

Invited Paper

Academy of Breastfeeding Medicine Founder's Lecture 2008: Breastfeeding—An Extrauterine Link Between Mother and Child

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Abstract

In addition to a near-optimal combination of nutrients for the growing infant, breastmilk contains a wide array of bioactive molecules that are known to protect the infant against infectious disease and modulate the composition of the indigenous intestinal microbiota. A growing number of factors that modulate the infant's immunophysiology have also been identified in breastmilk. We suggest that this early immunomodulation via breastmilk is vital for infant health and may explain the epidemiological data indicating that breastmilk reduces the risk of immunoinflammatory conditions in infancy and also later in life. The body of scientific data regarding the role of transforming growth factor- β in breastmilk in enhancing healthy immune maturation and reducing the risk of disease is reviewed in this article.

Introduction: Health Benefits of Breastfeeding

BREASTFEEDING FORMS a unique bond between the mother and her infant. Through an evolutionary process, breastmilk has developed to provide the neonate sufficient nutrients for growth and development. Breastmilk not only contains nutrients in close to optimal quantities but also factors that facilitate the uptake of vital nutrients such as calcium, iron, and vitamin B12 in the infant intestine.¹ In addition to its irreplaceable role in infant nutrition, epidemiological studies have provided compelling data suggesting that breastmilk provides protection against a variety of age-related conditions in the infant.

The role of breastmilk in reducing the risk of infectious disease in the infant is well established. In a prospective trial conducted in Mexico by Ruiz-Palacios et al.,² non-breastfed infants were 2.3 times more likely to suffer from diarrhea during the first 6 months of life than breastfed infants. In particular, breastfeeding protected against infection by *Campylobacter jejuni*. In a parallel fashion, infant morbidity and mortality resulting from diarrhea have been reported to be significantly reduced by breastfeeding in the Philippines³ (Table 1). This protection against diarrheal disease is crucial in developing

countries and may in part be mediated by the infant's reduced need to consume contaminated water. However, a significant reduction in the occurrence of infant death due to both diarrheal disease and respiratory tract infections by exclusive breastfeeding has been reported from Bangladesh, suggesting active protection by breastmilk factors.⁴

Necrotizing enterocolitis (NEC) is a devastating immunoinflammatory condition mostly affecting premature neonates. The pathophysiology of NEC is complex and involves compromised intestinal perfusion, immature and excessive inflammatory responsiveness by the mucosal immune system, and bacterial colonization and commencement of enteral feeding in conjunction with increased intestinal permeability in the immature intestine. According to a meta-analysis of clinical trials, consumption of breastmilk significantly reduces the incidence of NEC in neonates⁵ (Fig. 1). Consequently, minimal enteral feeding with breastmilk in the first days of life has become standard practice in the care of preterm infants in many centers.

In addition to providing protection against infectious disease and NEC in infancy, there are epidemiological data to suggest that breastfeeding may have more long-term consequences regarding child health by having an impact on the

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TABLE 1. SUMMARY DATA FROM A LARGE COHORT STUDY IN THE PHILIPPINES OF THE MORBIDITY AND MORTALITY OF INFANTS FED BY EITHER BREASTFEEDING OR FORMULA FEEDING

	Mode of feeding		Diarrhea cases			
			Morbidity		Mortality	
	Number	%	Number	%	Number	%
Breast-fed	6,408	66.60%	9	4.35%	0	0%
Mixed-fed	611	6.35%	8	5.80%	0	0%
Formula-fed	2,603	27.05%	121	89.35%	38	10%
Total	9,622		138		38	

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development of chronic ailments such as asthma and atopic disease or obesity. Atopic disease has a significant hereditary component, and the discrepant epidemiological data regarding the relationship between breastfeeding and the risk of atopic disease in the infant may be explained by confounding the genetic predisposition and differences in breastmilk composition.⁶ For obvious practical and ethical reasons it is impossible to conduct a randomized, let alone double-blind, trial assessing the long-term effects of breastfeeding on the incidence of atopic disease. Nonetheless, in an Australian prospective birth cohort study of more than 2,000 children, a shorter duration of exclusive breastfeeding has been reported to be associated with an increased risk of asthma and atopic sensitization.⁷ Interestingly, according to a meta-analysis based on six case-control studies, breastfeeding may also confer protection against celiac disease⁸ (Fig. 2).

The rising prevalence of obesity and related disorders such as type 2 diabetes are a major health concern in developed countries. Intriguingly, breastfed infants display increased weight gain during the first 6 months of life but appear to have a reduced risk of obesity in later life. This effect is dem-

onstrated in a cohort study of more than 15,000 children in the United States, in which breastfeeding protected against obesity in adolescence.⁹ A similar conclusion of an inverse association between breastfeeding and obesity in later life was drawn in a meta-analysis of 28 studies.¹⁰ Breastfeeding, particularly exclusive breastfeeding, has also been reported to be associated with lower blood cholesterol concentrations in adulthood in a systematic review of observational studies.¹¹ A meta-analysis of seven studies involving more than 76,000 subjects found that individuals who had been breastfed had a significantly lower risk of developing type 2 diabetes in later life.¹² These data demonstrate that the beneficial effects of breastfeeding may extend well beyond infancy and suggest that factors in breastmilk may prime the infant's immune system and metabolism during infancy in a way that promotes health decades later. The molecular basis of these effects is gradually being elucidated.

Protective Nutrients in Breastmilk

In addition to nutrients, breastmilk contains an impressive array of bioactive molecules, the teleological role of which is

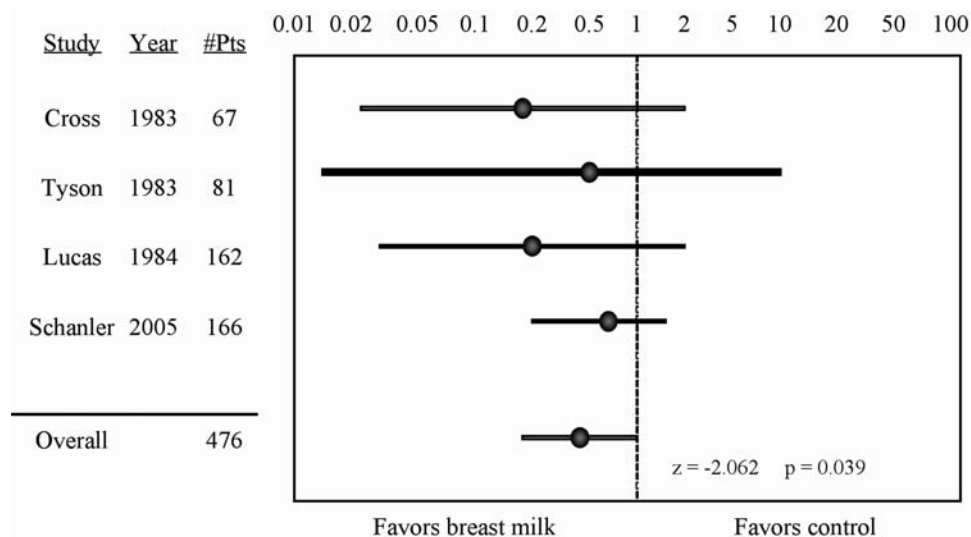


FIG. 1. Meta-analysis of four clinical studies examining the effect of breastfeeding on the incidence and severity of NEC. Reproduced with permission from McGuire and Anthony.⁵

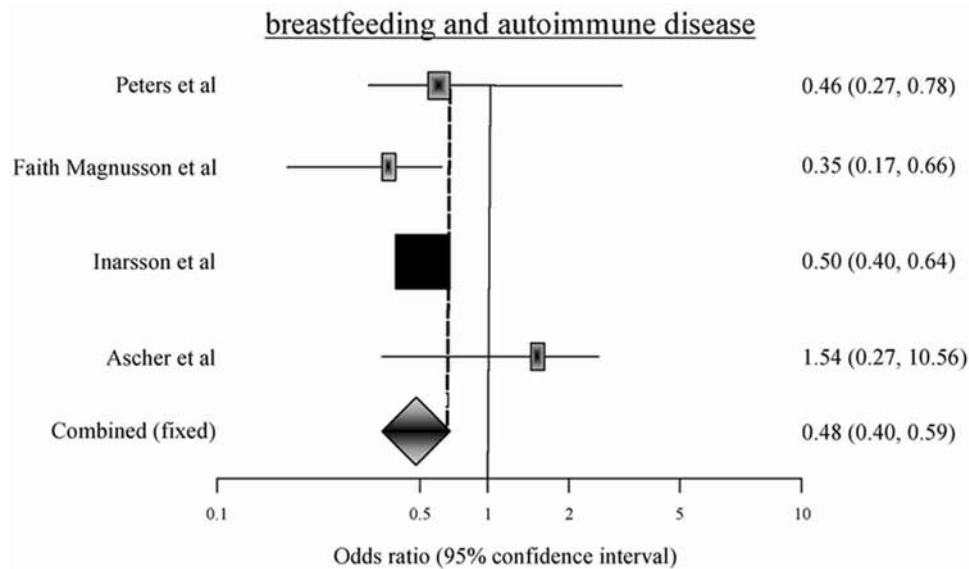


FIG. 2. Meta-analysis of four clinical studies examining the effect of breastfeeding on the incidence of celiac disease. Reproduced with permission from Akobeng et al.⁸

to provide the infant with exogenous support through the vulnerable period of early age. Functionally, these breastmilk components may roughly be divided into those providing passive immunoprotection against potentially pathogenic microbes and those having a direct impact on the infant's physiology by modulating mucosal immune responses. In addition, certain breastmilk components such as indigestible oligosaccharides modulate the infant's indigenous intestinal microbiota composition and thus indirectly have an impact on both intestinal mucosal barrier function and mucosal and systemic immune maturation.

Protection against pathogens

Immunoglobulins (Igs) were the first protective factors to have been identified in breastmilk and remain the most thoroughly investigated breastmilk immune components to date. Secretory IgA (SIgA), the most important Ig in breastmilk, is produced by maternal plasma cells and released into secreted milk.¹³ The antibodies bind microbial structures encountered on the mucosal surfaces of the mother, which include antigenic structures on both indigenous and pathogenic organisms and dietary antigens. SIgA is resistant to digestion and functions in the infant intestine by binding to antigens in the intestinal lumen.¹³ This renders potential pathogens less infective but may also modulate the establishment of the indigenous intestinal microbiota because the mother is the most important source of colonizing bacteria and breastmilk SIgA may be thought to recognize microbes of maternal origin. SIgA binding to dietary antigens may also reduce their allergenicity. Breastmilk SIgA concentrations are highest in colostrum and decrease in mature milk.¹⁴ Interestingly, IgA concentrations are particularly high in the breastmilk of mothers whose infants were born preterm.¹⁵ These phenomena illustrate how the composition of breastmilk varies over the course of lactation to meet the demands of the growing infant.

Certain breastmilk proteins such as κ -casein and α -lactalbumin, which are important nutritional sources of amino

acids for the infant, also display antimicrobial activity.¹ In addition, a number of bioactive breastmilk components whose primary function has been thought to be enhancing absorption of nutrients have been discovered to have antimicrobial properties and thus function also as protective nutrients. These include the iron-binding protein lactoferrin and haptocorrin, which binds vitamin B12.^{1,13}

Breastmilk contains an array of molecules primarily involved in innate antibacterial defense. Lysozyme, an enzyme capable of destroying Gram-negative bacteria by disrupting their cell walls, is found in significant quantities in breastmilk and also in the stool of breastfed infant.¹⁴ Additional directly antimicrobial innate immune molecules found in breastmilk include α - and β -defensins.^{16,17} Nondigestible glycans, most importantly oligosaccharides, may act as specific decoy receptor molecules for pathogenic microbes. Bacteria binding to breastmilk oligosaccharides are prevented from binding to intestinal epithelial cells, which is a crucial early step in bacterial attachment and pathogenesis of infectious disease.¹³

Impact on the indigenous intestinal microbiota

Beginning already at delivery, the neonate's sterile gastrointestinal tract is rapidly colonized with microbes. The indigenous intestinal microbiota are established during infancy, and this process is influenced by genetic, environmental, and dietary factors. It is well established that indigenous intestinal microbes provide the infant with vital stimuli that guide intestinal immune maturation and that disturbances in this process may result in development of atopic disease.^{18,19} Breastmilk has been reported to contain lactic acid bacteria,²⁰ but the significance of these microbes for intestinal microbiota composition in the infant is not known. Nonetheless, breastmilk also contains factors such as oligosaccharides¹³ and certain peptides²¹ that promote the growth of bifidobacteria. These data are in accordance with epidemiological data indicate that intestinal microbiota in breastfed infants is predominated by bifidobacteria, whereas

formula-fed infants display a more diverse intestinal microbiota composition.²²

Factors modulating intestinal immunity

As discussed above, breastfeeding appears to have an impact on later development of immunoinflammatory conditions such as atopic disorders and celiac disease. The molecular mechanisms underlying these long-term immunological effects of breastmilk are poorly understood. Nonetheless, scientific knowledge regarding the active immunomodulatory components in breastmilk is gradually expanding.

CD14 is a co-receptor for lipopolysaccharide, a structure in the outer membrane of Gram-negative bacteria, which regulates innate immune responses against these organisms.²³ Soluble (s)CD14 is found in substantial quantities in breastmilk,²⁴ and there are epidemiological data suggesting an inverse relationship between breastmilk sCD14 concentrations and the risk of developing atopic disease.^{25,26} LeBouder et al.²⁷ have reported that breastmilk modulates innate immune responses to bacterial antigens in human intestinal epithelial cells *in vitro*. This immunomodulation was demonstrated to be partially dependent on sCD14 in breastmilk. Taken together, these data provide evidence for the notion that breastmilk contains immunomodulatory molecules that may induce favorable changes in the infant's immune responsive phenotype that extend beyond the duration of breastfeeding.

In addition to innate immune receptors such as sCD14 and Toll-like receptors,²⁸ breastmilk contains cytokines, cytokine receptors, growth factors, and functional maternal immune cells that may modulate immune responses and intestinal maturation in the infant. Colostrum contains high concentrations of epidermal growth factor,²⁹ which has been shown to enter the systemic circulation in the infant³⁰ and therefore may play a role in inducing maturation in the infant intestine and other tissues. Interestingly, the concentration of epidermal growth factor is particularly high in breastmilk from mothers whose infants have been born prematurely.³¹

Transforming growth factor (TGF)- β , a cytokine found abundantly in breastmilk, has a wide variety of effects extending from regulation of cell proliferation and differentiation to modulation of immune responses.³² In humans, there are three TGF- β isoforms (TGF- β 1, - β 2, and - β 3), which are encoded by separate genes.³² TGF- β 1 is the predominant TGF- β isoform produced by immune cells, whereas TGF- β 2 is most abundant in breastmilk. The different TGF- β isoforms bind to the same receptor complex, but generalizing the data regarding one isoform and applying them to the others should be done cautiously.

TGF- β inhibits inflammatory responses in T cells³³ and intestinal epithelial cells³⁴ and modulates antigen-presenting cell function.³⁵ In addition, TGF- β plays an important role in the induction and function of regulatory T cells, which are central in establishment and maintenance of tolerance towards dietary antigens and indigenous intestinal microbes.^{32,36} In the intestinal mucosa, TGF- β enhances intestinal epithelial barrier function³⁷ by inducing IgA antibody production.³⁸

Because of the importance of TGF- β 2 in breastmilk, the latter half of this paper concentrates in reviewing the current knowledge of the role of breastmilk TGF- β in neonatal immune maturation and infant health.

Breastmilk TGF- β and Neonatal Immune Maturation

Experimental animal models

Based on work conducted using experimental animal models, TGF- β appears to be essential for healthy immune maturation in mammals. Mice homozygous for a null mutation in the TGF- β 1 gene ("TGF- β 1 knockout") exhibit an immunoinflammatory condition characterized by lymphocyte and macrophage infiltration into the heart, lungs, and salivary glands that leads to progressive wasting and eventually death at approximately 3 weeks of age.³⁹ Interestingly, Letterio et al.⁴⁰ have demonstrated that this process is delayed in TGF- β 1-deficient mouse pups born to heterozygous dams as a result of maternal transfer of TGF- β via the placenta and breastmilk, suggesting that maternal sources of TGF- β may profoundly modulate neonatal immunophysiology. The expression of TGF- β 1 in the neonatal intestine is initially very low according to a report based on work using a rat model. The mRNA for TGF- β 1 in healthy newborn rats is not detected before the age of 19 days and progressively increases thereafter.⁴¹ Extrapolating from these data, it may be suggested that breastmilk might act as an important exogenous source of TGF- β during early infancy when the mucosal and systemic immune responses are still immature. In line with this notion, it has been reported that rat pups fed formula devoid of TGF- β 2 exhibit more pronounced activation of both innate and adaptive immune systems in the intestine compared to those fed either formula supplemented with TGF- β 2 or breastmilk.⁴²

As alluded to above, TGF- β 2 appears to be the most abundant TGF- β isoform found in breastmilk, while TGF- β 1 is thought to be predominantly secreted by immune cells. There are data to suggest that responsiveness to TGF- β 2 is developmentally regulated and that the neonate may be evolutionarily adapted to immunoregulation by TGF- β 2 in breastmilk. To initiate intracellular signaling and induce its effects, TGF- β binds to a TGF- β receptor (TGFBR) II dimer on the target cell surface that then forms a complex with a TGFBR I dimer to initiate intracellular signaling.⁴³ A third receptor molecule, TGFBR III, is required for optimal responses to TGF- β 2 but not to TGF- β 1.⁴⁴ In the rat small intestine, the expression of TGFBR III and hence intestinal responsiveness to TGF- β 2 are confined to the neonatal period when the intestine is exposed to TGF- β 2 in breastmilk.⁴⁵ There are also data indicating that breastmilk may contain soluble TGFBR III and thus provides the infant with the receptor necessary for optimal responsiveness to TGF- β 2.⁴⁶

Recently, Verhasselt et al.⁴⁷ demonstrated in an elegant series of experiments that breastfeeding by dams exposed to inhaled allergen prevents subsequent development of allergic airway disease in a murine asthma model. The induction of allergen-specific tolerance was dependent on transfer of the allergen to the pup in breastmilk. Interestingly, the effect was abolished by exclusion of TGF- β in breastmilk by injecting the mother with TGF- β -specific monoclonal antibodies, indicating a necessary role for TGF- β in breastmilk-

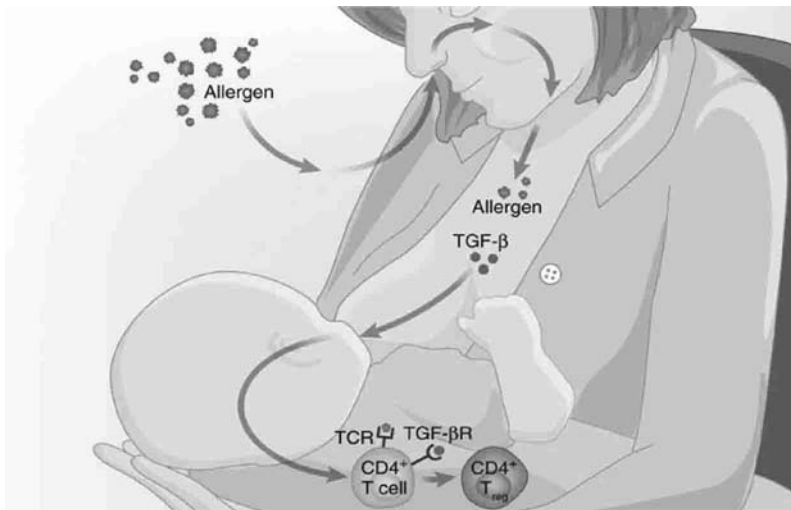


FIG. 3. Cartoon suggesting the role of breastfeeding in the expression of asthma in an animal model for asthma when the mother has the inhaled allergen in the breastmilk along with TGF- β . The combination of small quantities of allergen in the presence of TGF- β in breastmilk renders the suckling offspring permanently tolerant to the allergen, thus preventing the expression of asthma. Reproduced with permission from Puddington and Matson.⁶²

mediated tolerance induction. Penttila et al.⁴⁸ have also provided compelling evidence for the role of TGF- β in oral tolerance formation in the neonatal period (Fig. 3). Supplementation of cow's milk-based formula with TGF- β at a level comparable to that in breastmilk inhibits systemic allergic responses, including total IgE and cow's milk-specific IgG1 antibodies, compared to unsupplemented formula in allergy-prone rat pups. In a similar fashion, oral administration of TGF- β has been reported to reduce allergen-specific allergic-type immune responses in a high-dose oral tolerance murine model.⁴⁹

Immune maturation in the human infant

Assessing immune maturation in infants in clinical studies is difficult because of the lack of unambiguous indicators of maturation as well as obvious ethical issues regarding sample acquisition. Nevertheless, there are epidemiological data linking breastmilk TGF- β with favorable changes in both immune parameters and clinical outcomes. In a clinical study of 55 mother–infant pairs conducted in Japan, the concentrations of TGF- β 1 and TGF- β 2 in colostrum were associated with an increase in serum IgA concentration in the newborn infant during the first month of life.⁵⁰ Based on these data, the authors suggest that breastmilk TGF- β might serve as an exogenous trigger for IgA production in the immunologically naive neonate. This notion is compatible with an *in vitro* observation according to which TGF- β acts as a specific IgA class switch in human B cells.³⁸ It is of note that breastmilk TGF- β appears to be capable of modulating antigen-specific antibody responsiveness as well. Saarinen et al.⁵¹ assessed the association between breastmilk TGF- β 1 and cow's milk-specific antibodies in infants allergic to cow's milk. The concentration of TGF- β 1 in colostrum was positively correlated with cow's milk-specific IgA and IgG in the infants' sera. Moreover, the size of the skin prick test reaction to cow's milk reflecting IgE-mediated hyperresponsiveness in these infants was negatively associated with the level of TGF- β 1. In a similar fashion, TGF- β 2 concentration in colostrum has been reported to be associated with specific IgA responses to dietary antigens at 3 months of age.⁵²

Breastmilk TGF- β and the development of atopic disease in the infant

TGF- β in breastmilk may protect the infant against the development of atopic disease. High concentrations of both TGF- β 1 and TGF- β 2 in colostrum reportedly reduced the risk of developing atopic eczema during exclusive breastfeeding in high-risk infants.⁵² However, in a birth cohort study, concentrations of either TGF- β 1 or TGF- β 2 in colostrum were not associated with the risk of atopic sensitization or allergic disease by the age of 4 years.²⁶ This discrepancy may be explained by constitutional differences in the risk of developing atopic disease even though atopic heredity was taken into account in subgroup analyses in the latter study.

Oddy et al.⁵³ have reported the dose of TGF- β 1 that the child receives via breastmilk to be inversely associated with subsequent development of wheezing. In contrast, no association between breastmilk TGF- β 1 at 1 month of age and the occurrence of eczema, wheezing, or atopic sensitization was found in a study conducted in the Netherlands.⁵⁴ Unfortunately, data regarding breastmilk TGF- β 2 were not reported in either study.

In a randomized, double-blind, placebo-controlled trial of 62 mother–infant pairs, maternal consumption of the probiotic *Lactobacillus rhamnosus* GG was associated with a significant reduction of atopic eczema in high-risk infants by the age of 2 years.⁵⁵ In the same study, probiotic supplementation also markedly increased the concentration of TGF- β 2 but not that of TGF- β 1 in breastmilk collected when the infants were 3 months old. It may be suggested that the observed reduction in atopic eczema is causally related to the increase in breastmilk TGF- β 2, but other factors may also be at play. Recently, maternal supplementation with the probiotics *L. rhamnosus* and *Bifidobacterium lactis* was reported to increase the concentration of TGF- β 1 in breastmilk.⁵⁶ Interestingly, prenatal maternal consumption of another probiotic bacterium, *Lactobacillus reuteri*, has been reported to reduce the amount of TGF- β 2 in colostrum, and this reduction was associated with decreased risk of atopic sensitization in the infant at 2 years of age.⁵⁷ These discrepant data are difficult to reconcile even though there are major differences in

the studies regarding the probiotic strain used, timing of the intervention and sample collection, and clinical outcome measures.

Factors influencing breastmilk TGF- β concentrations

The amount of TGF- β in breastmilk may be influenced by a number of factors. Breastmilk concentrations of TGF- β ¹⁵⁸ and TGF- β ²⁵⁹ are reportedly lower in allergic mothers than in healthy mothers. As alluded to above, differences of this type in breastmilk composition may partially explain the discrepant epidemiological data regarding the protective effect of breastfeeding against allergic manifestations in the infant. Dietary and other environmental factors may also have an impact on breastmilk TGF- β concentrations. As discussed above, maternal supplementation with the probiotic *Lactobacillus* GG has been observed to significantly increase the amount of TGF- β 2 in breastmilk in a randomized, placebo-controlled trial, but the use of a different probiotic strain, *L. reuteri*, had the opposite effect.^{55,57}

Perplexingly, high concentrations of TGF- β 1 and TGF- β 2 in colostrum have recently been reported to be associated with shorter duration of breastfeeding along with maternal smoking and primiparity.⁶⁰ The authors suggest that breastmilk TGF- β 1 might play a causal role in early cessation of breastfeeding by having an impact on the involution of the mammary gland. Further studies are required to elucidate the matter.

Conclusions

Breastmilk confers the infant with indisputable short-term health benefits by providing balanced nutrition and reducing the occurrence of infectious disease and NEC. The long-term consequences of breastfeeding are more difficult to assess, but there are compelling epidemiological data to suggest that breastfeeding is associated with reduced risk of atopic disease as well as obesity and related metabolic disorders in later life. Based on these observations and accumulating mechanistic data, we suggest that breastmilk contains factors that are capable of programming the infant's developing immune system and metabolism into a health-promoting mode. Intriguing observations such as those indicating an association between breastfeeding and favorable cognitive development⁶¹ suggest that there are as of yet unknown effects of breastfeeding that future research should strive to uncover. The molecular mechanisms responsible for this early programming by breastmilk remains to be elucidated. In this review paper, we have examined breastmilk TGF- β as an example of a multifunctional immunomodulatory factor that has both short-term (e.g., direct anti-inflammatory effects, including the induction of IgA production) and long-term (e.g., protection against atopic disease) health effects.

The notion of breastfeeding inducing health benefits both in infancy and in later life underscores the importance of promoting breastfeeding in both developing and developed countries. Interventions that might promote the immunomodulatory properties of breastmilk such as maternal probiotic supplementation to increase breastmilk TGF- β 2 should also be explored. Lastly, identifying the molecular mechanisms of specific beneficial health effects might provide the opportunity to develop pharmaceutical means to combat diseases such as NEC in premature neonates.

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Disclosure Statement

No competing financial interests exist.

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