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Immunologic components in human milk and allergic diseases with focus on food allergy

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ABSTRACT

Human milk contains a wide range of immunomodulatory factors, including immunoglobulins, human milk oligosaccharides, cytokines, microbiome, innate factors and food antigens. Maternal diet can influence the content of human milk as it is well-established that dietary antigens can be secreted in human milk after maternal consumption, but whether these dietary antigens promote tolerance or sensitization in the infant is a subject of debate. This review summarizes the current literature on these immunologically active factors in human milk, including the microbiome, innate factors, and maternal diet-derived dietary antigens in the context of development of allergic diseases, with the focus on food allergy.

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Introduction

Atopic diseases are rapidly increasing worldwide with food allergy (FA) affecting 7.6% of children in the United States.¹ Genetics and environment can impact the development of atopic disease; the perinatal period is crucial as the gut microbiota is established and the immune system matures. Human milk (HM) has the ability to shape the maturing immune system. The benefits of HM include lessening the risk of atopic disease, including atopic eczema, asthma and respiratory allergies, with limited data on FA.²⁻⁴ There are many active components of HM that have been shown to play a role in or are associated with the development of allergic disease, including cytokines, immunoglobulins, growth factors, human oligosaccharides, and dietary antigens secreted in HM; their levels vary between mothers and may explain varying levels of protection. Other factors include milk microbiome, innate factors, defensins, lactoferrin, chemokines, growth factors, fatty acids such as long-chain

polyunsaturated fatty acid (PUFAs) and lactoperoxidase. The former have been recently extensively reviewed in the context of FA⁵, which we will briefly summarize here. The focus of this review will be on the microbiome and innate factors which have not been extensively reviewed, and maternal diet-derived dietary antigens present in HM, but commonly come up in discussions with breastfeeding mothers.

Human Milk Oligosaccharides (HMOs)

HMOs have many functions in the developing neonate, including shaping the gut microbiome composition, enhancing host epithelial and immune cell responses, and blocking attachment of microbial pathogens.⁶ The infant gut microbiome is influenced by many factors including mode of delivery, antibiotic use, gestational age, and mode of feeding (formula-fed or breastfed).⁷ Breastfed infants have significantly different composition of microbiome compared to formula fed infants.⁷⁻⁹ Breastfed infants have greater representation of *Bifidobacterium* and

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Lactobacillus and formula-fed infants have enrichment of *Clostridia* and *Escherichia coli*.^{8,9} The gut microbiome shifts when breastfeeding ceases to become similar to the adult microbiota with greater predominance of *Bacteroides*.⁹ HMOs are complex sugars that are unique to HM and provide selective fuel for the microbiome, specifically *Bifidobacterium*¹⁰, which explains the predominance of these bacteria in exclusively breastfed infants. Previous studies have shown that lower relative abundance of *Bifidobacteria*, such as *B. adolescentis* and *B. breve* is associated with FA in children with atopic dermatitis¹¹ and enrichment for specific microbes within the *Clostridia* class and *Firmicutes* phylum was associated with IgE-mediated FA.¹² A previous study has also shown a lower relative abundance of *Bifidobacteria* at 3 months of age to precede multi-sensitized atopy.¹³

Particular HMO profiles have also been associated with a greater risk of food sensitization¹⁴ and we have previously demonstrated that mothers of infants with cow's milk allergy (CMA) had lower amounts of specific HMOs, such as Lacto-N-fucopentaose III (LFNP-III) in their breast milk compared to mothers of infants without CMA.¹⁵ Lodge et al. identified specific HMO profiles associated with increased risk of allergic respiratory disease and decreased risk of food sensitization.¹⁶ HMO profiles may associate with the protection against FA through the infant gut microbiome or intestinal immune maturation.

Human milk cytokines

Cytokines are signaling molecules that facilitate communication between cells and play an integral role in guiding the immune system. HM contains a wide range of cytokines including transforming growth factor- β (TGF- β), interferon- γ (IFN- γ), interleukins (IL), and chemokines.^{17,18} Different factors can impact the cytokine content in HM. Higher levels of IL-6, IL-8 and TGF- β 1 were seen in women who grew up in a developing country compared to women born in a developed country.¹⁹ One cytokine of particular interest is TGF- β which is an anti-inflammatory cytokine that suppresses the Th2 and Th1-mediated pathways. TGF- β is abundant in HM.¹⁷ In neonatal mouse models, TGF- β plays a role in the maturation of the mucosal immune system and protection against asthma.²⁰ There is also evidence that TGF- β supports protection against atopic disease, particularly atopic dermatitis, and promotes specific IgA production in humans.²¹ Although data are conflicting, one systematic review of a dozen human studies found that TGF- β was associated with preventing atopic disease in infancy and early childhood.²²

There are fewer data on other cytokines.⁵ Higher levels of IL-13 in colostrum and mature milk were associated with lower risk of FA and eczema, respectively²³, whereas in another study increased levels of IL-2, CXCL10 and TNF- β associated with decreased risk of FA.²⁴ Lastly, increased levels of IL-1 β and IL-6 were shown to be associated with less atopic dermatitis²⁵ and CMA.²⁶

Immunoglobulins

Immunoglobulin A (IgA) is the most abundant immunoglobulin in HM followed by IgG and IgM.²⁷ Specific IgA secreted in

HM is created by lymphocytes that originated in the maternal gut and migrated to the mammary tissue through the entero-mammary link.²⁸ IgA reinforces the epithelial barrier and coats commensal bacteria to prevent microorganisms and antigens from inappropriately activating the immune system.²⁹ Colostrum from mothers of preterm infants contains higher protein content with higher amounts of immunoglobulins compared to term infants.²⁷

Maternal diet may influence immunoglobulin levels in HM. We found that restriction of CM in the maternal diet was associated with lower levels of cow's milk-specific IgA in HM.³⁰ High levels of total IgA and cow's milk-specific IgA in HM was, in turn, associated with protection against CMA.³¹ Mouse models have also suggested that maternal immunoglobulins and immune complexes in breast milk can promote neonatal tolerance induction.³² Mice were epicutaneously sensitized with ovalbumin and offspring of sensitized mice were protected against anaphylaxis and specific serum IgE sensitization. The mechanism of protection was via FcRn-dependent transfer of maternal allergen, immune complexes and IgG in breast milk to the offspring and subsequent development of allergen-specific T-regulatory cells in the offspring.³² These data show that transfer of maternal IgA or IgG-allergen immune complexes in breast milk may induce tolerance in the offspring.

Human milk microbiome

HM contains microbiota shown to be variable between mothers, likely due to differences in methods or detection and geographic locations.³³ The bacteria detected by culture methods include facultative *Streptococcus* and *Staphylococcus* spp, *Propionibacteria* and *Enterococcus*. The only viable gut-associated anaerobes were *Veillonella* and *Peptostreptococcus*. However, non-culture-dependent detection methods have found also members of the *Bacteroidetes* phylum, *Bifidobacteria* genus, and *Clostridia* class including *Blautia*, *Clostridium*, *Dorea*, *Eubacterium* and *Ruminococcus*. Also, butyrate producing *Coprococcus*, *Faecalibacterium*, *Roseburia* and *Subdoligranulum* have been detected. However the function of the HM microbiome is unknown. Recent studies comparing strains found in HM and infant stools as well as co-occurrence analyses suggest HM to act as a source of some bacterial species that colonize the infant gut.³⁴⁻³⁹ Although the breastfed microbiome appears less diverse than that of the formula-fed, breastfed may harbor more beneficial bacteria such as *Bifidobacteria* and inhibit the growth of harmful bacteria.³³ Similar mechanisms may exist in the infant gut ecosystem impacting induction of IgA, pro/anti-inflammatory epithelial responses and barrier function. One study associated maternal atopy to lower counts of *bifidobacterial* in milk which associated with lower counts in infant feces,⁴⁰ but no study exists assessing the association between HM microbiome and development of allergic diseases in the breastfed infant.

Innate Factors

Several components in HM have the capacity to influence innate immunity within the infant gut. Such factors include soluble toll-like receptors and co-receptors, HMOs, and

lactoferrin (LF). Soluble toll-like receptor 2 (sTLR2) and soluble co-receptor CD14 (sCD14) present in HM modulate microbe sensing. sTLR2 functions to decrease activation of membrane-bound TLR2 on enterocytes and immune cells, thus attenuating its inflammatory effects.⁴¹ *In vivo* murine models show that activators of TLR2 may inhibit oral tolerance, resulting in the development of FA.^{42,43} Thus, inhibition of TLR2 through sTLR2 may enhance oral tolerance and decrease the risk of FA. Activation of TLR2 and TLR4, a sensor of lipopolysaccharide (LPS), require the use of the co-receptor CD14. sCD14 in HM has been reported to have anti-inflammatory properties by preventing TLR4 signaling.⁴⁴ How sCD14 affects the establishment of oral tolerance in infants remains unknown. The overall anti-inflammatory effects of sTLR2 and sCD14 suggest a mechanism of tolerating microbes within the gut, thus paving the way for commensals to successfully colonize.

HMOs, discussed above, not only fuel the gut microbiome, but also play an essential role in modulating TLR signaling in the gut. Murine models of FA show that the absence of TLR4 results in skewing toward an allergic response, suggesting that increased TLR4 signaling may play a role in decreasing allergic responses and preventing allergic sensitization to food antigens.⁴⁵ One of the most prevalent HMOs, 2'-fucosyllactose (2'-FL) has the capacity to dampen enterocyte response to TLR4 activation *in vitro*, whereas LNFPIII enhances the pro-inflammatory TLR4 response *in vivo*⁴¹, implying that infants fed HM containing high levels of LNFPIII may be protected against the development of FA as shown by us.¹⁵ HMOs overall have been shown to promote the development of tolerance by inducing increased amounts of regulatory T cells (Tregs) and regulatory cytokine IL-10, which play a pivotal role in promoting the development of tolerance. Further studies demonstrated that this observation was dependent upon TLR4 signaling as well as signaling through a dendritic cell (DC) carbohydrate receptor.⁴⁶ This increased tolerogenic phenotype suggests that some HMOs may be protective against the development of FA. Overall, the interactions between HMOs and TLR4 have underlined the immunoregulatory capacity of HM on innate immunity.

One predominant glycoprotein in HM is LF. *In vitro* studies indicate that LF acts to neutralize LPS activity and inhibit inflammatory cytokine production.⁴¹ These anti-inflammatory effects may help infants tolerate their emerging gut microbiome. However, LF also possesses inflammatory properties via activation of an alternative TLR4 signaling pathway.⁴¹ In the context of the aforementioned murine models of FA, these inflammatory properties through the activation of TLR4 may be protective against the development of FA. Although LF plays dual roles, it has been described to possess a net anti-inflammatory effect *in vitro*.^{41,47,48} LF's net anti-inflammatory capacity is illustrated by its ability to protect against the development of intestinal infection and inflammation in preterm infants.⁴⁹ A study in pigs suggests that LF supplementation in their diet made them less susceptible to the development of FA.⁵⁰ Whether or not the impact of LF on TLR4 signaling contributes to this is unknown.

In summary, these HM components impact innate immunity and propensity toward the development of FA. Although some factors play a pro-FA role and others play an anti-FA

role, it is the net effect of these factors that is important in determining the overarching effect of HM on the development of FA. Further research is needed to identify exact mechanisms behind the effects of these HM innate factors on tolerance and FA development.

Presence of dietary antigens in human milk

Common allergenic proteins found in HM include proteins from CM⁵¹⁻⁵⁶, peanut⁵⁷⁻⁵⁹, wheat^{60,61} and hen's egg.^{56,62-64} These studies are discussed below and summarized in Table 1. Of note, specificity and sensitivity of these assays vary. Whether maternal consumption of highly allergenic foods promotes tolerance or sensitization in the infant is a subject of debate, as discussed below.

Maternal consumption of allergenic food during pregnancy and lactation and food allergy

Several retrospective studies have examined the association of maternal ingestion of allergenic foods during lactation and FA in children.⁶⁵⁻⁷¹ The studies investigating maternal consumption of peanut during lactation and the risk of peanut allergy in offspring have had mixed results. In a high-risk cohort of infants, peanut consumption during pregnancy was associated with likely peanut allergy, but this relationship was not found during lactation.⁶⁶ In a prospective cohort study, peripregnancy consumption of peanuts and tree nuts in nonallergic mothers was associated with decreased risk of peanut/tree nut allergy in offspring.⁷² One study found that maternal consumption of peanut while breastfeeding in combination with early introduction of peanut (<12 months) was protective against peanut allergy while either of these factors separately was associated with higher risk of peanut allergy.⁶⁷ Another study found that peanut consumption during lactation and pregnancy was associated with greater risk of peanut allergy in the infants; however, 75% of the cases had comorbid atopic dermatitis (AD) and the case controls did not⁶⁸ which is an important confounding variable as atopic dermatitis is known to increase the risk of FA.⁷³

Other food allergens including cow's milk (CM) and eggs have been investigated as well. A prospective trial of women with a strong family history of atopy investigated whether avoiding CM and eggs either during the last trimester of pregnancy and exclusive breastfeeding, only during breastfeeding, or no avoidance at all, impacted the sensitization to CM and egg in the infants.⁶⁹ No association between maternal avoidance of CM and eggs with sensitization of CM and eggs in the infants was found.⁶⁹ Hattevig et al. reported similar findings in a long-term follow-up at age ten years old where children of mothers who avoided egg, CM, and fish for 3 months after birth had no difference in the risk of atopic dermatitis, asthma, allergic rhinitis and clinical FA.⁷⁰

The mechanisms of protection were investigated by Metcalfe et al. who demonstrated that higher egg-containing maternal diet can increase infant egg-specific IgG4.⁶² IgG4 is thought to promote tolerance as evidenced in the increase after patients undergo immunotherapy.⁷⁴ We also previously demonstrated that maternal elimination of CM in the first three months of life is associated with lower CM-specific IgG4

Table 1 – Summary of studies measuring dietary antigen in human milk.

Study	No of mothers	Population	Antigen examined	Period of elimination prior to baseline	Timepoints	Levels detected***	Assay Detection Sensitivity	Timing of peak concentration	% mothers with detected antigen at any point	Atopic disease examined	Association found
Sorva et al. 1994	53	High risk, infants recruited for evaluation of CMA	BLG	24 h then consumption of 400 mL CM	0, 1, 2 h	0-7.84 ng/mL	0.002 ng/mL	Not stated	75	Infants with CMA	Varying patterns of BLG secretion seen in CMA
Host et al. 1988	19	9 mothers of CMA infants, 10 mothers of healthy infants	BLG	4 weeks for 1 sample, normal diet with 500 mL CM for 1 sample	4 h	4 week milk free: 0-10.5 ng/mL Normal diet w/ 500 mL CM: 0-45 ng/mL	0.3 ng/mL	n/a	21	Infants with CMA	Infants can react to milk w/out detected BLG
Host et al. 1990	20	10 atopic mothers, 10 healthy mothers	BLG	None, baseline obtained in morning prior to milk ingestion	0, 4, 8, 12, 24 h	<0.3-150 ng/mL	0.3 ng/mL	Median: 8 h	95	Mothers with or without atopy	Atopic status of mother did not correlate to BLG concentration
Jarvinen et al. 1999	26	16 mother of CMA infants, 10 mothers of healthy infants	BLG	2-4 weeks	0, 1, 2, 3, 4 h	2-4 week milk free: 0-0.52 ng/mL After CM challenge: 0-11.54 ng/mL	0.002 ng/mL	Not reported (mean time to symptoms was 21 h)	50 [47% undetected in mothers of CMA infants; 55% in mothers of healthy infants]	Infants with CMA	Infants can react to milk w/out detected BLG
Kilshaw & Cant 1984	22 for OA, 19 for BLG, 9 for OM	Healthy women	BLG, OA, OM	24 h then consumed ½ pint CM and 1 raw egg	0, 2, 4, 6 h	After challenge: OA: 0-6.17 ng/mL; BLG: 0-6.4 ng/mL; OM: 0-2.88 ng/mL	0.1 ng/mL-6.4 ng/mL	4 h	OA: 59 BLG: 53 OM: 78	n/a	n/a
Coscia et al. 2012	73	62 mother of term infants, 11 mothers of preterm infants	baS1 casein, ba1 casein	No elimination	0-4 days post delivery	n/a	n/a (used mass spectrometry)	n/a	n/a	n/a	BLG not found in colostrum; pre-term colostrum contained more ba1c
Metcalfe et al. 2016	92	General population	OA	6 week diet intervention Gp 1: egg-free diet Gp 2: 1-3 eggs/wk Gp 3: >4 eggs/wk	Samples done at 2,4,6 weeks into diet, Egg consumed 2-6 h prior to sample for gps 2&3	At 6 weeks (median values) Egg-free: 0.05ng/mL* 1-3eggs/wk: 0.05ng/mL* >4eggs/wk: 0.2 ng/mL	0.1-4ng/mL	6 weeks into dietary intervention	66	Presence of egg-specific IgG4 and IgE in infants	High consumption of eggs increases OV secretion in HM, High consumption of egg promotes IgG4 in infants (promote tolerance)
Cant et al. 1985	19	11 mothers of eczematous infants w/ positive egg SPT and 8 mothers	OA	24 h avoidance then consumed 1 raw egg	0, 2, 4, 6 h	0-4.0 ng/mL	Not stated	2-4 h post ingestion	74	AD egg sensitization	No association between amount OA found and whether control

Table 1 (continued)

Study	No of mothers	Population	Antigen examined	Period of elimination prior to baseline	Timepoints	Levels detected***	Assay Detection Sensitivity	Timing of peak concentration	% mothers with detected antigen at any point	Atopic disease examined	Association found
Verhasselt et al. 2020	88	of healthy infants with negative egg SPT Mothers with allergic disease	OA	No dietary intervention	3 and 6 months post delivery	**3 mo: 0.15 ng/mL 6 mo: 0.173 ng/mL	0.05 ng/mL	n/a	67	IgE-mediated egg allergy in infant	or infant with AD and positive egg SPT Presence of OA in HM associated with reduced risk of IgE-mediated egg allergy at 2.5 yrs of age
Palmer et al. 2005	41	Non-egg allergic mothers, 44% with atopy	OA	48 h egg free	0, 2, 4, 6, 8 h	Peak levels: No egg: 0.05ng/mL 1 raw egg: 1.08ng/mL 1/2 cooked egg: 2.24 ng/mL 1 cooked egg: 3.16ng/mL	0.1 ng/mL	2-6 h post ingestion most commonly, 2 participants peaked at 8h	76	Maternal history of atopy	Maternal atopy not associated with OA secretion; Dose-response exists between cooked egg ingestion and OV secretion
Chirido et al. 1998	49	Healthy, nonatopic mothers	GL	Unrestricted diet; Sub-group of 6 mothers: 3 d normal diet-> 3 d GF diet-> 3 d normal diet	During 2 d post-delivery and randomly	5-1200 ng/mL	Not stated	1 st d post-delivery and over 2 weeks; Subgroup: twice a day during diet protocol	100	n/a	GL levels higher in colostrum; No correlation established with gluten in diet and concentration
Troncone et al. 1987	53	Mothers of healthy term infants	GL	12 hours wheat free then consumption of 20g gluten	2-4 hours; subset of 6 mothers sampled at 0, 2, 4, 6 h	0-95ng/mL	5 ng/mL	2-4 h post ingestion	77(in first week of lactation)	n/a	n/a
Schocker et al. 2017	40	Cohort of healthy women	Ara h 2, Ara h 6	24 hours legume free then consumed 100 g dry roasted peanuts	0, varying time points from 1 hr to 12 hours	Ara h 2: 2.3-184 ng/mL Ara h 6: 1.1-79 ng/mL	Ara h 2: 1.3 ng/mL Ara h 6: 0.7 ng/mL	2 hours post ingestion	Ara h 2: 35 Ara h 6: 22.5		Ara h 2 and Ara h 6 have similar kinetics
Vadas et al. 2001	23	Healthy mothers without peanut or tree nut allergy	Ara h 1, Ara h 2	24 hours legume free diet then consumed 50 g (1/2 cup) dry roasted peanuts	0, 1, 2, 3, 4, 6, 8, 12 h	0-439 ng/mL (median peak 200 ng/mL)	Not stated	1-2 h post ingestion	48	n/a	Ara h 1 and Ara h 2 are secreted intact in HM

Table 1 (continued)

Study	No of mothers	Population	Antigen examined	Period of elimination prior to baseline	Timepoints	Levels detected***	Assay Detection Sensitivity	Timing of peak concentration	% mothers with detected antigen at any point	Atopic disease examined	Association found
Bernard et al. 2014	2	Non-allergic, non-atopic mother	Ara h 6	30g roasted peanut ingested on empty stomach or 150 min after peanut-free breakfast	0, 10 min-26 hr at various intervals	0-3.37 ng/ml	Not stated	Ara h 6 present within 10-20 minutes, peaks within first hour post ingestion	100	Mouse model of peanut sensitization with exposure to HM +/- peanut proteins	HM containing Ara h 6 promoted induction of oral tolerance in neonatal mice

Legend: BLG= β -lactoglobulin; CMA=cow's milk allergy; baS1 casein=bovine α -S1 casein; OA= ovalbumin; ba1 casein=bovine α -1 casein; Gp=group; SPT=skin prick test; AD= atopic dermatitis; GI=Glia-

din; d=day; GF=gluten free; n/a= not applicable

*below the lower level of detection for this assay and assigned 0.05 ng/mL by authors

**median levels shown

***all units have been converted to ng/mL for ease of comparison across studies

levels in infants and greater risk of CMA at 6 months of life.³⁰ Altering infant IgG4 with the maternal diet implies that maternal diet manipulation can promote tolerance in breastfed infants; however IgG4 may indicate exposure without implying tolerance. In addition, many mouse models indicate that food antigen exposure through maternal milk can promote tolerance in neonatal mice (as reviewed by Oyoshi and Jarvinen⁵); however, different mechanisms and outcomes may occur in mice and humans.

In summary, current data available about the effect of maternal diet on FA is variable without clear conclusions about whether maternal consumption of allergenic food during (pregnancy and) lactation is protective against or increases the risk of allergic disease. Maternal diet during pregnancy is linked to maternal diet during lactation and therefore is a confounding variable when analyzing the effect of maternal diet during lactation on FA in offspring. Many of these studies have varying definitions of exposure with different methods of assessing maternal consumption of the food of interest and do not always consider degree of breastfeeding (exclusive, duration, etc.). The outcomes vary with some relying on self-reported FA, serum specific IgE, and/or skin prick test which makes comparison across studies difficult. Most importantly, a critical observation about these studies is that maternal consumption of food is assumed to be the exposure of interest to the infant without direct measure of dietary antigen in the HM. However, there is high interindividual variation in the secretion of food proteins in HM as discussed in the next section of this review.

Cow's milk proteins in human milk

Cow's milk contains several proteins that are considered allergenic. Caseins and whey are the major proteins in cow's milk. β -lactoglobulin (BLG) is the most well studied allergenic protein in cow's milk, particularly with regards to its presence in HM, possibly because it does not exist endogenously in HM. Sorva et al. examined BLG in HM from mothers of infants with suspected CMA.⁵¹ HM samples measured at 1 and 2 hours after maternal consumption of 400 mL of fat-free CM during a supervised hospital stay showed higher levels of BLG in 51% of the mothers compared to baseline. Interestingly, BLG continued to decrease from the basal level in 28% of women after CM challenge, all of whom had infants with CMA⁵¹ suggesting they were slower metabolizers of CM, still clearing CM from >24h prior. These findings imply that women metabolize and secrete dietary proteins at varying rates. No detectable BLG was found in 25% of mothers.⁵¹ Host et al. found that only 21% of mothers had detectable BLG in HM 4 hours after a 500 mL CM ingestion.⁵² We measured BLG in 16 mothers of infants with challenge-proven CMA seen in a tertiary referral center and 10 mothers of healthy infants.⁵³ After a 2-4 week maternal elimination diet, mothers consumed CM and all but one of the infants with CMA had clinical manifestations of an immune response with eczema being the most common reaction.⁵³ BLG was not detected in almost half of the milk samples at the time of symptoms, however not all CM proteins such as caseins were measured.⁵³

Coscia et al. demonstrated that even colostrum contains CM proteins.⁵⁵ Bovine α -S1-casein was detected in colostrum

of mothers of term and preterm infants. BLG was not detected in human colostrum.

Egg proteins in human milk

The major allergenic proteins in hen's egg are ovomucoid and ovalbumin. Ovomucoid is heat resistant whereas ovalbumin is heat labile and has been studied more. In a study by Cant et al. ovalbumin was not detected in any baseline samples and was found in 74% of women after raw egg ingestion with the peak concentration occurring at 4 to 6 hours.⁷⁵ There was no difference found in ovalbumin concentration between the milk of mothers of infants with atopic dermatitis and egg sensitization vs mothers of healthy infants.⁷⁵

Palmer et al. also found a dose response relationship between the amount of egg ingested and quantity of ovalbumin in HM.⁶³ In a randomized, blinded, cross over trial women avoided egg for 48 hours and then consumed either no egg, one raw egg, or half or one cooked egg.⁶³ Consistent with previous studies^{56,62,75}, 24% of the women never had detectable ovalbumin in their breast milk.⁶³ No relationship between maternal atopy and frequency of detection of ovalbumin was found.⁶³ Interestingly, cooked egg consumption resulted in higher ovalbumin levels in HM compared to ingestion of raw egg.⁶³ A randomized controlled trial evaluated the effect of different quantities of egg consumption on the secretion of ovalbumin.⁶² For the first 6 weeks of lactation, participants were randomly assigned to either consume a high egg diet (>4 eggs per week), low-egg diet (1-3 eggs per week) or egg-free diet. Women in the high-egg diet group had significantly more ovalbumin in their breast milk compared to women in the no- to low-egg diets.⁶² Over 33% of women did not have detectable ovalbumin in their breast milk, including women in the high-egg diet group.⁶² Of note, only 23% of the mothers reported full compliance with the egg-free diet due to accidental ingestions of common egg-containing products.⁶² During the intervention, there was an increase in egg-specific IgG4 levels in infants that directly corresponded to increasing maternal egg consumption at 6 weeks into the intervention. Development of clinical allergy was not assessed and it is unclear whether IgG4 is a marker of tolerance or rather exposure. This trend was no longer present at 10 weeks after the intervention.⁶²

A recent observational infant cohort study found an association between presence of ovalbumin in a one-time HM sample (versus no detectable ovalbumin) and protection against IgE-mediated egg allergy at 2.5 years of age (RR 0.23; CI 0.06-0.85, $p=0.03$).⁷⁶ This may support the notion that dietary antigens in HM may promote tolerance in infants, although the study was small, did not assess exposure to egg over time, and randomized controlled studies are missing.

Wheat proteins in human milk

Gladin is a key allergenic protein found in wheat⁷⁷ and can be secreted in HM.^{60,61} Similar to other allergens, gladin concentrations peak in HM at 2 to 4 hours after consumption.⁶⁰ Colostrum contains higher gladin concentration compared to HM.⁶¹ Six mothers underwent a diet protocol with 3 days of

normal diet followed by 3 days of gluten-free diet, then returned to 3 days of normal diet. Four women had a decrease in gliadin during the gluten-free diet days whereas two women had a rise in gliadin.⁶¹ One woman had celiac disease and therefore followed a strict gluten-free diet and she did not have any detectable gliadin in the breast milk samples.⁶¹ It can be inferred that following a strict diet can alter the dietary antigens in breast milk whereas it is possible that unintentional ingestions of dietary antigens are common.

Peanut proteins in human milk

Ara h 2 is the most clinically important allergenic protein found in peanut followed by Ara h 1 and Ara h 3. Ara h 2 sensitization is associated with severe, potentially life-threatening reactions to peanut.⁷⁸⁻⁸⁰ Ara h 6 is another important allergenic protein found in peanut that shares homology with Ara h 2.⁸¹ Peanut protein is secreted in HM rapidly, detected as soon as 10 minutes after maternal consumption of peanut.⁵⁷ Between 22.5-48% mothers secrete peanut protein in HM.^{58,59} Ara h 1 and Ara h 2 peaked at 1-2 hours post-consumption of 1/2 cup roasted peanuts.⁵⁹ Of the 11 mothers with detectable peanut protein in HM, all but one mother cleared detectable peanut protein by 8 hours after consumption.⁵⁹ Consuming peanut with other food delayed secretion of peanut protein in HM and caused less peanut protein to be secreted.⁵⁷ Peanut protein secreted in HM was immunologically active binding IgE.⁵⁷

These studies highlight the difficult nature of drawing conclusions on the role of maternal diet and the induction of oral tolerance in infants. Maternal consumption of particular foods is an inconsistent and unreliable predictor for the presence of dietary antigen in HM as evidenced by the previously reviewed studies above where many mothers had no detectable antigen present in their breastmilk even after consumption of the foods of interest and others had detectable antigen levels after a period of "avoidance". It was noted in several studies that food antigens of interest were still present even after periods of attempted avoidance in 17-49% for milk^{51,82}, 7-65% for egg^{62,63}, 33% for wheat.⁶⁰ This highlights the need for using direct measurement of dietary antigens in HM in order to better characterize their role in oral tolerance induction in infants. There are many potential factors that play a role in whether a mother secretes dietary antigen in her breast milk which are highlighted in Table 2.

Table 2 – Factors affecting dietary antigen secretion in human milk.

Empty stomach vs consumed with other food ⁵⁷
Quantity and frequency of food antigen consumption ⁶²
Preparation of the food (cooked vs raw) ⁶³
Maternal intestinal permeability ⁵¹
Mammary epithelium permeability ⁸⁸
Varying rates of maternal metabolism
Frequency of breast emptying
Stage of lactation (colostrum vs mature milk) ⁵⁵

Questions that come up in the clinic relating to breastfeeding and immunomodulatory activity of breast milk

Current guidelines do not support the empiric elimination of highly allergenic foods during pregnancy and lactation.^{83,84} The question of whether maternal diet may be triggering symptoms in infants that may already be allergic comes up commonly. From the perspective of food allergy/intolerance, GERD and bloody stools, we have recently published some recommendations on how to workup an infant with different symptoms and when maternal elimination diets are needed.⁸⁵ The questions of how long it takes for mothers to clear their breast milk of particular food antigens while she begins strict elimination in her diet and whether she should stop breastfeeding in the meantime arise frequently. If an infant is experiencing symptoms of food-induced allergic proctocolitis (FPIAP) with blood-tinged stools, but otherwise thriving, we pose that breastfeeding be continued while mother eliminates suspected culprit foods in order to avoid disrupting the breastfeeding relationship with minimal risk to the infant since the allergens typically peak within hours of food ingestion. In only rare cases, very small amounts of the antigen may be present in HM at 24 hours after maternal ingestion of the food. Improvement in symptoms may take 48–96 hours^{86,87} as the gut epithelium takes time to heal.

A key observation is that the difficulty with compliance in following diets with avoidance of particular allergens (ex: egg-free, CM-free) is highlighted by the accidental ingestions in the studies we have previously outlined. Thorough, practical counseling to mothers on how to avoid relevant allergens is needed if avoidance of certain allergens is medically indicated. Nursing mothers of allergic infants who are symptomatic while exclusively breastfed should be properly counseled on label-reading and hidden sources of the food of interest. For example, many people do not realize CM proteins may be present in “milk-free” creamers that still contain whey or casein, baked goods, butter, etc. If assays that detected dietary antigens were readily available it would be optimal to help guide management of these infants and mothers. Furthermore, as observed in the aforementioned studies, many mothers do not secrete dietary antigens in HM. If a mother did not secrete the dietary antigens of interest, elimination of those foods in her diet would be unnecessary.

Conclusion

HM is comprised of many immunologically active components, which may have synergistic or even antagonizing effects. The sum of these effects requires further study. A common clinical question is whether dietary antigens in HM induce oral tolerance or allergic sensitization in infants. Studies that rely on maternal diet as a proxy for dietary antigens in HM have mixed results, likely as a result of the high inter-individual variation in dietary antigen secretion and issues of adherence. Current guidelines do not support the empiric elimination of highly allergenic foods during pregnancy and lactation; however, they do not comment on whether mothers should intentionally ingest large or small amounts of highly allergenic foods. With randomized studies lacking, further studies are needed to better guide physicians and mothers to prevent allergic disease.

Declaration of Competing Interest

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