

Review

Immunomodulatory effects of breast milk on food allergy

Kirsi M. Järvinen, MD, PhD^{*}; Hayley Martin, BS[†]; Michiko K. Oyoshi, PhD[‡]^{*} Division of Pediatric Allergy and Immunology & Center for Food Allergy, University of Rochester School of Medicine and Dentistry, Rochester, New York[†] Department of Public Health Sciences, University of Rochester School of Medicine and Dentistry, Rochester, New York[‡] Division of Immunology, Boston Children's Hospital and the Departments of Pediatrics, Harvard Medical School, Boston, Massachusetts

Key Messages

- Breastfeeding is recommended to prevent the development of allergic diseases; however, studies have not adequately assessed the role of human milk in food allergy.
- Potential benefits against food allergy may differ between infants given differences in immunomodulatory composition of human milk between mothers (differences not captured in epidemiologic studies).
- The protection against allergy development provided by human milk may be attributable to the effect on the infant gut microbiome or via direct effects on the immune system.
- High levels, ratios, or combinations of certain human milk immune factors (IgA, cytokines, oligosaccharides) are associated with reduced risk of food allergy in the infant; it remains uncertain whether these are directly protective or whether biomarkers of transferred protection. The role of food antigens in human milk in initial sensitization or tolerance induction is unclear.
- Animal studies highlight potential mechanisms of protection provided by antigens, transforming growth factor β , and immunocomplexes, yet their relevance is poorly understood in humans.
- Studies evaluating the effect of breastfeeding and human milk composition on food allergy are needed.

ARTICLE INFO

Article history:

Received for publication January 28, 2019.

Received in revised form April 23, 2019.

Accepted for publication April 23, 2019.

ABSTRACT

Objective: To summarize the literature on immunomodulatory effects of breast milk on sensitization and possible mechanisms of action.**Data Sources:** Animal and human studies in PubMed that assessed breastfeeding or breast milk composition in food allergy.**Study Selections:** All recent studies and some older key publications focusing on this topic.**Results:** Human milk composition is highly variable among mothers, which can affect the developing infant immune system. Human milk also affects the infant gut microbiome, which is associated with food allergy. High levels of human milk immune factors (IgA, cytokines, oligosaccharides) are associated with reduced risk of food allergy in the infant; it remains uncertain whether these are directly protective or biomarkers of transferred protection. Animal studies highlight potential mechanisms of protection provided by antigens, transforming growth factor β , and immunocomplexes, yet their relevance is poorly understood in humans. The role of food antigens in human milk in initial sensitization or tolerance induction is unclear.**Reprints:** Kirsi M. Järvinen, MD, PhD, University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave, Box 611, Rochester, NY; E-mail: Kirsi_jarvinen-seppo@URMC.Rochester.edu.**Disclosures:** None. **Funding Sources:** Dr Järvinen was supported by award U01AI131344 from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health and award R21002516 from the National Center for Advancing Translational Sciences. Dr Martin is a trainee in the Medical Scientist

Training Program funded by 5T32 GM007356. Dr Oyoshi is supported by award R01AI142872 from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health and the Food Allergy Research & Education Inc, the HOPE APFED/ARTrust Pilot Grant, the William F. Milton Fund, National Center for Advancing Translational Sciences grant 8UL 1TR000170 from the Harvard Catalyst Clinical and Translational Research Center, and the Boston Children's Hospital Pediatric Associates Award.

<https://doi.org/10.1016/j.anai.2019.04.022>

1081-1206/© 2019 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Conclusion: The protection against allergy development provided by human milk may be attributable to the effect on the infant gut microbiome or direct effects on immune system. Studies evaluating the effect of breastfeeding and human milk composition on food allergy are needed.

© 2019 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

Not only is human milk (HM) considered the ideal nutrition source for term infants during the first 6 months of life by providing nutrients needed for growth and development,¹ but it also provides antimicrobial and immunomodulatory factors that provide defense against infections.^{2,3} Therefore, breastfeeding provides continued exposure to the mother's immune system during the crucial window of the first few months of life, when the infant's immune system is constantly developing. However, the immunomodulatory makeup of HM has not been well characterized and demonstrates a great deal of variability among mothers, which implies that the benefit of breastfeeding to the breastfed infants may differ among dyads. This variability can have an effect on the degree to which breast milk can provide benefit in individual mother-infant pairs (Fig 1).

Breastfeeding is currently recommended as primary prevention for allergic diseases, including food allergy (FA).^{4–6} A systematic review concluded that there is a protective effect of exclusive breastfeeding against cow's milk allergy (CMA) in early childhood among high-risk infants.⁷ However, more recent studies did not find an association between FA and breastfeeding, although they concluded that the evidence on FA had high heterogeneity and low quality,⁸ insufficient to draw conclusions about the effect of breastfeeding on FA.⁹ The most recent population-based study from Australia reported no association between breastfeeding and length of breastfeeding with FA at 1 year (likely underpowered for CMA).¹⁰ Unfortunately, to date, most studies have/lacked adequate statistical power to assess the effect of breastfeeding on FA or have not evaluated this association at all (or only assessed food sensitization) because of methodologic problems. Randomized clinical trials are lacking for ethical reasons, and the definitions of breastfeeding and allergic outcomes vary greatly among existing studies.

The only randomized trial on the topic, the Promotion of Breastfeeding Intervention Trial (PROBIT) from Belarus, designed to promote breastfeeding duration and exclusivity of breastfeeding,

documented a reduction in gastrointestinal infections and eczema in the 12 months in the intervention group.¹¹ The 6-year follow-up in this cohort found that this protective effect was no longer observable; however, FA was not assessed.¹² Some of the discrepancy among studies may be explained by differences in HM composition, which has not been accounted for in the broad definition of breastfeeding. Breastfeeding in general may protect against wheezing and eczema by protecting against early-life (viral) infections,⁸ but protection against FA might require more defined composition of immune factors from HM. In addition, the mechanisms by which HM affords protection are largely unknown. Gene-environment interaction have also been shown to affect protection. The association between breastfeeding and food sensitization modified by single-nucleotide polymorphisms in the interleukin (IL) 12 receptor $\beta 1$, Toll-like receptor (TLR) 9, and thymic stromal lymphopoietin genes: for example, breastfeeding increases the risk of food sensitization in children carrying the GG genotype but decreases the risk in children carrying the GT/TT genotype of the IL-12 receptor $\beta 1$.¹³

Breast milk originates from secretory cells within the lactating breast. Most HM components, including lactose, milk lipids, and a variety of milk proteins, are produced in the lactating cells.¹⁴ Beyond the major macronutrient components, HM also contains human milk oligosaccharides (HMOs), immune cells, cytokines, chemokines and hormones, immunoglobulins, critical growth factors, active enzymes (including peroxidases and lysozymes), lactoferrin and additional secretory components, soluble CD14, TLR2, and tumor necrosis factor (TNF) receptor, along with maternal diet-derived food antigens and its own microbiome made up of bacteria and viruses.^{15,16} Previous studies have evaluated many of these HM components and their association with allergy development in offspring. This review discusses the most recent (last ≥ 10 years) and selected key older studies found in PubMed that have assessed the association between breastfeeding and FA in infants and young children and summarizes existing animal and human studies that have evaluated the association of human milk immunologically

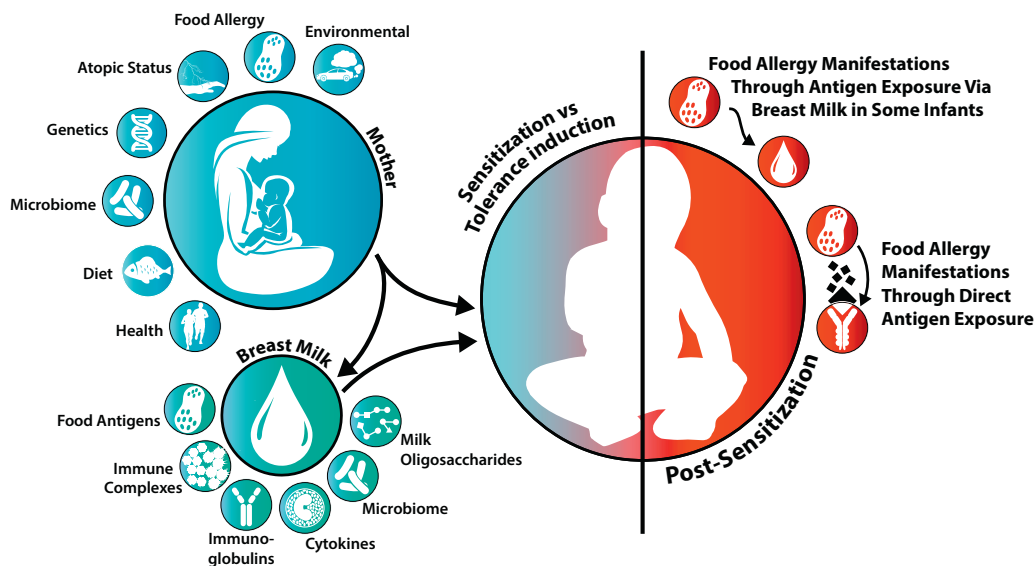


Figure 1. Maternal and breast milk factors that influence the development of the neonatal immune system.

Table 1
Studies on the Association Between Maternal Allergen Exposure and Tolerance in Offspring

Study	Mouse strain	Allergen	Maternal sensitization, route and timing	Maternal allergen exposure, route, and timing	Cross-fostering by sensitized mothers	Offspring outcome	Maternal impact on offspring allergies	Comments
Verhasselt et al., ³² 2008	BALB/c	OVA	NA	Aerosol, lactating	Yes	Asthma	Protection	Protection is milk TGF- β dependent
Mosconi et al., ³³ 2010	BALB/c	OVA	IP, preconceptional	Aerosol, lactating	Yes	Asthma	Protection	Protection is milk IC dependent
Turfkruyer et al., ⁴² 2016	BALB/c	OVA	NA	Oral, lactating	No	Asthma	Protection	Inefficient neonatal tolerance is attributable to vitamin A deficiency
Hamada et al., ³⁴ 2003	BALB/c	OVA	IP, preconceptional	Aerosol, preconceptional and pregnancy	No	Asthma	Asthma exaggeration	Lack of maternal allergen exposure during lactating
Macchiaverni et al., ³⁵ 2014	BALB/c	<i>Dermatophagoides pteronissinus</i>	IN, preconceptional	IN, lactating	Yes	Asthma	Sensitization	
Järvinen et al., ³⁶ 2015	C3H/HeJ	Peanut	Ad libitum, preconceptional	Ad libitum, pregnancy, and lactating	No	FA	No impact	
Bernard et al., ³⁷ 2014	BALB/c	Peanut	NA	NA	No	FA	Protection	Human milk containing peanut and peanut-IC prevents sensitization in mice
Lopez-Exposito et al., ³⁸ 2009	C3H/HeJ	Peanut	Oral, preconceptional	Oral, pregnancy, and lactating	No	FA	Protection	
Ohsaki et al., ³⁹ 2018	BALB/c	OVA, peanut	Epicutaneous, preconceptional	Epicutaneous, pregnancy, and lactating	Yes	FA	Protection	Milk IC-offspring FcRn axis induces neonatal tolerance
Rekima et al., ⁴³ 2017	BALB/c	OVA	IP, preconceptional	Oral, lactating	Yes	FA	Protection	Milk allergen and TGF- β supplementation protect offspring against FA

Abbreviations: FA, food allergy; FcRn, neonatal crystallizable fragment receptor; IC, immune complex; IN, intranasal; IP, intraperitoneal; NA, not applicable; OVA, ovalbumin; TGF- β , transforming growth factor β .

active components with FA in offspring and their effect on infant gut microbiome. These components include allergens, immunoglobulins, immune complexes, cytokines, chemokines, growth factors, and HMOs and exclude enzymes, lactoferrin, soluble CD14, soluble TLR2, and soluble TNF receptor, where no data are available in association with FA.

Breast Milk and Infant Gut Microbiome

Some of the effect of breastfeeding on the infant immune system may be attributable to its effect on infant gut microbiome. In addition to prenatal environment, delivery mode, antibiotic treatment, and diet, the infant microbiome composition is significantly influenced by breastfeeding.^{17,18} Among breastfed infants, microbiome diversity is initially lower compared with formula-fed infants, given that HM creates an intestinal environment that gives selective advantage to highly adapted intestinal microbiota by providing HMOs. The dominant microbes of breastfed infants (*Bifidobacteria*, *Lactobacilli*, *Enterobacteriaceae*) are surpassed by *Clostridium* and *Bacteroides* species after cessation of breastfeeding and introduction of complementary foods.^{19–22} Cohort studies, including the Wayne County Health, Environment, Allergy, and Asthma Longitudinal Study, found that breastfeeding is one of the most influential factors in the development of the infant microbiome.¹⁸ In humans, breastfeeding is a major source of beneficial bacterial species,²³ indicating the essential role of this food source in determining the microbiota in early life that may affect the later development of allergic diseases, including FA. Kourosh et al.²⁴ found that children with IgE-mediated FA had significantly different microbial composition, particularly among *Clostridia* species, compared with control subjects. Fieten et al.²⁵ in a study conducted in children with atopic dermatitis, found fewer than 2 *Bifidobacterium* species (*Bifidobacterium breve*, *Bifidobacterium adolescentis*) in addition to lower numbers of *Faecalibacterium prausnitzii*, and *Akkermansia muciniphila* in the fecal microbiome of children with concomitant FA compared with those without FA. Finally, Fazlollahi et al.²⁶ found a higher α -diversity of microbial flora in egg-allergic children compared with non-FA controls. These studies and a recent review²⁷ highlight that alterations in gut microbiome may be a key factor in the development of FA.

The effect of microbiome on the infant immune system is likely attributable to the short-chain fatty acids (SCFAs), including propionate, butyrate, and acetate, that are a byproduct of microbial metabolism and have anti-inflammatory functions.²⁸ A recent study found that SCFAs were altered in children who are atopic.²⁸ Similarly, in a mouse model, researchers found that higher levels of the SCFAs acetate and butyrate may inhibit the development of FA by inducing tolerance via CD103⁺ dendritic cells.²⁹ In studies of term infants, although overall SCFA levels are higher in formula-fed compared with breastfed infants, the highest levels of acetate are observed in exclusively breastfed infants.^{28,30} Taken together, these studies support the hypothesis that one mechanism of FA protection conferred via breastfeeding may be through the influence of HM on the infant gut microbiome that leads to reduction in proinflammatory processes.

Insights From Animal Models

Animal models of FA have been developed to explore the mechanisms involved in the development of diseases to normally harmless food allergens.³¹ These models of FA have been used to dissect how maternal factors influence the susceptibility of offspring to allergies. This section focuses on the role of breast milk factors in the development of experimental FA in offspring.

Table 2
Recent Studies Evaluating Maternal Diet including or excluding Allergenic Foods During Lactation (During or Not During Pregnancy) and FA/Sensitization in Offspring

Study (location)	No. Of patients ^a	General population or high risk for atopy	Exposure	Outcome	Adequate statistical adjustment ^b	Study design	Effect of intake during pregnancy	Effect of intake during lactation	Comments
Peanut									
Du Toit et al, ⁶⁶ 2008 (United Kingdom and Israel)	176	General population	Infant and maternal consumption vs no consumption between countries (Israel vs United Kingdom) (grams and frequency monthly)	FA (self-report followed by SPT, IgE, or OFC)	No	Retrospective	No association	Protective	Early infant exposure to peanut showed strongest protective effect
Fox et al, ⁶³ 2009 (United Kingdom)	443	2 Control groups: a general population group and a high-risk (egg allergy) group	Maternal consumption vs no consumption (grams per week)	FA ($\geq 95\%$ predictive value on SPT, IgE or OFC)	Yes	Case-control	No association	No association	Maternal effect disappeared when adjusted for household peanut exposure
Des Roches et al, ⁶⁷ 2010 (Canada)	403	General population	Maternal consumption (frequency)	FA (clinical history plus positive SPT or IgE test result)	Yes	Case-control	Increased risk	Increased risk	
Sicherer et al, ⁶⁴ 2010 (United States)	503	High risk	Maternal consumption (≥ 2 times per week vs < 2 times per week)	High level sensitization (IgE, ≥ 5 kU/L)	Yes	Retrospective	Increased risk	No association	
Frazier et al, ⁷² 2014 (United States)	8205	General population	Maternal consumption (servings per week) of peanuts and tree nuts during peripregnancy period (exposure captured via survey completed closest to index birth)	Physician reviewed self-report FA diagnosis		Prospective cohort	Protective		Did not evaluate lactation and pregnancy separately. Greatest benefit seen in mothers without peanut/tree nut allergy
Pitt et al, ⁶⁵ 2017 (Canada)	342	High risk	Maternal consumption while lactating (ever vs never)	Sensitization (positive SPT result)	Yes	Prospective cohort (nested)	Not assessed	Protective ^c	
Cow's milk, egg, and others									
Herrmann et al, ⁶⁸ 1996 (Germany)	99-120	High risk	Maternal unrestricted vs restricted diet (cow's milk and egg) during pregnancy and lactation, vs lactation only	Sensitization (IgE, ≥ 0.35 kU/L)	No	Non- randomized comparison	No association	No association	
Hattevig et al, ⁶⁹ 1999 (Sweden)	115	High risk	Maternal unrestricted diet during lactation vs restricted diet (cow's milk, egg, fish)	Sensitization (SPT and IgE ≥ 0.35 PRU/mL)	Yes	Non- randomized comparison	Not assessed	No association	
Nwaru et al, ⁷⁰ 2011 (Finland; DIPP Nutrition Study, 19980-2000)	1018	High risk for type 1 diabetes (all with HLA-DQB1)	Maternal consumption of foods (z scores) during lactation (milk and egg)	Cow's milk, egg, and wheat sensitization (IgE, ≥ 0.35 kU/L)	Yes	Prospective cohort	Not assessed	No association	
Tuokkola et al, ⁷¹ 2016 (Finland; DIPP Nutrition Study, 1997-2004)	2820	High risk for type 1 diabetes (all with HLA-DQB1)	Maternal consumption of milk during pregnancy and lactation (first vs second quartiles and third and fourth vs second and third quartiles)	Cow's milk allergy ^d (physician diagnosis or self-report)	Yes	Prospective cohort	Protective	No association	Using the same cohort as above study does

Abbreviations: DIPP Nutrition Study, The Finnish Type 1 Diabetes Prediction and Prevention Study; FA, food allergy; NS, not significant; OFC, oral food challenge; SPT, skin prick test.

^aNumber of patients included in lactation specific analyses.

^bIncludes (at minimum) adjustment for atopic status of family and/or patient if different between comparison groups.

^cAmong group with maternal peanut consumption during lactation and introduction to infants before 12 months, no adjusted effect estimate reported for peanut consumption during lactation without interaction with direct ingestion because this was highly significant ($P = .003$).

^dRegistry-obtained physician diagnosis used to justify cost of non-cow's milk formula or self-report if breastfed or diagnosed at older than 1 year.

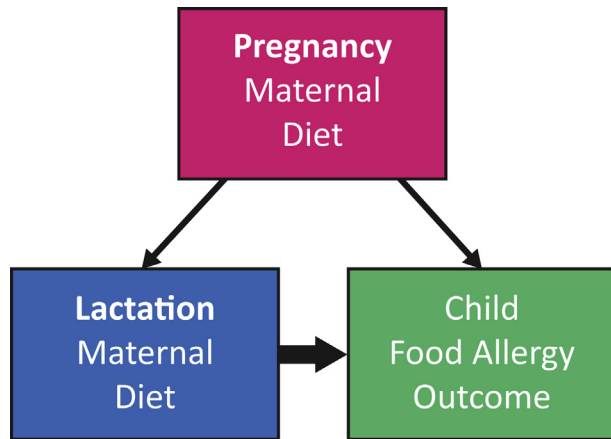


Figure 2. To estimate the effect of maternal diet during lactation on food allergy outcomes in the offspring (bold arrow), it is necessary to control for the effect of maternal diet during pregnancy. Without statistically controlling for this variable given its association to both the outcome (child food allergy) and the exposure (maternal diet during lactation), the resulting effect estimate would not reflect the true association of interest.

Allergen, Immunoglobulins, and Immune Complexes

The concept of tolerance induction by allergen in breast milk has been shown in experimental asthma.³² Aerosol allergen (ovalbumin; [OVA]) exposure of wild-type (WT) mothers during breastfeeding resulted in an allergen transfer to offspring through milk and the induction of allergen specific, transforming growth factor (TGF) β -dependent tolerance toward allergic airway inflammation. Naïve offspring adopted and nursed by preconceptually sensitized WT mothers with OVA and alum adjuvant intraperitoneally exposed to OVA aerosols during breastfeeding received maternal immune complexes (ICs) (allergen and allergen specific IgG) via milk and exhibited a long-lasting allergen-specific protection from asthma.³³ In contrast, earlier studies have indicated maternal transmission of asthma susceptibility to offspring³⁴ from WT mothers preconceptually immunized with OVA and alum and exposed to OVA aerosol during pregnancy. Because OVA-sensitized mothers were *not* exposed to OVA during breastfeeding, these data imply that allergen transfer via milk is critical for successful tolerance induction in offspring. Respiratory allergen from house dust mite is present in HM and primes for allergic sensitization in experimental asthma,³⁵ suggesting that maternal exposure to environmental allergen reflected in milk may facilitate allergic sensitization rather than protection in offspring. In conclusion, these asthma studies imply that milk-borne allergen, ICs, and TGF- β may induce tolerance in offspring against asthma, whereas insufficient milk-borne allergen exposure or specific environmental allergen exposure of offspring may promote asthma.

Although the mechanisms of experimental asthma may not simply be applicable to experimental FA, these observations led to the hypothesis that allergens, immunoglobulins, and ICs in milk might facilitate the induction of tolerance to food allergens in neonates. On the subject of FA, we found that preconceptional peanut exposure of C3H/HeJ mothers (a substrain of C3H mice that lacks a functional Toll-like receptor 4 that recognizes lipopolysaccharide) resulted in the generation of varying levels of maternal immunoglobulins in serum and breast milk. However, maternal peanut exposure preconceptionally or during pregnancy and breastfeeding had no effect on offspring rates of peanut allergy assessed by oral and intraperitoneal peanut challenge in a model where peanut allergy was elicited by oral immunization with peanut and cholera toxin (CT).³⁶ These findings support the recommendations of no dietary restrictions for pregnant and breastfeeding mothers.

Bernard et al³⁷ found that HM that contained peanut allergens induced partial oral tolerance in peanut-sensitized young mice, supporting a beneficial role of milk-born allergen in decreasing disease susceptibility in offspring. A stronger immunization of C3H/HeJ mothers with oral peanut/CT and peanut exposure during pregnancy and breastfeeding indeed reduced peanut allergy risk in offspring. Offspring of preconceptionally peanut/CT-sensitized mothers exhibited IgG1-mediated anaphylaxis in response to oral peanut challenge (first exposure). Interestingly, when sensitized mothers also received peanut/CT during pregnancy and breastfeeding, offspring were protected from anaphylaxis after first peanut challenge as well as active peanut/CT sensitization and oral peanut challenge. These results suggest that maternal immune responses during pregnancy and breastfeeding are critical in the reduction of disease susceptibility in offspring.

We recently delineated the mechanisms of neonatal tolerance induction against FA, mediated by maternal IgG-ICs and neonatal crystallizable fragment receptor (FcRn).³⁹ Maternal allergen sensitization through the epicutaneous route⁴⁰ prevented food anaphylaxis and allergen specific IgE in offspring after epicutaneous sensitization and oral challenge of offspring with allergen. This protection was mediated by FcRn-dependent transfer of maternal allergen, IgG, and ICs via breast milk and induction of allergen-specific T-regulatory cells in offspring. Neonatal tolerance was induced in offspring of naïve mothers who were fostered immediately after birth and nursed by OVA-sensitized mothers. The induction of tolerance was also observed in offspring of naïve mothers supplemented with IgG-ICs during breastfeeding. Induction of oral tolerance in offspring required FcRn-dependent antigen presentation by CD11c⁺ dendritic cells. Human breast milk that contained OVA, IgG, and ICs induced tolerance in humanized FcRn mice, providing particularly important evidence of the potential clinical relevance of our findings in humans. Jointly, these findings from our mouse model indicate that interactions of offspring FcRn and maternal IgG-ICs are key components of the induction of T-regulatory cell responses and regulation of food-specific tolerance in the neonatal period. This study provided experimental evidence of the key role played by maternal allergen-specific immunoglobulins in milk in establishing effective tolerance that prevents FA in offspring. These effects extend well past the previously described roles of maternal antibodies and FcRn in the provision of passive immunity. In addition, such food-specific IgG antibodies are also induced during oral immunotherapy in humans and act through Fc γ RIIb to suppress IgE-mediated hypersensitivity.⁴¹ Strategies that favor maternal IgG responses might prove useful in the prevention of FA in offspring.

Vitamin A and TGF- β

In humans, increased risk of allergy during early childhood indicates insufficient immune regulation in this period of life. In addition, in mice, neonates (ie, first week) are refractory to oral tolerance elicited by maternal allergen transfer via milk because of a physiologic vitamin A deficiency.⁴² Unsensitized BALB/c WT mothers were fed OVA in the first, second, and third weeks of or throughout breastfeeding, and tolerance induction in offspring to OVA-induced airway inflammation was assessed. Oral tolerance induction was fully efficient only starting in the third week of life. Insufficient tolerance in 1-week-old neonates was associated with a reduction in gut barrier, retinaldehyde dehydrogenase expression by mesenteric lymph node CD103⁺ neonatal dendritic cells, and serum retinol levels compared with those in adult mice. Vitamin A supplementation rescued neonatal defects and resulted in sufficient tolerance induction in 1-week-old mice. Oral tolerance was dependent on interferon (IFN) γ . These results may suggest vitamin A supplementation together with maternal allergen transfer via

milk as possible interventions for allergy prevention in the neonatal stage of life.

To assess the duration of oral tolerance induced by breast milk toward FA, BALB/c WT mothers were preconceptually immunized intraperitoneally with OVA/alum then fed OVA during breastfeeding while nursing offspring from unsensitized mothers.⁴³ Breastfeeding by OVA-sensitized mothers resulted in a decrease in frequencies of allergic diarrhea and serum levels of mast cell protease 1 after repeated oral OVA challenges in 6-week-old but not in 13-week-old offspring. Supplementation with TGF- β after weaning till 12 weeks prolonged protection against diarrhea and improved gut barrier in 13-week-old mice breastfed by OVA-sensitized mothers. Although the precise mechanisms responsible for allergic diarrhea are not fully understood, expansion of intestinal mast cells is a common phenomenon in patients with FA^{44,45} and has been associated with disease severity in experimental FA models.^{46,47} Breastfeeding by allergen-sensitized mothers together with offspring TGF- β supplementation may provide long-lasting prevention of allergic diarrhea and intestinal mast cell expansion.

Food specific IgG and food allergen and ICs are present in sera and breast milk of healthy humans.^{48–53} Allergen-sensitized female mice in these studies above are not necessarily allergic or atopic but were used to investigate the role of maternal allergen and allergen specific immunoglobulins in milk in influencing offspring allergies. Analysis of how atopic status of mothers may influence tolerance induction or FA in offspring in humans will be an important future issue to be addressed. One of the critical advantages of using mouse models to study the effect of breast milk on the development of FA in offspring is that sensitization or tolerance can be induced to specific allergens under controlled environmental conditions within defined genetic backgrounds at specific timing, which is not possible in humans. This aspect of mouse models allows extensive and precise investigations into the mechanisms involved in maternal-fetal interaction during the breastfeeding period, such as identification of possible triggers of food sensitization, as well as pathways involved in the induction of tolerance toward FA. Increasing animal studies underscore the critical role of breast milk in the induction of tolerance toward FA in offspring. Relevant findings from mouse models of FA and asthma described above are summarized in Table 1.

Human Milk ICs and FA

Dietary Allergen and Maternal Diet

Debate over whether the presence of food antigens in HM results in sensitization or tolerance in infants is ongoing. The detection of specific dietary antigens in HM after maternal consumption has been well established for a wide variety of food proteins, including OVA,^{54,55} β -lactoglobulin,^{56,57} gliadin,⁵⁸ and peanut.^{37,59–61} Although maternal ingestion of dietary allergens can induce symptoms in some sensitized infants,⁶² this is certainly not always the case. Furthermore their role in the initial sensitization to foods remains unclear, and the currently available evidence does not support restriction of the maternal diet during pregnancy or lactation.⁵

To account for variation in maternal dietary patterns, several studies have evaluated the association between intake of allergenic foods during lactation and FA outcomes in offspring^{63–72} (Table 2). Studies evaluating peanut consumption during lactation reported mixed findings; 2 studies^{63,64} reported no association, 2 studies^{65,66} found reduced risk of peanut sensitization or FA, and 1 study⁶⁷ found that maternal consumption during lactation increased the infant's risk of peanut allergy. One study on maternal peanut and tree nut consumption during the peripregnancy period found that higher levels of consumption in nonallergic mothers resulted in

protection from peanut and tree nut allergy in the infant.⁷² This study did not report the independent effect of consumption during lactation, however. Studies evaluating cow's milk, egg, and other allergens found no association between maternal diet during lactation and risk of allergy or sensitization.^{68–71} A number of methodologic considerations may explain the discrepant findings among studies. Definitions of exposure (estimates of maternal consumption during lactation, along with breastfeeding intensity and duration) and outcome (oral food challenge–diagnosed FA, self-report FA, sensitization on skin prick test, or allergen specific IgE) are not used systematically among studies, making associations difficult to compare directly. Many of these studies have relatively small sample sizes, relying on small numbers of events (FA detection) for statistical analysis. Several of the studies found no association between maternal diet during lactation and FA outcomes; however, very small numbers of individuals in these studies experienced these outcomes,^{68,69} generating significant concerns about the risk of type II error. Another important factor to consider is that the high concordance between maternal diet during pregnancy and lactation further complicates evaluating these associations separately^{64,73,74}; however, statistical control for maternal diet during pregnancy is likely necessary for obtaining an unconfounded effect estimate of the association of interest. If we assume that maternal diet during pregnancy is associated with FA risk in the offspring and that maternal diet in pregnancy is also tightly associated with maternal diet during lactation, then this variable meets the classic definition of a confounder and should be adjusted for in statistical models (Fig 2). Randomized controlled trials would be an optimal tool for eliminating these statistical concerns. Given the large variability in findings reported and numerous methodologic concerns, observational studies that evaluate maternal diet during lactation (and do not consider the composition of HM) thus far have not been able to conclusively determine the role played by maternal dietary antigens in HM in the development or prevention of FA.

One of the major limitations of studies that do not measure dietary antigens and rely on maternal dietary intake as a proxy measure is potentially significant misclassification of a child's exposure to these antigens via HM. Several reports have found large interindividual variation in food allergens in HM and have demonstrated that a nontrivial proportion of women had undetectable levels of food allergens after consumption of these foods. Following cow's milk consumption, 15% to 47% of individuals had no detectable β -lactoglobulin in HM.^{56,57} More than 25% of mothers in egg consumption arms of a randomized trial demonstrated no detectable OVA in their milk,⁵⁴ and in a randomized crossover trial, 24% of mothers never demonstrated detectable OVA in their milk.⁵⁵ After peanut consumption, 52% of women had no detectable peanut proteins,⁶¹ and 72% had no detectable Ara h 2, the most potent allergenic peanut protein, in breast milk.⁵⁹ Beyond the presence or absence of detectable antigen, for women with detectable levels of food antigens in their HM, the timing of the onset of detection, the peak concentration, and the duration throughout which the food protein is detectable also varied greatly among individuals. The sources of variability in these factors (timing, concentration, and duration of detection) are likely multifactorial and are unrelated to mammary epithelial permeability,⁵⁴ suggesting that perhaps the variability may be in part attributable to differences in maternal intestinal permeability to these antigens.

Maternal dietary intake affects infant immune markers that likely alter the risk of FA development. Metcalfe et al⁵⁴ found that high-dose egg exposure in the mother resulted in higher levels of specific IgG4, a marker of exposure and possible immune tolerance in breastfed infants, compared with mothers in low or no egg arms of the trial. Similarly, our study found that mothers who restricted cow's milk intake during lactation had infants with lower levels of specific

Table 3

Studies Assessing the Association Between Cytokine Levels in HM and FA in Infants

Study (location)	No. of patients	Duration of follow-up	Cytokines assessed	FA
Saariinen et al, ⁹¹ 1999 (Finland)	6209	12 mo	TGF- β 1, TGF- β 2	Higher TGF- β 1 levels in colostrum are associated with infants who develop IgE-mediated cow's milk allergy vs non-IgE-mediated cow's milk allergy; healthy controls were found in between
Bottcher et al, ⁹² 2003 (Sweden)	53	2 y	IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-16, IFN- γ , TGF- β 1, TGF- β 2, RANTES, eotaxin	No significant association
Snijders et al, ⁹³ 2006 (the Netherlands)	315	2 y	IL-12 or TGF- β 1 (IL-10 undetectable)	No significant association
Kuitunen et al, ⁹⁴ 2012 (Finland)	364 (colostrum) and 321 (BM)	2 y	IL-10, TGF- β 1	No significant association
Järvinen et al, ⁸⁸ 2015 (Finland)	145	2 y	IL-1 α , IL-1 β , IL-6, IL-10, PDGF-BB, CCL27, VEGF, TSLP, CCL11, CXCL10, and CXCL11, CCL22, TGF- β 1, TNF- α and - β , CCL1, CCL17, IL-31, eotaxin 3, CXCL9, IL-5, GM-CSF, and IL-12p70	Higher levels of IL-1 β , IL-6, IL-10, and TGF- β 1 in HM showed association with cow's milk tolerance
Munblit et al, ⁹⁵ 2017 (United Kingdom, Russia, and Italy)	398	6 mo	IL-2, IL-4, IL-5, IL-10, IFN- γ , IL-12, IL-13, HGF, TGF- β 1, TGF- β 2, TGF- β 3	Higher IL-13 levels associated with protection, otherwise no significant association
Berdi et al, ⁹⁶ 2018 (France)	263	5 y	Eotaxin, CX3CL1, CXCL1, IL-8, CXCL10, CCL7, CCL22, CCL2, CCL3, CCL4, RANTES, IFN- α 2, IFN- γ , IL-10, IL-12p40, IL-12p70, IL-15, IL-17A, IL-1 receptor agonist, IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-13, soluble CD40 ligand, TNF- α , TNF- β , EGF, FGF-2, G-CSF, Flt3L, GM-CSF, PDGF-AA, PDGF-BB, TGF- α , VEGF	Higher levels of CXCL10, TNF β and IL-2 associated with protection against FA

Abbreviations: BM, bone marrow; EGF, epidermal growth factor; FA, food allergy; FGF, fibroblastic growth factor; Flt3L, FMS-like tyrosine kinase 3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HGF, hepatocyte growth factor; HM, human milk; IFN, interferon; IL, interleukin; PDGF, platelet-derived growth factor; RANTES, regulated upon activation, normal T cell expressed, and secreted; TGF, transforming growth factor; TSLP, thymic stromal lymphopoietin; and VEGF, vascular endothelial growth factor.

IgG4.⁷⁵ These data suggest that the effect of dietary antigens in HM on immune markers of exposure in offspring have demonstrated benefit among individuals with detectable levels of food antigen.

Taken together, findings from existing epidemiologic studies evaluating the association between maternal diet during lactation and FA outcomes in offspring are limited in their explanatory power because of the inability to control for wide interindividual variation concentration of food antigens in HM. Importantly, the maternal diet during lactation influences HM composition in ways that antigen concentration along with food specific immunoglobulins in humans.⁷⁵ This may be confounded by maternal diet during pregnancy, which itself may be protective.⁷⁶ Additional large-scale studies that carefully evaluate the concentration of dietary antigens in HM, and the associated risk of allergic disease will be needed to clearly elucidate these complex associations.

IgA

IgA is the fifth most prevalent component in HM, with smaller amounts of IgG and IgM in HM.⁷⁷ Present mostly in secretory form, secretory IgA controls gut microbiota and intestinal homeostasis.⁷⁸ Plasma cells in the lamina propria of the gut produce mucosal IgAs, which are transported by the polymeric immunoglobulin receptor across the epithelium.⁷⁹ B cells that originate from the maternal gut migrate to the mammary tissue via the enteromammary link and secrete specific IgA that enters HM.⁸⁰ This process is regulated by the mucosal vascular addressin MadCAM-1, a protein that interacts with both the mucosa-associated CCR10⁸¹ and the gut-targeting receptor $\alpha_4\beta_7$ -integrin.⁸² Given the interaction between these components, it is hypothesized that the specificity of IgA in a mother's milk is a reflection of the antigenic exposure her immune system experiences to dietary proteins in her gut.

Consistent with prior reports,^{83,84} in a prospective birth cohort oversampled for factors that confer high risk of FA development, we found that increased levels of both total IgA⁸⁵ and cow's milk

specific IgA⁷⁵ in HM were associated with protection against CMA. Maternal exposures dictated by geography, microbial burden, and contact with animals (specifically farm animals and cats) are likely major determinants of the levels and specificity of the IgA observed in HM.⁷⁷ We also found that a restriction of cow's milk from the maternal diet was associated with reduced levels of cow's milk specific IgA levels in HM when compared with mothers with unrestricted diets,⁷⁵ implying that the dietary antigenic exposure of the maternal immune system via the gastrointestinal tract shifts the antibody specificity observed in HM. This could be the reason why maternal diets restricting highly allergenic foods may not be advantageous in prevention of FA. We also found that the IgA in HM had different specificity to cow's milk antigens than the IgA in serum, indicating that distinct antibody-producing lymphocytes likely contribute to HM and serum IgA, thereby providing further evidence of the enteromammary link.⁸⁶ This evidence suggests that maternal diet influences the specific IgA composition of HM. An active connection between immune tissues in the maternal gut and the mammary gland results in a HM-specific IgA profile influenced by the mother's dietary exposures.

Although the precise function of HM IgA remains unclear, it is believed to provide passive protection until infant production of IgA commences after birth.⁸⁷ During lactation, IgAs also influence the microbiome composition of the infant, conferring protection to colitis in adulthood.⁷⁸ In summary, the IgA found in HM is shaped by many factors, including maternal exposures that vary based on diet, geographic location, and exposure to microbes, among others. These immunoglobulins are likely an important factor in the prevention of CMA in the infant. No studies have assessed the effect of immunoglobulins or antibodies in other FA.

Cytokines, Chemokines, and Growth Factors

HM contains numerous immunomodulating cytokines that stimulate or suppress immune cells.⁷⁷ Whereas some are found in

relatively low concentrations in HM, others, such as TGF- β , are found in larger quantities.⁸⁸ Interindividual variation in HM cytokine levels may stem from differences in microbial exposures; TGF- β , IFN- γ , and IL-10 levels vary based on maternal country of origin and country of residence.⁷⁷

TGF- β is a key regulatory cytokine involved in the inhibition of T_H1 and T_H2 pathways and, thus far, is the most well-studied soluble factor in HM. Considering all 3 isoforms, TGF- β is the predominant cytokine in HM, the most common isoform being TGF- β 2.⁷⁷ TGF- β is involved in the induction of production of specific IgA and intestinal epithelial cell maturation.⁸⁹ A 2010 review by Oddy and Rosales⁹⁰ included 12 studies conducted in humans and reported that 8 of the studies found that higher levels of TGF- β 1 or TGF- β 2 resulted in lower risk of atopic outcomes in the first years of life; however, the results from other studies differed. A summary of association between TGF- β and the development of FA can be found in Table 3. Although 3 studies did not find an association between TGF- β 1 (and in 1 study also TGF- β 2) levels in milk and FA, 2 studies found that higher levels were associated with a lower risk of FA. Among the latter 2 studies, the first study of FA found that mothers of infants with IgE-mediated CMA had lower TGF- β 1 concentrations in colostrum than mothers of infants with non-IgE-mediated CMA and that the concentration of TGF- β 1 in healthy controls fell between these 2 groups⁹¹ (Table 2). The second study found a protective association between a group of several proinflammatory and regulatory cytokines (including TGF- β 1) in HM and CMA.⁸⁸ On the basis of these studies, the role of TGF- β levels on FA development has not been conclusively determined and may differ among different types of FA. Additional studies specifically evaluating FA are needed to better understand the influence of TGF- β on this process.

New and ongoing studies on the effect of additional cytokines and chemokines present in HM on the development of atopic conditions have produced variable results.⁷⁷ Several authors report an absence or very low concentrations of several of these bioactive substances, including IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IFN- γ , CCL5, CXCL8, CXCL10, and TNF- α . These studies found no association between these compounds and atopy development.⁷⁷ Table 2 summarizes the current literature evaluating the associations between cytokines and FA development.^{72,88,91–96} Our study found that protection against CMA was associated with the levels of IL-1 β , IL-6, IL-10, and TGF- β 1 in HM.⁸⁸ It is not possible to determine from this study whether these chemokines and cytokines are biomarkers of the mechanism that confers protection from CMA or if they are directly related to the process of protection. However, given that these factors promote differentiation of T_H17 cells, the production of IgA, and crosstalk between the ROR γ t⁺ innate lymphoid cell 3 population and gut macrophages,⁸⁸ it is plausible that the chemical factors we detected in HM samples are directly involved in promoting protective immunomodulation. Another study reported that increased IL-13 concentration in both colostrum and mature HM were associated with lower risk of FA and eczema, respectively, by parent report. These authors found no significant association among levels of IL-2, IL-4, IL-5, IL-10, IL-12, and IFN- γ with either of these conditions or wheeze.⁹⁵ Finally, a recent study using the case-cohort design among the Early Versus Delayed Enteral Feeding to Treat People With Acute Lung Injury or Acute Respiratory Distress Syndrome (EDEN) birth cohort found a positive association between the concentration of IL-2, CXCL10, and TNF- β and reported FA in childhood.⁹⁶ Higher concentrations of IL-1 β and IL-17 were found in mothers who reported increased physical activity.⁷⁷ Overall, maternal atopic status was not systematically associated with cytokine levels in HM.⁸⁸ Overall, these studies found the strongest association between high levels of TGF- β and perhaps some other regulatory and inflammatory cytokines and decreased FA risk, but data are few and to some extent conflicting.

HMOs

HMOs are abundant sugar chains found exclusively in HM. During the period of exclusive breastfeeding, the infant's gut microbiome feeds primarily off of these HMOs, which promotes the growth of *Bifidobacterium* and *Bacteroides* species, leading to their predominance in the gut microbiome of exclusively breastfed infants.⁹⁷ The infant is unable to digest these HMOs, which originate as lactose molecules that are modified by a series of glycosyltransferases, enzymes, which are encoded by the genes responsible for the Lewis blood groups and secretor status. Two fucosyltransferases, FUT2 (secretor gene) and FUT3 (Lewis gene), are responsible for the addition of fucose. Mothers express a unique set of enzymes and synthesize their own variety of HMOs based on which allelic variants they express. Resulting from this genetic variation, HM demonstrates significant interindividual heterogeneity in HMO composition, both in terms of the oligosaccharides present and their relative abundance. This variation indicates that breastfed infants are exposed only to certain HMOs in their mother's milk. A total of 15% to 25% (varying by ethnic background) of mothers lack a functional FUT2 enzyme (FUT2^{-/-})^{98,99} and are labeled nonsecretors, given that they lack all HMOs containing α 2-linked fucose.¹⁰⁰ Infants who receive HM from nonsecretor mothers have microbiota with later establishment of bifidobacteria predominance.¹⁰¹ The study by Sprenger et al.¹⁰⁰ found that infants who were born by cesarean section and fed by nonsecretor mothers had an increased risk of developing IgE-associated atopic dermatitis. This study did not evaluate the risk of FA development and did not consider the concentration of individual HMOs. In our study, infants fed HM that contained low concentrations of lacto-N-fucopentaose III had a higher risk of developing CMA compared with infants who received HM with higher levels of this oligosaccharide.¹⁰² Similarly, in a report from the Canadian Healthy Infant Longitudinal Development (CHILD) study, authors evaluated the association between HMO profiles and food sensitization at 12 months and found that the presence of specific HMOs conferred reduced risk of food sensitization in the infant.¹⁰³ Taken together, these findings suggest that specific HMO profiles may result in protection from the development of FA and that this protection may be conferred via their effect on infant gut microbiome.

The protection observed in these studies may be attributable to the effect of HMOs on gut microbiome or direct effects on immune cells. Certain HMOs have demonstrated anti-inflammatory characteristics and promote the maturation of the gastrointestinal immune system. Some HMOs inhibit intestinal cell growth, whereas others interact with dendritic cells via the lectin receptor dendritic cell-specific intercellular adhesion molecule 3–grabbing non-integrin.⁷⁷ Additional studies with a focus on the specific HMO composition of HM will be required to more clearly elucidate the role of these compounds in mechanisms of atopic disease development and prevention.

Conclusions

The composition of immunologically active substances and significant interpersonal variation of HM have yet to be fully described. Past epidemiologic studies have often failed to account for these variables and have historically operationalized HM exposure crudely in ways that may obscure associations of interest. In addition, the outcome definitions for FA also vary from study to study, with some authors using the gold standard oral food challenge, others relying on clinical diagnosis, and still others reporting associations with evidence of sensitization. Because of these challenges, in combination with a number of factors that have historically not been accounted for, including wide variation in biologically active components in HM, differences in maternal diet, microbial

exposures, and geographic differences, it is not surprising that findings have been inconsistent in the literature thus far. Because of ethical considerations given the established benefits of breastfeeding, large-scale randomized trials with assignment to infant diet type are lacking, which makes simultaneously controlling for all of these factors challenging. To date, most studies have lacked the statistical power to properly evaluate the association between breastfeeding and FA or have avoided FA as an outcome altogether because of these challenges.

Improved understanding of maternal-offspring interactions from animal models, in parallel with human studies, will contribute to the development of novel strategies through breastfeeding to prevent and treat FA. The challenges with animal studies include its limited applicability to human systems. In rodents, FcRn functions in the neonatal period, transporting maternally derived IgG in milk across the epithelial barrier in the gut, whereas in humans, FcRn transports robustly maternal IgG to the fetus across placenta.¹⁰⁴ Furthermore, the composition of breast milk differs between these species; for example, milk oligosaccharides are much more complex in human than in mouse. In addition, the ratio of IgA to IgG differs in human and murine milk. These and other differences may limit the ability to fully translate findings in animal studies to those mechanisms that are biologically similar, although the strengths of animal models include being able to answer specific questions that are difficult or unethical to address in humans.

The studies reviewed highlight the complex, variable composition of HM and the effect that differences in HM characteristics may have on FA development or prevention. The biologically active components of HM may either directly affect FA risk or perhaps indirectly influence this process through an effect on gut microbiota or other still unknown mechanisms. Dietary allergens in HM in combination with immunoglobulins may provide a tolerogenic environment, explaining the reduced risk seen in some studies. On the basis of all the information available to date, it is clear that it is critical to consider the combination of each of these factors and that the effect of this complex system considered together is likely greater than the sum of each individual part. To enhance our understanding of this system, future studies must evaluate the networks of bioactive components in HM, assess their effects on the development of the infant immune system and microbiome as a system, and recognize that this field is still in its infancy. Clarification of the role of these HM components may lead to key targets for the primary prevention and potential treatment of FA. Large prospective cohort studies that include careful characterization of exposure to these key HM components, as well as careful outcome consideration, would provide critical evidence regarding mechanisms and targetable factors in the development of atopic diseases.

References

1. Victora CG, Bahl R, Barros AJ, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *Lancet*. 2016;387:475–490.
2. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev*. 2012;CD003517.
3. Bachrach VR, Schwarz LR. Breastfeeding and the risk of hospitalization for respiratory disease in infancy: a meta-analysis. *Arch Pediatr Adolesc Med*. 2003;237–243.
4. Fleischer DM, Spergel JM, Assa'ad AH, Pongracic JA. Primary prevention of allergic disease through nutritional interventions. *J Allergy Clin Immunol Pract*. 2013;1:29–36.
5. Muraro A, Halken S, Arshad SH, et al. EAAACI food allergy and anaphylaxis guidelines. Primary prevention of food allergy. *Allergy*. 2014;69:590–601.
6. Greer FR, Sicherer SH, Burks AW. 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*. 2008;121:183–191.
7. van Oudijk J, Kull I, Borres MP, et al. Breastfeeding and allergic disease: a multidisciplinary review of the literature (1966–2001) on the mode of early feeding in infancy and its impact on later atopic manifestations. *Allergy*. 2003;58:833–843.
8. Lodge CJ, Tan DJ, Lau MX, et al. Breastfeeding and asthma and allergies: a systematic review and meta-analysis. *Acta Paediatr*. 2015;104:38–53.
9. de Silva D, Geromi M, Halken S, et al. Primary prevention of food allergy in children and adults: systematic review. *Allergy*. 2014;69:581–589.
10. Goldsmith AJ, Koplin JJ, Lowe AJ, et al. Formula and breast feeding in infant food allergy: a population-based study. *J Paediatr Child Health*. 2016;52:377–384.
11. Kramer MS, Chalmers B, Hodnett ED, et al. Promotion of Breastfeeding Intervention Trial (PROBIT): a randomized trial in the Republic of Belarus. *JAMA*. 2001;285:413–420.
12. Kramer MS, Matush L, Vanilovich I, et al. Effect of prolonged and exclusive breast feeding on risk of allergy and asthma: cluster randomised trial. *BMJ*. 2007;335:815.
13. Hong X, Wang G, Liu X, et al. Gene polymorphisms, breast-feeding, and development of food sensitization in early childhood. *J Allergy Clin Immunol*. 2011;128:374–381 e372.
14. Gao X, McMahon RJ, Woo JG, Davidson BS, Morrow AL, Zhang Q. Temporal changes in milk proteomes reveal developing milk functions. *J Proteome Res*. 2012;11:3897–3907.
15. Andreas NJ, Kampmann B, Mehring Le-Doare K. Human breast milk: a review on its composition and bioactivity. *Early Hum Dev*. 2015;91:629–635.
16. Järvinen KM, Bergmann KE, Bergmann R. Breast—always best? In: Wahn U, Sampson HA, eds. *Allergy, Immunity and Tolerance in Early Childhood: The First Steps of the Atopic March*. Amsterdam, the Netherlands: Elsevier; 2015:235.
17. Azad MB, Konya T, Maughan H, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *CMAJ*. 2013;185:385–394.
18. Levin AM, Sitarik AR, Havstad SL, et al. Joint effects of pregnancy, sociocultural, and environmental factors on early life gut microbiome structure and diversity. *Sci Rep*. 2016;6:31775.
19. Bergstrom A, Skov TH, Bahl MI, et al. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. *Appl Environ Microbiol*. 2014;80:2889–2900.
20. Backhed F, Roswall J, Peng Y, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe*. 2015;17:690–703.
21. Voreades N, Kozil A, Weir TL. Diet and the development of the human intestinal microbiome. *Front Microbiol*. 2014;5:494.
22. Subramanian S, Huq S, Yatsunenkov T, et al. Persistent gut microbiota immaturity in malnourished Bangladeshi children. *Nature*. 2014;510:417–421.
23. Pannaraj PS, Li F, Cerini C, et al. Association between breast milk bacterial communities and establishment and development of the infant gut microbiome. *JAMA Pediatr*. 2017;171:647–654.
24. Kourosh A, Luna RA, Balderas M, et al. Fecal microbiome signatures are different in food-allergic children compared to siblings and healthy children. *Pediatr Allergy Immunol*. 2018;29:545–554.
25. Fieten KB, Totte JEE, Levin E, et al. Fecal microbiome and food allergy in pediatric atopic dermatitis: a cross-sectional pilot study. *Int Arch Allergy Immunol*. 2018;175:77–84.
26. Fazlollahi M, Chun Y, Grishin A, et al. Early-life gut microbiome and egg allergy. *Allergy*. 2018;73:1515–1524.
27. Bunyavanich S. Food allergy: could the gut microbiota hold the key? *Nat Rev Gastroenterol Hepatol*. 2019;16:201–202.
28. Kumari M, Kozyskyj AL. Gut microbial metabolism defines host metabolism: an emerging perspective in obesity and allergic inflammation. *Obes Rev*. 2017;18:18–31.
29. Tan J, McKenzie C, Vuillermin PJ, et al. Dietary fiber and bacterial SCFA enhance oral tolerance and protect against food allergy through diverse cellular pathways. *Cell Rep*. 2016;15:2809–2824.
30. Bridgman SL, Azad MB, Field CJ, et al. Fecal short-chain fatty acid variations by breastfeeding status in infants at 4 months: differences in relative versus absolute concentrations. *Front Nutr*. 2017;4:11.
31. Oyoshi MK, Oettgen HC, Chatila TA, Geha RS, Bryce PJ. Food allergy: Insights into etiology, prevention, and treatment provided by murine models. *J Allergy Clin Immunol*. 2014;133:309–317.
32. Verhasselt V, Milcent V, Cazareth J, et al. Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma. *Nat Med*. 2008;14:170–175.
33. Mosconi E, Rekima A, Seitz-Polski B, et al. Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development. *Mucosal Immunol*. 2010;3:461–474.
34. Hamada K, Suzuki Y, Goldman A, et al. Allergen-independent maternal transmission of asthma susceptibility. *J Immunol*. 2003;170:1683–1689.
35. Macchiaverni P, Rekima A, Turfkruyer M, et al. Respiratory allergen from house dust mite is present in human milk and primes for allergic sensitization in a mouse model of asthma. *Allergy*. 2014;69:395–398.
36. Järvinen KM, Westfall J, De Jesus M, et al. Role of maternal dietary peanut exposure in development of food allergy and oral tolerance. *PLoS One*. 2015;10:e0143855.

37. Bernard H, Ah-Leung S, Drumare MF, et al. Peanut allergens are rapidly transferred in human breast milk and can prevent sensitization in mice. *Allergy*. 2014;69:888–897.
38. Lopez-Exposito I, Song Y, Jarvinen KM, Srivastava K, Li XM. Maternal peanut exposure during pregnancy and lactation reduces peanut allergy risk in offspring. *J Allergy Clin Immunol*. 2009;124:1039–1046.
39. Ohsaki A, Venturelli N, Buccigrosso TM, et al. Maternal IgG immune complexes induce food allergen-specific tolerance in offspring. *J Exp Med*. 2018;215:91–113.
40. Bartnikas LM, Gurish MF, Burton OT, et al. Epicutaneous sensitization results in IgE-dependent intestinal mast cell expansion and food-induced anaphylaxis. *J Allergy Clin Immunol*. 2013;131:451–460.e451–456.
41. Burton OT, Logsdon SL, Zhou JS, et al. Oral immunotherapy induces IgG antibodies that act through FcγRIIb to suppress IgE-mediated hypersensitivity. *J Allergy Clin Immunol*. 2014;134:1310–1317.e1316.
42. Turfkruyer M, Rekima A, Macchiaverni P, et al. Oral tolerance is inefficient in neonatal mice due to a physiological vitamin A deficiency. *Mucosal Immunol*. 2016;9:479–491.
43. Rekima A, Macchiaverni P, Turfkruyer M, et al. Long-term reduction in food allergy susceptibility in mice by combining breastfeeding-induced tolerance and TGF-β-enriched formula after weaning. *Clin Exp Allergy*. 2017;47:565–576.
44. Hogan SP, Wang YH, Strait R, Finkelman FD. Food-induced anaphylaxis: mast cells as modulators of anaphylactic severity. *Semin Immunopathol*. 2012;34:643–653.
45. Caffarelli C, Romanini E, Caruana P, Street ME, de' Angelis G. Clinical food hypersensitivity: the relevance of duodenal immunoglobulin E-positive cells. *Pediatr Res*. 1998;44:485–490.
46. Brandt EB, Strait RT, Hershko D, et al. Mast cells are required for experimental oral allergen-induced diarrhea. *J Clin Invest*. 2003;112:1666–1677.
47. Burton OT, Darling AR, Zhou JS, et al. Direct effects of IL-4 on mast cells drive their intestinal expansion and increase susceptibility to anaphylaxis in a murine model of food allergy. *Mucosal Immunol*. 2013;6:740–750.
48. Husby S, Oxelius VA, Teisner B, Jensenius JC, Svehag SE. Humoral immunity to dietary antigens in healthy adults. Occurrence, isotype and IgG subclass distribution of serum antibodies to protein antigens. *Int Arch Allergy Appl Immunol*. 1985;77:416–422.
49. Schwarz A, Panetta V, Cappella A, Hofmaier S, Hatzler L, Rohrbach A, et al. IgG and IgG4 to 91 allergenic molecules in early childhood by route of exposure and current and future IgE sensitization: results from the Multicentre Allergy Study birth cohort. *J Allergy Clin Immunol*. 2016;138:1426–1433.e12.
50. Hirose J, Ito S, Hirata N, Kido S, Kitabatake N, Narita H. Occurrence of the major food allergen, ovomucoid, in human breast milk as an immune complex. *Biosci Biotechnol Biochem*. 2001;65:1438–1440.
51. Rumbo M, Chirido FG, Anon MC, Fossati CA. Detection and characterization of antibodies specific to food antigens (gliadin, ovalbumin and beta-lactoglobulin) in human serum, saliva, colostrum and milk. *Clin Exp Immunol*. 1998;112:453–458.
52. Hochwallner H, Alm J, Lupinek C, et al. Transmission of allergen-specific IgG and IgE from maternal blood into breast milk visualized with microarray technology. *J Allergy Clin Immunol*. 2014;134:1213–1215.
53. Schwarz A, Panetta V, Cappella A, et al. IgG and IgG4 to 91 allergenic molecules in early childhood by route of exposure and current and future IgE sensitization: results from the Multicentre Allergy Study birth cohort. *J Allergy Clin Immunol*. 2016;138:1426–1433 e1412.
54. Metcalfe JR, Marsh JA, D'Vaz N, et al. Effects of maternal dietary egg intake during early lactation on human milk ovalbumin concentration: a randomized controlled trial. *Clin Exp Allergy*. 2016;46:1605–1613.
55. Palmer DJ, Gold MS, Makrides M. Effect of cooked and raw egg consumption on ovalbumin content of human milk: a randomized, double-blind, crossover trial. *Clin Exp Allergy*. 2005;35:173–178.
56. Sorva R, Makinen-Kiljunen S, Juntunen-Backman K. Beta-lactoglobulin secretion in human milk varies widely after cow's milk ingestion in mothers of infants with cow's milk allergy. *J Allergy Clin Immunol*. 1994;93:787–792.
57. Høst A, Husby S, Osterballe O. A prospective study of cow's milk allergy in exclusively breast-fed infants. Incidence, pathogenetic role of early inadvertent exposure to cow's milk formula, and characterization of bovine milk protein in human milk. *Acta Paediatr Scand*. 1988 Sep;77(5):663–670.
58. Chirido FG, Rumbo M, Anon MC, Fossati CA. Presence of high levels of non-degraded gliadin in breast milk from healthy mothers. *Scand J Gastroenterol*. 1998;33:1186–1192.
59. Schocker F, Baumert J, Kull S, Petersen A, Becker WM, Jappe U. Prospective investigation on the transfer of Ara h 2, the most potent peanut allergen, in human breast milk. *Pediatr Allergy Immunol*. 2016;27:348–355.
60. Schocker F, Scharf A, Kull S, Jappe U. Detection of the Peanut Allergens Ara h 2 and Ara h 6 in human breast milk: development of 2 sensitive and specific sandwich ELISA assays. *Int Arch Allergy Immunol*. 2017;174:17–25.
61. Vadas P, Wai Y, Burks W, Perelman B. Detection of peanut allergens in breast milk of lactating women. *JAMA*. 2001;285:1746–1748.
62. Jarvinen KM, Makinen-Kiljunen S, Suomalainen H. Cow's milk challenge through human milk evokes immune responses in infants with cow's milk allergy. *J Pediatr*. 1999;135:506–512.
63. Fox AT, Sasieni P, du Toit G, Syed H, Lack G. Household peanut consumption as a risk factor for the development of peanut allergy. *J Allergy Clin Immunol*. 2009;123:417–423.
64. Sicherer SH, Wood RA, Stablein D, et al. Maternal consumption of peanut during pregnancy is associated with peanut sensitization in atopic infants. *J Allergy Clin Immunol*. 2010;126:1191–1197.
65. Pitt TJ, Becker AB, Chan-Yeung M, et al. Reduced risk of peanut sensitization following exposure through breast-feeding and early peanut introduction. *J Allergy Clin Immunol*. 2018;141:620–625.e621.
66. Du Toit G, Katz Y, Sasieni P, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J Allergy Clin Immunol*. 2008;122:984–991.
67. DesRoches A, Infante-Rivard C, Paradis L, Paradis J, Haddad E. Peanut allergy: is maternal transmission of antigens during pregnancy and breastfeeding a risk factor? *J Invest Allergol Clin Immunol*. 2010;20:289–294.
68. Herrmann ME, Dannemann A, Gruters A, et al. Prospective study of the atopy preventive effect of maternal avoidance of milk and eggs during pregnancy and lactation. *Eur J Pediatr*. 1996;155:770–774.
69. Hattevig G, Sigurs N, Kjellman B. Effects of maternal dietary avoidance during lactation on allergy in children at 10 years of age. *Acta Paediatr*. 1999;88:7–12.
70. Nwaru BI, Erkkola M, Ahonen S, et al. Maternal diet during lactation and allergic sensitization in the offspring at age of 5. *Pediatr Allergy Immunol*. 2011;22:334–341.
71. Tuokkola J, Luukkainen P, Tapanainen H, et al. Maternal diet during pregnancy and lactation and cow's milk allergy in offspring. *Eur J Clin Nutr*. 2016;70:554–559.
72. Frazier AL, Camargo Jr CA, Malspeis S, Willett WC, Young MC. Prospective study of peripregnancy consumption of peanuts or tree nuts by mothers and the risk of peanut or tree nut allergy in their offspring. *JAMA Pediatr*. 2014;168:156–162.
73. Des Roches A, Bégin P, Infante-Rivard C, Paradis J, Paradis L. Peanut allergy and the impact of maternal consumption during pregnancy and breastfeeding. *J Allergy Clin Immunol*. 2011;128:248–249.
74. Sicherer SH, Wood RA, Stablein D, et al. Reply. *J Allergy Clin Immunol*. 2011;128:249–250.
75. Jarvinen KM, Westfall JE, Seppo MS, et al. Role of maternal elimination diets and human milk IgA in the development of cow's milk allergy in the infants. *Clin Exp Allergy*. 2014;44:69–78.
76. Bunyavanich S, Rifas-Shiman SL, Platts-Mills TA, et al. Peanut, milk, and wheat intake during pregnancy is associated with reduced allergy and asthma in children. *J Allergy Clin Immunol*. 2014;133:1373–1382.
77. Rajani PS, Seppo AE, Jarvinen KM. Immunologically active components in human milk and development of atopic disease, with emphasis on food allergy, in the pediatric population. *Front Pediatr*. 2018;6:218.
78. Rogier EW, Frantz AL, Bruno ME, et al. Secretory antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and host gene expression. *Proc Natl Acad Sci U S A*. 2014;111:3074–3079.
79. Kaetzel CS. Cooperativity among secretory IgA, the polymeric immunoglobulin receptor, and the gut microbiota promotes host-microbial mutualism. *Immunol Lett*. 2014;162:10–21.
80. Hanson LA, Korotkova M. The role of breastfeeding in prevention of neonatal infection. *Semin Neonatol*. 2002;7:275–281.
81. Morteau O, Gerard C, Lu B, et al. An indispensable role for the chemokine receptor CCR10 in IgA antibody-secreting cell accumulation. *J Immunol*. 2008;181:6309–6315.
82. Tanneau GM, Hibrand-Saint Oyant L, Chevalerey CC, Salmon HP. Differential recruitment of T- and IgA B-lymphocytes in the developing mammary gland in relation to homing receptors and vascular addressins. *J Histochem Cytochem*. 1999;47:1581–1592.
83. Machtinger S, Moss R. Cow's milk allergy in breast-fed infants: the role of allergen and maternal secretory IgA antibody. *J Allergy Clin Immunol*. 1986;77:341–347.
84. Savilahti E, Tainio VM, Salmenpera L, et al. Low colostral IgA associated with cow's milk allergy. *Acta Paediatr Scand*. 1991;80:1207–1213.
85. Jarvinen KM, Laine ST, Jarvenpää AL, Suomalainen HK. Does low IgA in human milk predispose the infant to development of cow's milk allergy? *Pediatr Res*. 2000;48:457–462.
86. Seppo AE, Savilahti EM, Berin MC, Sampson HA, Jarvinen KM. Breast milk IgA to foods has different epitope specificity than serum IgA-Evidence for entero-mammary link for food-specific IgA? *Clin Exp Allergy*. 2017;47:1275–1284.
87. Renz H, Brandtzaeg P, Hornef M. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. *Nat Rev Immunol*. 2011;12:9–23.
88. Jarvinen KM, Suarez-Farinas M, Savilahti E, Sampson HA, Berin MC. Immune factors in breast milk related to infant milk allergy are independent of maternal atopy. *J Allergy Clin Immunol*. 2015;135:1390–1393.e1391–1396.
89. Rautava S, Lu L, Nanthakumar NN, Dubert-Ferrandon A, Walker WA. TGF-β2 induces maturation of immature human intestinal epithelial cells and inhibits inflammatory cytokine responses induced via the NF-κB pathway. *J Pediatr Gastroenterol Nutr*. 2012;54:630–638.
90. Oddy WH, Rosales F. A systematic review of the importance of milk TGF-β on immunological outcomes in the infant and young child. *Pediatr Allergy Immunol*. 2010;21:47–59.
91. Saarinen KM, Vaarala O, Klemetti P, Savilahti E. Transforming growth factor-β1 in mothers' colostrum and immune responses to cows' milk proteins in infants with cows' milk allergy. *J Allergy Clin Immunol*. 1999;104:1093–1098.
92. Bottcher MF, Jenmalm MC, Björkstén B. Cytokine, chemokine and secretory IgA levels in human milk in relation to atopic disease and IgA production in infants. *Pediatr Allergy Immunol*. 2003;14:35–41.
93. Snijders BE, Damoiseaux JG, Penders J, et al. Cytokines and soluble CD14 in breast milk in relation with atopic manifestations in mother and infant (KOALA Study). *Clin Exp Allergy*. 2006;36:1609–1615.

94. Kuitunen M, Kukkonen AK, Savilahti E. Impact of maternal allergy and use of probiotics during pregnancy on breast milk cytokines and food antibodies and development of allergy in children until 5 years. *Int Arch Allergy Immunol*. 2012;159:162–170.
95. Munblit D, Treneva M, Peroni DG, et al. Immune components in human milk are associated with early infant immunological health outcomes: a prospective three-country analysis. *Nutrients*. 2017;9:E532.
96. Berdi M, de Lauzon-Guillain B, Forhan A, et al. Immune components of early breastmilk: association with maternal factors and with reported food allergy in childhood. *Pediatr Allergy Immunol*. 2019;30(1):107–116.
97. Yu ZT, Chen C, Newburg DS. Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes. *Glycobiology*. 2013;23:1281–1292.
98. Castanys-Munoz E, Martin MJ, Prieto PA. 2'-fucosyllactose: an abundant, genetically determined soluble glycan present in human milk. *Nutr Rev*. 2013;71:773–789.
99. Erney RM, Malone WT, Skelding MB, et al. Variability of human milk neutral oligosaccharides in a diverse population. *J Pediatr Gastroenterol Nutr*. 2000;30:181–192.
100. Sprenger N, Odenwald H, Kukkonen AK, Kuitunen M, Savilahti E, Kunz C. FUT2-dependent breast milk oligosaccharides and allergy at 2 and 5 years of age in infants with high hereditary allergy risk. *Eur J Nutr*. 2017;56(3):1293–1301.
101. Lewis ZT, Totten SM, Smilowitz JT, et al. Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants. *Microbiome*. 2015;3:13.
102. Seppo AE, Autran CA, Bode L, Jarvinen KM. Human milk oligosaccharides and development of cow's milk allergy in infants. *J Allergy Clin Immunol*. 2017;139:708–711.e705.
103. Miliku K, Robertson B, Sharma AK, et al. Human milk oligosaccharide profiles and food sensitization among infants in the CHILD Study. *Allergy*. 2018;73(10):2070–2073.
104. Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. *Nat Rev Immunol*. 2007;7:715–725.