



Restricting Cow's Milk in the Maternal Diet Reduces the Titers of β -Lactoglobulin-Specific IgG Antibodies in Human Milk

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Abstract

Background: Human milk antibodies specific to allergen enhance immunological tolerance in neonates by educating their immature mucosal immunity. The impact of restricting food allergens in diet and maternal factors on the levels of allergen-specific antibodies in human milk remains unclear. We aimed to evaluate the influence of the maternal avoidance diet of cow's milk on the titers of IgG, SIgA/IgA, and IgM specific to β -lactoglobulin (BLG) in human milk.

Materials and Methods: Human milk samples were collected from 26 women nonrestricting cow's milk and 11 women restricting cow's milk. The titers of IgG, SIgA/IgA, and SIgM/IgM specific to BLG were measured using ELISA.

Results: BLG-specific IgG titers were 2.9-fold higher in women nonrestricting cow's milk than those restricting cow's milk in their diet ($p=0.026$), but BLG-specific SIgA/IgA and SIgM/IgM titers were comparable between these two groups. BLG-specific IgG was positively correlated with BLG-specific SIgA/IgA titers in milk from mothers nonrestricting cow's milk ($p=0.0007$) but did not correlate for mothers restricting cow's milk. BLG-specific SIgA/IgA titer decreased with increasing postpartum time in milk from women restricting cow's milk ($p=0.019$). Type of delivery, infant gender, maternal age, and probiotic intake did not influence the BLG-specific antibody titers.

Conclusions: This study reveals that the secretion of BLG-specific IgG in human milk increases in women nonrestricting cow's milk compared with women restricting cow's milk. The role of breast milk allergen-specific antibodies on the neonatal gut (crosstalk with immune and epithelial cells) remains to be investigated.

Keywords: breastfeeding, immunological tolerance, allergens, food antigens, cow's protein-free diet, neonatal gut

Introduction

THERE IS EVIDENCE THAT HUMAN MILK IgG and IgA provide natural protection from allergic reactions in infants.^{1–3} Human milk antibodies are critical in educating immature mucosal immunity and developing allergen tolerance in neonates. High allergen-specific IgG in mother's milk enhanced the protection against allergic sensitization at 5 years of postnatal age.² IgG could improve neonatal tolerance

by binding to allergens and transporting them through the neonatal epithelial cells to be delivered to antigen-presenting cells for presentation to T cells.⁴ This presentation allows shaping the neonatal immune responses to recognize allergens as not dangerous for the host.

Vertical transport of maternal IgG postnatally through the neonatal gut epithelial layer as facilitated by the neonatal Fc receptor (FcRn) has been described in murine models⁵ but not shown to exist in humans. In contrast, human FcRn transports

maternal IgG to the fetus antenatally across the placenta.⁵ However, human milk antibody binding antigen likely happens in humans,⁶ contributing to the epithelial barrier function and immune exclusion.

As seen in IgG, high cow's milk-specific IgA concentrations in human milk were associated with protection against cow's milk allergy in early childhood.^{1,3} Lactating women drinking cow's milk were associated with higher levels of secretory IgA (SIgA) in human milk than those who did not drink cow's milk.¹ These observations could be related to the antigenic activation in the maternal gut and the transfer of antibodies specific to food antigen in the human milk.⁷ β -Lactoglobulin (BLG) is the most abundant whey protein in cow's milk and is absent in human milk, which provides a marker for the presence of antibodies specific to the cow's milk.¹

Lower levels of BLG-specific IgA were associated with a higher risk of developing atopic dermatitis and food sensitization in early childhood.⁸ In the absence of allergen-specific antibodies, infants could be more exposed to "free" allergen that promote the development of allergies.⁹ Antibodies attached to allergens may attenuate the neonatal immune response against these antigens and educate their immature gut.¹⁰ Although few studies reported an association between human milk IgG or IgA and the development of allergic disease at an early age, some studies found no association.^{11,12} These contractional studies could be due to other maternal and infant factors influencing the antibody levels in human milk, but those are still not well known.

The impact of restricting food allergens in diet and maternal factors (mode of delivery, maternal age, infant gender, and probiotic supplementation) on the levels of human milk IgG and SIgA specific to allergens remains unclear. In addition, no study has evaluated the effect of maternal restriction diet on human milk secretory IgM (SIgM) specific to allergens in human milk. In this study, we focused on the maternal restriction of cow's milk, which was the most often restricted food reported by lactating women.

To examine the influence of maternal restriction diet on the levels of allergen-specific antibodies in human milk, we compared the titers of IgG, SIgA/IgA, and SIgM/IgM specific to BLG in milk between women nonrestricting cow's milk and those restricting cow's milk. We hypothesized that BLG-specific antibody titers are higher in milk from women drinking cow's milk than in milk from women restricting cow's milk. These results can provide knowledge on the impact of maternal diet on the secretion of allergen antibodies in human milk.

Materials and Methods

Study design and participants

Mothers Milk Cooperative (Boulder City, NV) approved the participants and collected their written consent to use their milk for research purposes. The institutional review board (IRB00012424) of Medolac Laboratories (Boulder City, NV) approved the milk collection for this study. Donors completed a survey to report their maternal avoidance diet, including the restriction of cow's milk. Donors with cow's milk restriction were also avoiding all dairy proteins in their diet. The type of delivery and probiotic intake were also reported (Table 1). The inclusion criteria were donors who completed a health questionnaire and the survey related to maternal restriction diet. The exclusion criteria were donors who were smoking, taking medications, or other drugs.

Human milk collection

Human milk samples (50 mL) were collected at home with clean electric breast pumps into sterile plastic containers and stored immediately at -20°C in deep freezers. The participants cleaned their breasts with a wet washcloth (no soap or alcohol) before pumping. Human milk samples were frozen and transported in insulated boxes to Medolac Laboratories, where they were kept frozen and stored at -80°C until the ELISA measurements. Frozen samples were rapidly thawed at 37°C and centrifuged at 1,301 g for 20 minutes at 4°C .

Antibody titers specific to BLG

The area under the curve (AUC) of antibodies specific to BLG was determined using ELISAs as described in our recent studies^{13,14} with some modifications. In brief, ELISAs were recorded with a microplate reader (SpectraMax iD5, Molecular Devices, Sunnyvale, CA). Clear flat-bottomed microplates were coated with 100 μL of BLG from bovine milk (L3908-250MG; Sigma-Aldrich, St-Louis, MO) at 20 $\mu\text{g}/\text{mL}$ in 1 $\times$ phosphate-buffered saline (PBS). Microplates were incubated overnight at 4°C . After incubation, plates were washed three times using PBS 1 $\times$ with 0.05% Tween-20 detergent (PBST) and 200 μL of blocking buffer (PBST with 3% of bovine serum albumin fraction V) was added in all wells for 1 hour at room temperature.

Human milk supernatants were diluted from 5 $\times$ to 40 $\times$ for SIgA/IgA and from 2.5 $\times$ to 20 $\times$ for IgG and SIgM/IgM in serial 2-fold dilutions in blocking buffer. For each step (addition of 100 μL standards/samples and secondary antibodies at 1 $\mu\text{g}/\text{mL}$), washing and incubation for 1 hour at room

TABLE 1. DEMOGRAPHIC DESCRIPTION OF LACTATING WOMEN WHO DONATED HUMAN MILK IN THIS STUDY

Demographics	Nonrestricting cow's milk (n=26)	Restricting cow's milk (n=11)	p
Postpartum age, months ^a	8 $\pm$ 3 (3–12)	7 $\pm$ 3 (4–11)	0.99
Gestational age at birth, weeks ^a	38 $\pm$ 2 (36–41)	38 $\pm$ 2 (36–42)	0.48
Infant gender, n	13 males: 13 females	6 males: 5 females	0.81
Maternal age, years ^a	33 $\pm$ 4 (25–41)	33 $\pm$ 4 (27–39)	0.85
C-section, n (%)	5 (19.2)	4 (36.4)	0.28
Probiotic intake, n (%) ^b	16 (61.5)	4 (36.3)	0.17

^aData are presented as mean $\pm$ SD (min and max).

^bProbiotics taking were Greek yogurt and/or capsules by donors. SD, standard deviation.

temperature were performed. The detection was completed using goat antihuman IgM mu-chain HRP for total IgM (SIgM/IgM), goat antihuman gamma-chain HRP for IgG, and goat antihuman alpha-chain HRP for total IgA (SIgA/IgA) (Abcam, Cambridge, MA). Interpolation of a standard curve (sigmoidal, 4PL) was created using four points (in duplicate) for each sample, and AUC was calculated using GraphPad Prism (version 9).

Statistical analysis

Mann–Whitney test was used to compare the BLG-specific antibody titers in milk between women nonrestricting cow's milk and those restricting cow's milk. Mann–Whitney test was also used to determine the effect of probiotic intake, infant gender, and type of delivery. Linear regression was performed to evaluate the effect of postpartum time and maternal age on the antibody titers in both groups.

Results

Maternal demographics

We recruited 26 women nonrestricting cow's milk and 11 women restricting cow's milk. The maternal age, postpartum time, gestational age, infant gender, maternal age, type of delivery, and probiotic intake were comparable and not significantly different between the two groups (Table 1).

Maternal restriction diet on food allergen-specific antibodies in human milk

To determine whether the maternal restriction diet influences the antibody levels specific to BLG in human milk, we compared the BLG-specific antibody titers in human milk between mothers restricting cow's milk and those nonrestricting cow's milk. Most interestingly, the titer of BLG-specific IgG was 2.9-fold higher in women nonrestricting cow's milk than in those restricting cow's milk in their diet ($p=0.026$, Fig. 1a), but BLG-specific SIgA/IgA and SIgM/IgM titers were comparable between these two groups

(Fig. 1b, c). Our results indicate that lactating women drinking cow's milk in their diet increased the levels of IgG specific to BLG in human milk compared with lactating women restricting cow's milk.

Moreover, BLG-specific IgG was positively correlated with BLG-specific SIgA/IgA titers in milk from women nonrestricting cow's milk ($p=0.0007$, Fig. 2a), but did not correlate with SIgM/IgM. In contrast, no correlation was observed between BLG-specific IgG and SIgA/IgA in milk from mothers restricting cow's milk (Fig. 2b).

Effect of maternal factors on food allergen-specific antibody titers

BLG-specific IgG titer tended to increase across postpartum time in milk from mothers nonrestricting cow's milk ($p=0.10$, Fig. 3a), but postpartum time did not influence SIgA/IgA or SIgM/IgM. In contrast, BLG-specific SIgA/IgA titer decreased with increasing postpartum time in milk from women restricting cow's milk ($p=0.019$, Fig. 3b), whereas no change was observed for IgG or SIgM/IgM. The titers of BLG-specific IgG, SIgA/IgA, and SIgM/IgM were comparable between lactating women taking probiotics and those who were not ingesting probiotics (Supplementary Fig. S1a–c). Infant gender (Supplementary Fig. S2a–c) and mode of delivery (Supplementary Fig. S3a–c) did not influence the antibody titers against BLG.

Discussion

This study aimed to determine the impact of maternal restriction diet on the levels of antibodies specific to food allergens in human milk. For the first time, we demonstrated that milk from women nonrestricting cow's milk had a higher BLG-specific IgG titer than that from women restricting cow's milk in their diet. Our results support the findings that BLG-specific IgG1 and IgG4 levels in blood were higher in infants who were breastfed by mothers with no cow's milk elimination than those who were breastfed by mothers with cow's milk elimination.¹



FIG. 1. Effect of restricting cow's milk in the maternal diet on the titers of antibodies specific to BGL in human milk. Violin plots show the AUC for (a) BLG-specific IgG; (b) BLG-specific secretory IgA (SIgA)/IgA; (c) BLG-specific secretory IgM (SIgM)/IgM in human milk from women nonrestricting cow's milk ($n=26$) and women restricting cow's milk in their diet ($n=11$). AUCs were calculated using four serial dilutions in duplicate for each milk sample. The asterisk indicates $p < 0.05$. Differences between the two groups were assessed by Mann–Whitney test (nonparametric test). AUC, area under the curve; BLG, β -lactoglobulin; ns, not significant.

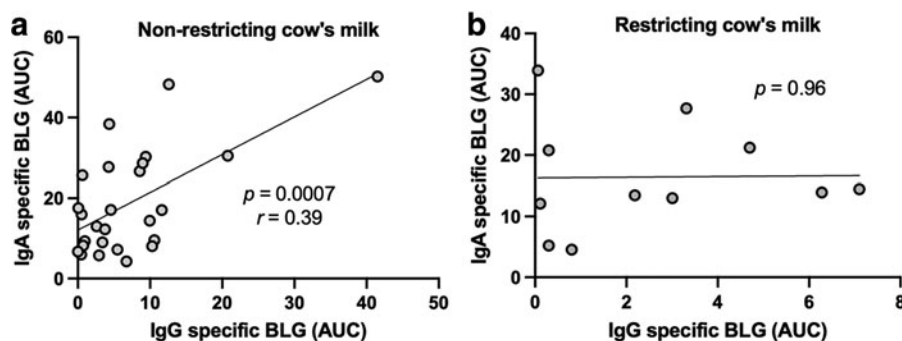


FIG. 2. Effect of maternal restriction diet on the correlation between the titers of IgG and secretory IgA (SIgA)/IgA specific to BLG in human milk. Linear regression between BLG-specific IgG and SIgA/IgA in milk from (a) women nonrestricting cow's milk ($n=26$) and (b) women restricting cow's milk in their diet ($n=11$). BLG, β -lactoglobulin.

Infants from mothers with high human milk allergen-specific IgG levels did not produce IgE, suggesting that allergen-specific IgG could prevent the development of allergic sensitization.² Therefore, higher ingestion of BLG-specific IgG levels in breastfed infants could enhance neonatal oral tolerance induction. A maternal diet with cow's milk proteins may increase the levels of BLG-specific IgG in human milk.

We observed that BLG-specific IgG titers were positively correlated with SIgA/IgA titers in human milk from mothers nonrestricting cow's milk, but this correlation was absent in mothers restricting cow's milk. Moreover, BLG-specific SIgA/IgA titer decreased with increasing postpartum time only in milk from women restricting cow's milk but not in milk from those nonrestricting cow's milk. We also found no significant difference for BLG-specific SIgA/IgA or SIgM/IgM titers between women restricting cow's milk and those drinking cow's milk. In contrast, Järvinen et al.¹ demonstrated that maternal cow's milk avoidance was associated with lower levels of cow's milk-specific IgA levels (including BLG and casein) in human milk but did not determine the levels of cow's milk-specific IgG or IgM.

In contrast, Fälth-Magnusson¹⁵ observed that IgA and IgG did not differ between mothers on strictly cow's milk-free diet and mothers taking cow's milk. These differences in findings could be related to other factors modulating antibody secretion (in human milk) and immune response. Maternal avoidance diets could reduce the exposure of food antigens in the infant's immune system, making the neonate more at risk of lacking oral tolerance induction and sensitization and developing allergic diseases.¹ A study is needed to elucidate the role of breast milk allergen-specific antibodies on the neonatal gut, including the crosstalk between immune and epithelial cells.

We showed that the type of delivery, infant gender, maternal age, and probiotic intake did not influence the antibody titers specific to BLG. These results indicate that these maternal factors might have less impact on the antibody specific to BLG than the maternal diet with cow's milk. Moreover, the titer of BLG-specific IgG and SIgA/IgA strongly varied between lactating women independently of maternal restriction diet, suggesting the presence of individual factors influencing the secretion of antibodies in human milk.

Previous studies reported that the type of delivery and probiotic supplementation influenced the development of mucosal immunity and allergies.^{16–18} Type of allergen, allergen exposure, maternal allergy, environmental conditions (farm exposure), population investigated, and genetic predisposition can also account for the variation of allergen-specific antibodies between women.²

The limitations of this study were the small sample size and self-report of cow's milk restriction from the donors. We also did not know how long the mothers started avoiding cow's milk and dairy products.

In summary, our study is the first to provide a detailed investigation of SIgA/IgA, IgG, and SIgM/IgM specific to BLG in milk from mothers nonrestricting and restricting cow's milk. Our results indicate that women who drink cow's milk had a more significant increase in the secretion of BLG-specific IgG in human milk than women with a restriction of cow's milk. A larger cohort study is needed to identify maternal and infant factors associated with the upregulation of antibodies specific to allergens in human milk and the infant's gut. A study is needed to investigate the importance of human milk antibodies in enhancing the neonatal immune response to develop tolerance to allergens and prevent allergic reactions.

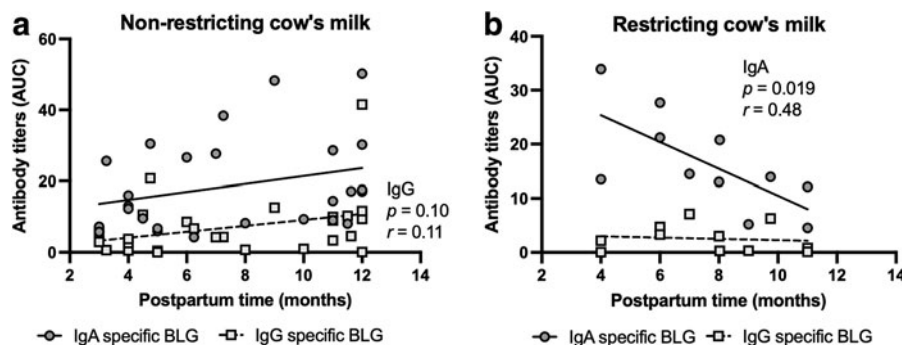


FIG. 3. The effect of maternal restriction diet and postpartum time on the titers of IgG and secretory IgA (SIgA)/IgA specific to BLG in human milk. Linear regression between postpartum time and BLG-specific IgG or SIgA/IgA in milk from (a) women nonrestricting cow's milk ($n=26$) and (b) women restricting cow's milk in their diet ($n=11$). BLG, β -lactoglobulin.

Authors' Contributions

V.D-M. conceptualized and designed the study, carried out the statistical analysis, drafted the article, and approved the final article as submitted. V.D-M. has primary responsibility for the final content. V.D-M. and S.L. carried out ELISA analyses. S.L. approved the final article as submitted.

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Disclosure Statement

V.D-M. and S.L. were employees at Medolac Laboratories.

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Supplementary Material

Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3

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