophages can secrete IL-12. The immune environment in utero is regulated predominantly by Th2-cell predominance [85]. Restoration of a balance between Th2-type and Th1-type responses post-natally may be orchestrated, by being exposed to IL-12 from the milk of a breastfeeding mother [86].

One study identified IL-12 in 62% of BM [86] samples, but there was no association between IL-12 in BM and maternal atopic status or maternal illness/infection. There was no significant difference in the level of IL-12 in colostrum of allergic compared with non-allergic mothers [83].

IL-13 was originally described as a cytokine that inhibits inflammatory cytokine production [87, 88]. Similar to IL-4, it promotes IgE production but also regulates eosinophilic inflammation, mucus secretion, and airway hyper-responsiveness in asthma patients. At the moment, it is unclear what regulates IL-4/IL-13 receptor expression in BM, and this affects the functional activity of IL-13 [89]. We also do not know what role IL-13 plays in colostrum. There are few studies focused on IL-13 detection in colostrum. Bottcher et al. [80] [68] detected IL-13 in only 12 of 48 (25%) colostrum samples, but the concentration correlated with the levels of IL-4, IL-5, and IL-10. Prokesova and co-authors, in contrast, found IL-13 in almost every sample tested [50]. This may be due to ethnic and environmental differences in the population groups (from Sweden and Czech Republic) or differences in methodology.

IL-15 is secreted by various cells but predominantly by mononuclear phagocytes. It has structural similarity with IL-2 and activates natural killer cell proliferation, cytotoxicity, and cytokine production and regulates natural killer cell/macrophage interaction [90]. This cytokine is known to play a key role in anti-HIV responses by stimulating both CD8 T cells and natural killer cells [91]. The single study of IL-15 concentrations in BM showed an association with a decreased risk of HIV transmission, and it was hypothesized that IL-15-mediated immunity may protect against HIV transmission during breastfeeding [92].

IFN- γ is a Th1 cytokine and has extensive and diverse immune-regulatory effects on many cells including an inhibitory effect on Th2-cells. IFN- γ has been found in colostrum and BM [93, 94], but biological activity of this immune modulator remains to be determined. Bottcher and co-authors detected IFN- γ in less than 10% of their samples [80], and Rudloff et al.

[82] failed to detect any in 42 samples. In contrast, Prokesova et al. [50] found IFN- γ in almost every sample which again raises the question why cytokine presence in colostrum varies so much from one study to the other but could relate to differing environmental influences.

There is no clear evidence so far that any of interleukins detected in BM have preventive or causative effect on allergy/atopy development in early childhood.

TGF- β is a family of regulatory cytokines that have pleiotropic functions in various cell lineages involved in many physiological and pathological processes such as embryogenesis, carcinogenesis, and the immune response [95]. At present, three members of the TGF- β family have been found (TGF- β 1, TGF- β 2, and TGF- β 3). TGF- β 1 is the most prevalent form expressed in the immune system [94].

A number of studies have shown that high TGF- β levels in human milk are associated with the prevention of allergic diseases during infancy. A study performed in Finland in 1999 found that the concentration of TGF- β was higher in colostrum than in mature milk and infant's serum and higher in infants with allergic disease onset after the weaning period comparing to babies with an onset of allergy prior to weaning indicating an allergy delaying effect of TGF- β in colostrum [52].

A systematic review summarizes the associations between TGF- β in human milk and immunological outcomes in infants and children [96]. Eight of 12 studies from this review reported an association between higher TGF- β 1 or 2 levels in colostrum or BM and reduced risk of atopic outcomes in the infant. The authors suggest that TGF- β found in human milk is involved in maintaining homeostasis in the intestine, regulating inflammation, promoting oral tolerance and thereby reducing allergy development [96]. A high level of heterogeneity among the published studies makes interpretation difficult with large differences in study populations, design and methodology, TGF- β isoform type assayed, time when milk samples were collected, age at which outcome was measured, and finally, lack of consistency in the immunological outcomes measured and methods used. Ando and co-authors showed that orally administered TGF- β can mediate its activity in the intestinal mucosa and enhance the induction of oral tolerance to a high-dose dietary antigen [97], and Nakao suggests that oral administration of TGF- β simultaneously with an allergen might potentially be a useful approach for the primary prevention of allergic diseases in infants [98].

A soluble component of CD14 (sCD14) is found in very high concentrations in human milk. Increased levels of circulating sCD14 correlate with infection and autoimmunity [99]. Polymorphisms in the promoter

region of the DNA encoding CD14 have been associated with reduced levels of circulating sCD14 and in turn are inversely correlated with total IgE levels [100]. Savilahti et al. [72] studied a large cohort of 4676 children in four groups, with either long or short exclusive breastfeeding (3.5 months or 2 weeks) who were subsequently classified for the presence or absence of atopy. Children with atopic symptoms and IgE sensitization at the age of 4 years received colostrum with a significantly lower concentration of sCD14 than those without symptoms and IgE sensitization. Jones et al. [8] found that BM sCD14 levels at 3 months were lower in mothers of infants who subsequently developed eczema than those without eczema; however, another study failed to confirm these findings [101]. The difference in findings may be partially explained by difference in CD14 genotype as it has been recently shown that breastfeeding was associated with a decreased risk of atopic sensitization in children with CT/CC genotype (OR 0.667, 95% CI 0.463 – 0.960) [102].

Oligosaccharides are the third largest fraction in BM after lactose and lipids. Their exact physiological role remains unknown. Oligosaccharides are closely related to the Lewis blood group and four different milk groups have been described depending on their specific Lewis blood group-dependent oligosaccharide patterns [103]. It is known that colostrum contains more oligosaccharides than mature BM [104]. Oligosaccharides have various properties, including being prebiotic, anti-adhesive, glycome modifying and anti-inflammatory, which can directly or indirectly influence infant immune responses [105, 106]. As an illustration of these effects, Ward et al. [107] showed that oligosaccharides intensified the growth of *Bifidobacterium infantis* to a much higher degree than inulin or glucose. Human milk oligosaccharides also inhibit attachment of intestinal bacteria to the surface of the intestinal epithelial cells, and direct evidence for the relevance of this comes from clinical trial findings that the addition of certain oligosaccharides to cow's milk formula reduces infection risk in infants [108, 109].

Among the oligosaccharides, the monosaccharide N-acetylglucosamine (a component of several oligosaccharides) is important for the growth of *Bifidobacterium bifidum* [110], which has been found in higher proportion in healthy infants faeces samples in comparison with those who were allergic [111].

Polyunsaturated fatty acids (PUFA)

There is some evidence that PUFAs may play a role in immune development [112]. Some studies have shown that lower levels of n-3 PUFA and/or higher concentrations of n-6 PUFA in the serum of atopic infants and in their mother's BM [113–115] were associated with a

Table 3. Relationship between BM immune composition and infant immune development – observational studies

Author and year	Country and numbers	Immune modulators measured	Main outcomes
Saarinen 1999 [76]	Finland 108 infants with cow's milk allergy; 207 healthy controls	IgA, IgM, Cow's milk specific antibodies, IL-6, IFN- γ , TGF- β 1	↓ TGF- β 1 in colostrum – infants with IgE-mediated cow's milk allergy. Other factors did not differ
Kalliomaki 1999 [52]	Finland 27 infants with eczema	TGF- β 1, TGF- β 2	↓ TGF- β 1 and TGF- β 2 in colostrum – infants with preweaning onset eczema compared with postweaning onset
Jones 2002 [8]	UK 8 infants with eczema; 21 without	sCD14	↓ sCD14 levels in infants with eczema
Bottcher 2003 [68]	Sweden 53 infants	IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-16, IFN- γ , TGF- β 1, TGF- β 2, RANTES, cotaxin, SIgA	No difference in mediator levels with respect to atopy, allergic symptoms, or salivary IgA in the first 2 years of life
Oddy 2003 [148]	Australia 243 infants	TGF- β 1, IL-10, TNF- α , sCD14	Prolonged breastfeeding and ↑ TGF- β 1 in breast milk at 2 weeks were associated with reduced infant wheezing
Reichardt 2004 [113]	Germany 218 infants	n-3 and n-6 polyunsaturated fatty acids	No association between fatty acid levels and infant atopic eczema.
Savilahti 2005 [72]	Finland 228 children	total IgA, IgA antibodies to cow's milk, casein, sCD14, and TGF- β 1, TGF- β 2	↑ colostrum linoleic acid (n-6) associated with high milk IgE. ↓ colostrum docosapentaenoic acid (n-3) associated with high total IgE at 1 year ↓ colostrum IgA casein antibodies and ↓ sCD14 concentration were associated with atopy. ↓ colostrum IgA cow's milk antibodies and sCD14 were associated with atopy and allergic symptoms (eczema, allergic rhinitis, conjunctivitis, asthma) by 4 years
Rigotti 2006 [149]	Italy 22 infants	TGF- β 1, IL-10	TGF- β and IL-10 did not influence immunological outcomes at the age of 6 months
Snijders 2006 [83]	Netherlands 307 infants	TGF- β 1, IL-10, IL-12, sCD14	None of the studied immune factors in breast milk at 1 month were associated with infant's atopic outcomes
Bottcher 2008 [75]	Sweden (colostrum and mature milk from women treated with <i>L. reuteri</i> (n = 54) or placebo (n = 55) from 36-week gestation until labour)	Total IgA, SIgA, TGF- β 1, TGF- β 2, IL-10, TNF, sCD14, Na/K ratios	Infants receiving BM with low levels of TGF- β 2 were less likely to become sensitized or develop eczema during their first 2 years
Lowe 2008 [155]	Australia 194 infants	n-3 and n-6 polyunsaturated fatty acids	↑ n-3 fatty acid in colostrum was associated with increased risk of infantile atopic eczema, while total n-3 concentration in BM was associated with increased risk of non-atopic eczema. ↑ total n-6 fatty acid in colostrum was associated with increased risk of childhood rhinitis. There was no evidence of associations between fatty acid profile and risk of asthma.
Tomicic 2010 [30]	Sweden, Estonia 39 Estonian and 60 Swedish infants	SIgA, IL-4, IL-10, IL-13, IFN- γ , TGF- β 1, TGF- β 2	↑ IL-13 in colostrum was associated with allergic sensitization during infancy in Swedish cohort, but not in the Estonian cohort.
Thijis 2011 [156]	Netherlands 315 infants	n-3 polyunsaturated fatty acids	↑ n-3 PUFAs in breast milk at 1 month are associated with atopic dermatitis, parent reported eczema and sensitization at 1 year of age
Kuitunen 2012 [73]	Finland 364 infants	Total IgA, IgA antibodies to cow's milk, casein, β -lactoglobulin, ovalbumin, TGF- β 2, IL-10	↑ OVA IgA antibodies in colostrum were associated with atopy at 2 years. ↓ OVA IgA antibodies in BM were a risk factor for eczema by 2. ↑ TGF- β 2 in BM at 3 months was associated with allergic disease and eczema at 2 years

higher risk of allergic disease. Others found a positive correlation between elevated n-3 long-chain-PUFA levels in colostrum and food or inhalant allergen sensitization and atopic eczema in infants at 6, 12, and/or 24 months of age [116]. The relationship between dietary linoleic acid and arachidonic acid, the intake of n-3 PUFA, and the modifications in enzyme expression and activity appear to have a substantial impact on allergy manifestation. However, more randomized controlled intervention studies confirming gene–environment interaction are required to establish the relationship between the availability of n-3 and n-6 LC-PUFA during breastfeeding and atopy development in children [117].

Methodological consideration for future studies

The vast majority of the studies performed on human colostrum and/or BM composition employed enzyme-linked immunosorbent assay (ELISA). Different research groups employ a range of sampling methods and

processing prior to laboratory tests, and currently, there is no ‘gold standard’ technique. Some publications provide centrifugation details in rpm, but because rotor radius is important the actual force used to centrifuge milk samples may vary considerably between studies. Moreover, as the levels of several immune mediators in breast milk are closely correlated with one another, the use of more sophisticated statistical analysis techniques may be necessary, to allow the characterization of a limited number of ‘lacto-types’ which can then be related to outcomes in the infants [118, 119].

The overall conclusion from all the human milk immune modulator studies is that there is a need to establish a ‘gold standard’ in methodology for phenotyping mothers selected, characterizing the environment, timing of sampling, sample processing, assays, and analysis.

For many of the immune-modulatory components present in BM, there is only limited direct evidence for a role in the development of infant intestinal immunity or allergic disease. However, some BM immune components

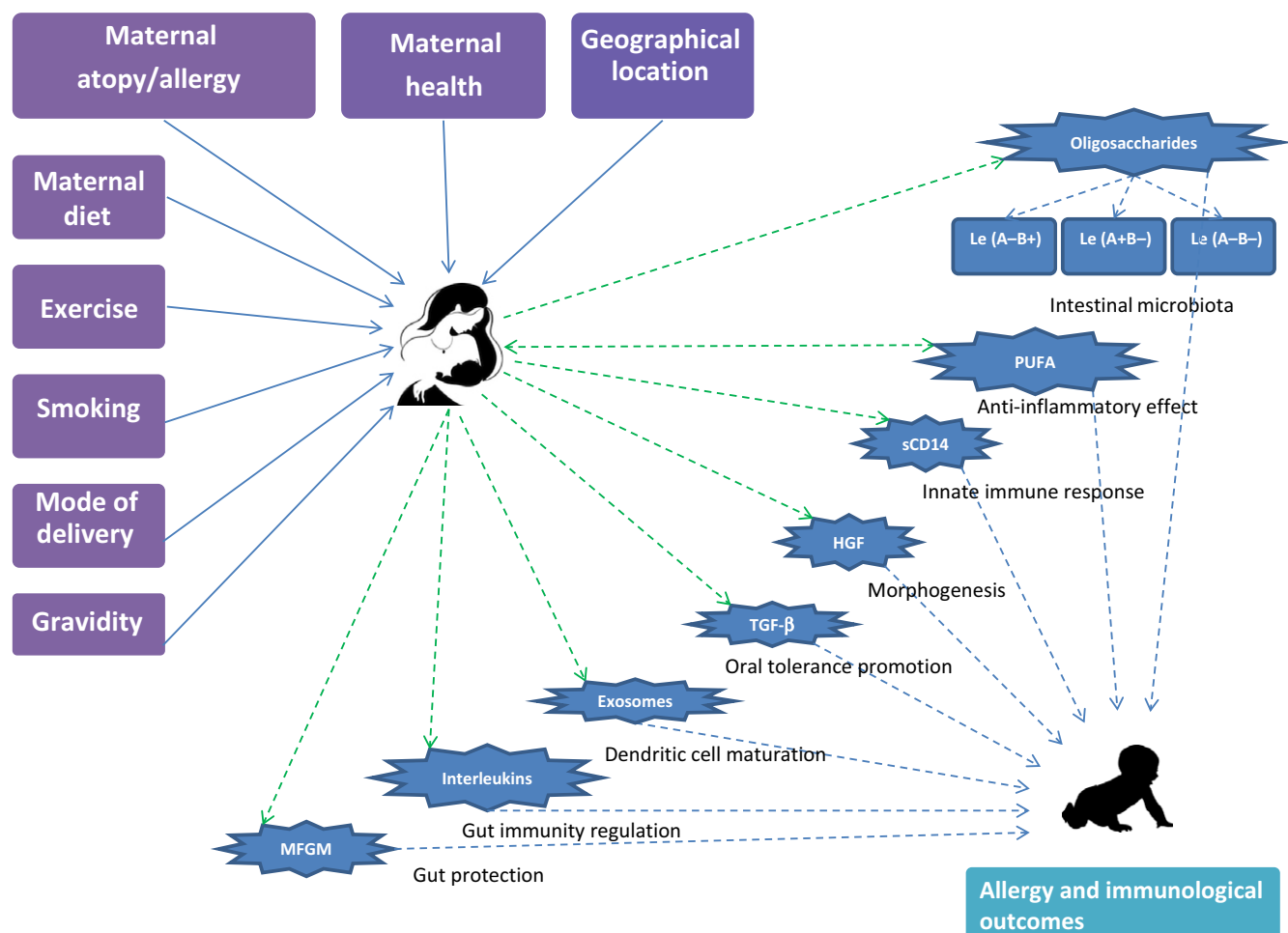


Fig. 1. Maternal and environmental factors influence breast milk composition and subsequently result in Allergic and Immunological outcomes.

are known to be important factors in infant immune development, or important stimuli of immune responses generally. In Table 3, we summarize the evidence that variations in levels of breast milk immune mediators are associated with altered allergic disease risk. The evidence is perhaps strongest for TGF- β , which has known biological effects on intestinal immune responses, is actively secreted into breast milk, and has been shown in several studies of breast milk composition to differ significantly between populations.

Maternal factors and environmental exposures which may influence infant health via altered BM constituents

Several factors with the potential to influence infant immune development may be modifiable. There is evidence that a number of different exposures can influence maternal breast milk and colostrum constituents, and even a limited body of evidence that such interventions can alter infant immune outcomes. Below, and in Fig. 1, we summarize some of the exposures/maternal variables which have been associated with altered breast milk or colostrum composition, with or without direct evidence of effects on infant immune outcomes.

Tobacco smoke

It is well established that tobacco smoke is immunotoxic, but the effect of smoking on the immunological function of the mammary gland of smoking mothers has not been well studied. Prenatal tobacco smoke exposure can reduce colostrum IL-1 α [120], and tobacco smoke also reduces both total lipid content and absolute amounts of the fatty acids linoleic, arachidonic, α -linolenic and docosahexaenoic acid in BM at 1 month [121].

Gestation

The effects of premature delivery on BM composition have been highlighted in one study [122], where IL-6, TGF- β 1, and TGF- β 2 were higher in colostrum following preterm compared with term delivery. The cohort size was small, with 10 prematurely and 22 term-delivered women, so further studies are needed to confirm this finding.

Physical activity

Increasing metabolic-equivalent tasks, and thus caloric expenditures, can increase pro-inflammatory cytokines in the BM [123]. This was shown in a cross-sectional study, using self-reported data and an unvalidated exercise instrument so again these observations require confirmation. If a relationship between maternal physical

activity and BM composition is confirmed, then this is one possible mechanism through which modern lifestyles with reduced physical activity might lead to altered infant immune development.

Maternal diet

Maternal diet during pregnancy and lactation could significantly influence allergy development. Maternal diet can influence breast milk fatty acid and immune mediator composition [124–126]. Urwin et al. [127] showed that salmon consumption during pregnancy leads to a higher proportion of individual and total n-3 PUFA in BM during early lactation and a lower ratio of n-6: n-3 PUFA as well as lower sIgA levels.

Dunstan and co-authors showed that giving fish oil to atopic pregnant women led to lower infant cytokine response to common allergens, and less severe atopic dermatitis by 1 year compared with olive oil-supplemented controls [128]. In contrast, Johansson and co-authors suggest that diet is not so important in that women with a combination of eczema and respiratory allergy had lower BM levels of several PUFAs and lower ratio of long-chain n-3/n-6 PUFAs than healthy women, despite similar fish consumption [129]. Recent data suggest that two FADS haplotypes are strongly associated with the levels of PUFAs. A and D haplotypes including rs174546 may explain differences in the ability to synthesize n-3 and n-6 PUFAs [130].

Probiotic supplementation during pregnancy and lactation also may influence atopy development. Prescott et al. [131] showed that giving *L. rhamnosus* HN001 or *Bifidobacterium lactis* HN019 resulted in a higher levels of TGF- β 1 and IgA in BM compared with placebo and Dotterud showed that maternal supplementation with *Lactobacillus rhamnosus* GG, *L. Acidophilus* La-5, and *Bifidobacterium animalis* subsp. *lactis* Bb-12 from 36-week gestation to 3 months post-natally during breastfeeding reduced infant eczema [132]. Similarly, Rautava found reduced eczema in women supplemented with either the combination of the probiotics *Lactobacillus rhamnosus* LPR and *Bifidobacterium longum* BL999 or the combination of *Lactobacillus paracasei* ST11 and *Bifidobacterium longum* BL999 during the last 2 months of pregnancy and the first 2 months of breastfeeding [133]. A recent dietary intervention study by Hoppu et al. [124] on 125 Finnish mothers suggests that diet (favourable fat intake (SFA, MUFA, PUFA)) on its own and diet in conjunction with probiotic intake can influence cytokine and fatty acid composition in BM. They found higher levels of n-3 fatty acids and α -linolenic acid in BM in both dietary intervention groups and concentrations of IL-2, IL-4, IL-10, and TNF- α was higher in BM of mothers in diet and probiotic group. Kuitunen and co-authors found that probiotic intervention has been associated with

Table 4. Effect of maternal atopy/allergy and maternal environment on BM immune composition

Author and year	Country and numbers	Immune modulators measured	Exposure of interest	Main outcomes
Islam 2006 [146]	Bangladesh (colostrum from 105 mothers)	IgA, IgM, IgG, peripheral immune cells	Age, BMI, parity	Maternal age, BMI, parity, and income level did not correlate with immunoglobulin concentration in colostrum
Hoppu 2012 [124]	Finland (colostrum and/or 1 month BM samples from 125 mothers)	IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ , n-3 polyunsaturated fatty acids	Fatty acid supplementation	↑ TNF- α , IL-10, IL-4, and IL-2 both dietary intervention groups compared with controls; ↑ γ -linolenic acid in the diet/probiotic group in comparison with diet/placebo group
Urwin 2012 [127]	UK (BM from 123 mothers)	sCD14, TGF- β 1, TGF- β 2, sIgA, fatty acid composition	Fatty acid supplementation	Salmon consumption during pregnancy ↓ n-6 PUFA: n-3 PUFA ratio. ↓ sIgA in BM in the salmon group. All immune factors ↓ in BM between days 1 and 28
Amoudruz 2009 [31]	Sweden (32 born in Sweden and 32 migrated)	IL-6, IL-8, TGF- β 1, TGF- β 2, sCD14	Habitat	Mothers from developing countries ↑ IL-6, IL-8, and TGF- β 1 in BM
Holmlund 2010 [153]	Sweden, Mali (30 from Mali, 32 Swedish immigrants, and 33 native Swedish)	TGF- β 1, sCD14	Habitat	Women from Mali and immigrant women from Sweden ↑ TGF- β 1 than BM from native Swedish women. Mali women ↑ sCD14 in BM compared with other two groups
Peroni 2010 [137]	Italy (colostrum and 1 month BM n = 45 farming environment; n = 69 urban environment)	TGF- β 1, IL-10	Habitat	Exposure to farming environment has been associated with higher concentrations of TGF- β 1 and IL-10 in BM compared with mothers from urban environment
Tomicic 2010 [30]	Sweden, Estonia (colostrum and 1 month BM from 39 Estonian and 60 Swedish mothers)	sIgA, IL-4, IL-10, IL-13, IFN- γ , TGF- β 1, TGF- β 2	Habitat	↓ TGF- β 2 and ↑ sIgA, IL-10, and IFN- γ in BM from Estonian mothers compared with Swedish mothers
Striker 2004 [69]	Brazil (colostrum from 82 mothers having vaginal delivery, caesarean section with labour or elective caesarean section)	IgA, IgG, IgM	Labour type	Occurrence of labour together with surgical stress induces higher IgA concentrations in colostrum of women submitted to caesarean section with labour
Rudloff 1999 [82]	Germany (milk from 19 allergic and 23 non-allergic mothers)	IL-4, IL-10, IFN- γ , MIP-1 α	Maternal atopy/allergy	Concentrations of pro-inflammatory markers and cytokines in BM did not differ significantly between allergic and non-allergic mothers
Bottcher 2000 [80]	Sweden (colostrum and 1 month BM from 19 allergic and 20 non-allergic mothers)	IFN- γ , IL-4, IL-5, IL-6, IL-10, IL-13, TGF- β 1, TGF- β 2	Maternal atopy/allergy	↑ IL-4, IL-5 and IL-13 in colostrum from allergic mothers compared with non-allergic
Laiho 2003 [125]	Finland (BM from 43 allergic and 51 non-allergic mothers)	TGF- β 2, TNF- α , IL-4, IL-10, prostaglandin E2, cysteinyl leukotrienes, fatty acid composition	Maternal atopy/allergy	↓ TGF- β 2 in BM of Allergic mothers. Other cytokines concentrations and fatty acid composition have not been different between the groups. Positive association has been observed between TGF- β 2 and proportion of polyunsaturated fatty acids

Table 4 (continued)

Author and year	Country and numbers	Immune modulators measured	Exposure of interest	Main outcomes
Prokesova 2006 [50]	Czech Republic (colostrum and BM from 21 allergic and 21 non-allergic mothers)	IL-4, IL-5, IL-6, IL-10, IL-13, IFN- γ , TGF- β	Maternal atopy/allergy	No significant difference in colostrum of healthy and allergic mothers. $\uparrow$ IL-4 and $\downarrow$ IL-10 observed in BM of allergic mothers
Rigotti 2006 [149]	Italy (BM from 13 allergic and nine non-allergic mothers)	TGF- β 1, IL-10	Maternal atopy/allergy	$\downarrow$ TGF- β 1 in BM in allergic mothers compared to non-allergic. IL-10 level did not differ much between allergic and non-allergic mothers
Snijders 2006 [83]	Netherlands (1 month BM from 182 allergic and 125 non-allergic mothers; 123 sensitized and 164 non-sensitized mothers)	TGF- β 1, IL-10, IL-12, sCD14	Maternal atopy/allergy	$\uparrow$ sCD14 levels in mothers with a positive vs. negative allergic history and in mothers who were sensitized vs. non-sensitized. IL-10 has not been detected
Sidor 2008 [158]	Poland (colostrum and 1 month BM from 12 allergic mothers and 30 non-allergic mothers)	β -casomorphin-5, β -casomorphin-7	Maternal atopy/allergy	Content of β -casomorphin-5 in colostrum of control group was three times higher than allergics
Marek 2009 [81]	Poland (colostrum from 30 allergic mothers and 46 non-allergic)	IL-4, IL-10, TGF- β 1	Maternal atopy/allergy	$\uparrow$ TGF- β 1 in allergy group. IL-10 was present in colostrum of all women, and median IL-10 concentration did not differ between the allergy and control groups. IL-4 level did not differ between the two groups, but was detectable more often in colostrum of allergic than non-allergic mothers
Rautava 2002 [152]	Finland (BM from 30 women taking probiotics and 32 taking placebo)	TGF- β 1, TGF- β 2	Probiotic	Giving probiotics to pregnant and lactating women $\uparrow$ TGF- β 2 in their milk compared with mothers receiving placebo
Bottcher 2008 [75]	Sweden (colostrum and mature milk from women treated with <i>L. reuteri</i> ($n = 54$) or placebo ($n = 55$)) from 36-week gestation until labour)	Total IgA, SIgA, TGF- β 1, TGF- β 2, IL-10, TNF, sCD14, Na/K ratios	Probiotic	<i>L. reuteri</i> supplementation during pregnancy was associated with $\downarrow$ TGF- β 2 and slightly $\uparrow$ levels of IL-10 in colostrum
Prescott 2008 [131]	New Zealand (1 week, 3 month, and 6 month BM from 34 mothers receiving <i>Lactobacillus rhamnosus</i> HN001; 35 mothers – <i>Bifidobacterium lactis</i> HN019 and 36 taking placebo beginning 2 – 5 weeks before delivery and continuing for 6 months into lactation)	IL-6, IL-13, IFN- γ , TNF- α , IL-10, TGF- β 1, IgA sCD14	Probiotic	$\uparrow$ TGF- β 1 in early 1 week BM from groups taking probiotics. $\uparrow$ IgA in BM from both the <i>B. lactis</i> HN019 and the <i>L. rhamnosus</i> HN001 group
Boyle 2011 [77]	Australia (7 days and 28 days BM samples from 38 mothers receiving placebo and 35 receiving probiotic)	Total IgA, sCD14, TGF- β	Probiotic	Prenatal probiotic (<i>Lactobacillus rhamnosus</i> GG) supplementation was associated with $\downarrow$ BM soluble CD14 and IgA levels

Table 4 (continued)

Author and year	Country and numbers	Immune modulators measured	Exposure of interest	Main outcomes
Kuitunen 2012 [73]	Finland (364 colostrum and 321 BM samples)	Total IgA, IgA antibodies to cow's milk, casein, β -lactoglobulin, ovalbumin, TGF- β 2, IL-10	Probiotic	Probiotic intervention (<i>Lactobacillus rhamnosus</i> GG, <i>L. rhamnosus</i> , <i>Bifidobacterium breve</i> , <i>Propionibacterium freudenreichii</i> ssp. <i>shermanii</i> JS) was associated with $\uparrow$ IL-10 and $\downarrow$ casein IgA levels in BM and TGF- β 2 in colostrum $\downarrow$ IL-1 α concentrations in smokers' colostrum compared with non-smoking mothers. IL-1 α , β -endorphin, leptin levels in transitional milk did not differ significantly
Zanardo 2005 [120]	Italy (colostrum and 10th day BM from 42 smoking and 40 non-smoking mothers)	IL-1 α , β -endorphin, leptin	Smoking	

increased IL-10 levels and decreased casein IgA levels in BM and TGF- β 2 levels in colostrum [73]. Boyle et al. [77] found that prenatal treatment with *Lactobacillus rhamnosus* GG was not sufficient to prevent eczema. However, total IgA levels at day 28 and sCD14 at day 7 in BM were lower in the probiotic group.

Together, these data all support the principle that maternal dietary composition can influence BM immune composition, although the precise nature of the effects of different dietary components and the mechanisms through which they influence BM composition need further investigation. Current data suggest that there is not enough evidence to recommend antigen avoidance to high-risk women during both pregnancy and lactation [134].

Gravidity

There are very few data on gravidity in relation to immune modulators in human milk, although increased IgA and IgM were found in colostrum of primiparous compared with multiparous women [69]. The 'hygiene hypothesis' remains the leading explanation for the high prevalence of immune-mediated diseases in the affluent world, caused by abnormal early immune development [135]. The inverse relationship between risk of atopic diseases, associated with a Th2 immune response, and early life exposure to microbial exposures promoting Th1 responses, as measured by surrogates, such as birth order or sibship size, early attendance at day care, and early exposure to pets or other animals, is well described [136]. One possibility is that these exposures modulate breast milk immune composition and thereby influence infant immune development. If there is an effect of gravidity on BM immune composition, then microbial exposures which impact on BM immune mediator levels [137] may explain these differences in mediator levels in relation to gravidity. Thus, mothers breastfeeding higher birth-order infants will be exposed to a wider array of organisms from their other children, and these will affect milk cytokine levels as shown in some probiotic trials.

Habitat

Studies of the effect of the maternal environment, or maternal atopy/allergic status on BM immune composition are summarized in Table 4. Variability in BM composition has been shown to be important in other settings – for example, BM from diabetic mothers can lead to a higher risk of obesity in offspring compared with BM from non-diabetic milk donors [32]. In the context of allergy, it is clear that BM of mothers living in different settings, and even of mothers in the same

setting but with different childhood exposures, differs significantly [30, 31]. The pathway for this latter finding is thought to be that enteral exposures of the mother during her own infancy prime her intestinal immune cell development such that when immune cells migrate from the maternal intestine to the mammary gland, there is altered BM composition [138].

At present, it is not possible to have specific guidelines on a particular diet modification and/or lifestyle. Healthy diet and exercise may benefit mother as well as child development.

Conclusion

In this review, we have seen that mode of milk feeding can influence infant immune development and allergic health outcomes, albeit with some inconsistencies between studies. We have described some of the wide range of BM constituents which can potentially influence infant immune development and identified areas for further investigation in determining their precise relationship with infant allergic outcomes. Finally, we have seen that maternal variables, especially allergic status and diet, can influence some of the BM constituents and also influence allergic health outcomes [132]. We have also identified important methodological concepts for future studies of BM composition. There is a tantalizing prospect that sup-

plementation of the maternal diet during lactation may be a simple, safe intervention that could promote infant immune development and lifelong health. In the first instance, it is crucial to establish relationships between ethnicity, environmental diversity, maternal health and microbiome and BM immune constituents, and their influence on allergy development in children. This knowledge can give us a much clearer idea of possible beneficial effects of maternally administered immunoactive supplements as an allergy prevention strategy.

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Conflict of interest

The authors declare no conflict of interest.

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