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Themed Issue: The Gut Microbiome and Immunity: How it is Shaped by Early Life

BRIEF REPORT

Infant gut immunity: a preliminary study of IgA associations with breastfeeding

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Secretory immunoglobulin A (IgA) plays a critical role in gut mucosal immune defense. Initially provided by breastmilk, IgA production by the infant gut is gradually stimulated by developing gut microbiota. This study reports associations between infant fecal IgA concentrations 4 months after birth, breastfeeding status and other pre/postnatal exposures in 47 infants in the Canadian Healthy Infant Longitudinal Development cohort. Breastfed infants and first-born infants had higher median fecal IgA concentrations (23.11 *v.* 9.34 μg/g protein, $P < 0.01$ and 22.19 *v.* 8.23 μg/g protein, $P = 0.04$). IgA levels increased successively with exclusivity of breastfeeding (β-coefficient, 0.37, $P < 0.05$). This statistical association was independent of maternal parity and household pets. In the absence of breastfeeding, female sex and pet exposure elevated fecal IgA to levels found in breastfed infants. In addition to breastfeeding, infant fecal IgA associations with pre/postnatal exposures may affect gut immunity and risk of allergic disease.

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Introduction

Immunoglobulin A (IgA), in its secretory form (sIgA), is critical to the gut mucosal immune defense system in early life. It plays a key role in immune exclusion of pathogens and the development of oral tolerance to commensal gut bacteria.[1] Although IgA has been detected in the feces of breastfed infants as early as the first few weeks of life,[2,3] an infant's ability to produce IgA during this time period is limited.[4] Passive immunity is provided to the nursing infant via IgA and other antimicrobial peptides in breastmilk, particularly in colostrum.[5] Breastmilk also provides a source of live bacteria that assist in the early establishment of the intestinal microbiota.[6] As evident in animal and human studies, a mutualism exists between intestinal IgA and commensal gut bacteria, whereby microbial exposure stimulates IgA production in the infant and in turn IgA regulates the composition of the microbiota.[7–9] Infant IgA production is delayed in exclusively formula-fed infants and has been shown to be consistently lower in these infants throughout the 1st year of life.[2,3] Epidemiological evidence suggests that delayed maturation of the infant's immune system and its ability to produce IgA during early life may contribute to the development of atopy.[10–12] The primary aim of this study was to examine the association between breastfeeding and infant fecal IgA concentration in early life. A secondary aim was to determine whether associations with infant fecal IgA were modified by the fetal or postnatal environment.

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Methods

Study design

The study involved a sub-sample of 47 infants (36–46 weeks gestation) from the Vancouver and Winnipeg sites of the Canadian Healthy Infant Longitudinal Development (CHILD) national population-based birth cohort[13] (www.canadianchildstudy.ca). We selected the first 47 infants for whom fecal samples were available for analysis and who had complete data on breastfeeding status. Mothers of the infants involved in our study were enrolled during pregnancy between September 2008 and January 2009. Infant stool samples were collected at mean age of 3.9 months (range 2.9–5.3) using a standard protocol as part of a scheduled home visit. Samples were refrigerated in the home immediately following collection and during transport and then stored at −80°C for later use. At this time mothers were asked to report on breastfeeding status using a standardized questionnaire. Breastfeeding was

categorized as any breastfeeding (yes/no) and by degree of breastfeeding exposure (none, partially breastfed, exclusively breastfed). Information on other covariates were obtained from hospital records (infant sex, mode of delivery, birth weight and gestational age) or through standardized questionnaires completed by mothers (maternal age, current maternal allergy or asthma, smoking during pregnancy, number of older biological children as a proxy for parity, furry household pets). Maternal weight status [body mass index, weight (kg)/height (m^2)] was calculated from height and weight measured at the 1 year postpartum clinic visit. Written informed consent was obtained from parents at enrollment.

Analysis of fecal samples

Infant fecal IgA was measured using an enzyme-linked immunosorbent assay (Bethyl Laboratories Human IgA kit, TX, USA) according to the manufacturer's instructions and expressed as average micrograms of IgA per gram of total protein (total protein determined by standard colorimetric bicinchoninic acid (BCA) protein assay). All samples were assayed in duplicate. The manufacturer's Accessory Kit supplied all buffers, plates and tetramethylbenzidine and the color was allowed to develop for 10 min and then stopped with 50 µl of 0.2 M sulfuric acid (Fisher Scientific, AB, Canada). Absorbance was read at 450 nm using a scanning spectrophotometer (Molecular Devices, CA, USA). Samples with a coefficient of variation >10% were repeated.

Statistical analysis

Spearman correlation was used to examine the association between fecal IgA and age at fecal collection, gestational age and birth weight. Univariate non-parametric statistics (Mann–Whitney *U*-test, Kruskal–Wallis test) were used to describe associations between fecal IgA (as a continuous variable) and breastfeeding, as well as associations between IgA and other covariates (sex, maternal age, maternal allergy or asthma, maternal smoking in pregnancy, maternal weight status, birth mode, parity, household pets). To assess non-linear relationships, the same associations were tested using IgA as a binary variable ('high IgA' yes/no representing the highest tertile of IgA compared with tertiles 1 and 2 combined and 'low IgA' yes/no representing the lowest IgA tertile compared with tertiles 2 and 3 combined) using Pearson χ^2-test. Crude associations between breastfeeding and IgA concentration were also tested using linear regression (log IgA) and logistic regression models. To assess the potential confounding or effect modification by prenatal factors (sex of the fetus and maternal age, parity and smoking, overweight and allergy status, birth mode, exposure to pets) or postnatal environmental exposures (household pets) on the associations between breastfeeding and IgA (see Fig. S1 Directed Acyclic Graph in supplementary data), models were adjusted for each covariate separately. As 87% of households in the CHILD cohort owned pets during the index pregnancy and when their infants were 3 months old, this variable is listed as both a pre and postnatal exposure. Variables were retained in final models at a *P*-value of 0.05 or if they caused a ⩾10% change in the estimate for breastfeeding. Covariates that did not improve the model fit or were correlated with other variables in the model were excluded. Smoking during pregnancy was not included in linear or logistic models as too few women smoked (only 3 out of 46) and estimates were therefore unreliable. All analysis was conducted using IBM SPSS version 22.

Results

Fecal IgA was measured in 47 infants from the Vancouver and Winnipeg sites of the CHILD study (Table S1). Median IgA was 14.28 µg/g protein (interquartile range 7.13–30.19) in fecal samples obtained from these infants. In all, 46 % of infants were exclusively breastfed, 28% partially breastfed and 26% not breastfed; 81% of first-born infants were breastfed *v.* 67% of infants with siblings. Breastfed infants (any breastfeeding *v.* none) and infants from primiparous mothers had higher median IgA (23.11 *v.* 9.34 µg/g protein, $P < 0.01$ and 22.19 *v.* 8.23 µg/g protein, $P = 0.04$, respectively). Concentrations of IgA rose successively with exclusivity of breastfeeding (P for trend = 0.02; Fig. 1). In all, 43% of infants breastfed at 3 months had IgA in the highest tertile compared with only 8% of those that were not breastfed ($P = 0.03$). No statistically significant differences in fecal IgA levels were found according to infant sex, maternal allergy, maternal smoking and maternal weight status. Fecal IgA concentration was not associated with age at which fecal samples were collected (Spearman correlation $P > 0.05$; Fig. S2).

The extent of breastfeeding (none, partial, exclusive) was associated with a significant linear increase in infant fecal IgA.

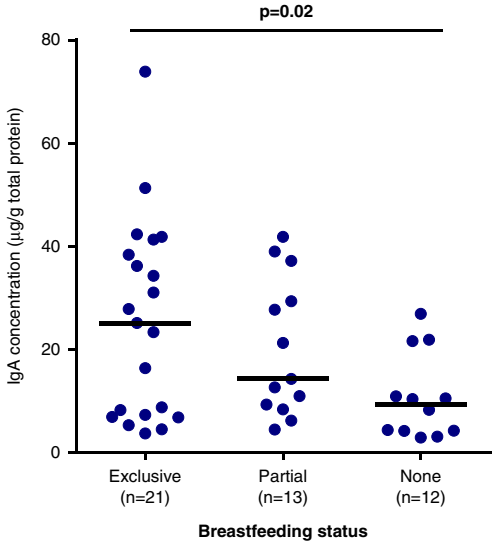

Fig. 1. Fecal immunoglobulin A (IgA) according to breastfeeding status ($n = 46$). Bars represent median values. Comparison by Spearman correlation. Fecal IgA and breastfeeding status measured at mean age of 4 months.

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This relationship remained after adjustment for pets and parity ($P = 0.03$; Table 1) but was diminished in strength. IgA levels were higher among first-born infants following adjustment for breastfeeding status ($P < 0.07$; Table 1) but there was no evidence of an interaction between parity and extent of breastfeeding. There was however evidence of effect modification by household pets and sex (P for interaction 0.04 and 0.06, respectively). The association between breastfeeding and IgA was only evident in male infants; in the absence of pets, breastfeeding was associated with increased IgA compared with non-breastfed infants, whereas there was no association between breastfeeding and IgA in infants living in a household with pets (Fig. S3).

Odds of having IgA in the highest tertile was 8.3 times greater in breastfed compared with non-breastfed infants (95% CI 0.96, 70.1, $P = 0.06$). After adjustment for parity and household pets, the association between breastfeeding and high fecal IgA was no longer evident. No interaction effects between breastfeeding status, and maternal parity, infant sex or household pets were seen in logistic regression models.

Discussion

In this pilot study of 47 infants born in two urban centers in Canada, breastfeeding was associated with higher IgA levels in their stools at the mean age of 4 months. Breastfed infants had a eight-fold greater odds of having fecal IgA levels in the highest tertile. Breastfeeding status was associated with a statistically significant linear increase in fecal IgA, such that levels in partially breastfed infants fell in between those of exclusively breastfed and exclusively formula-fed infants. This successive rise in fecal IgA concentrations with increased breastfeeding practice was not unexpected as breastmilk is the main source of IgA for infants during the first few months after birth.[14] However, maternal parity was also associated with infant fecal IgA levels and the strength of breastfeeding associations varied according to infant sex and presence of household pets.

The highest levels of IgA were found in exclusively breastfed infants, consistent with observations in other Western countries.[2,3,15,16] Despite this, we found median fecal IgA concentrations in non-breastfed infants amounted to over one-third of concentrations found in exclusively breastfed infants, suggesting that infants at 3–5 months of age are capable of endogenous production of IgA.[3] There was a wide range of IgA values observed within feeding groups, particularly those that were exclusively breastfed. Large variations in breastmilk IgA content have been attributed to environmental and maternal factors including country of residence,[11] parity,[5,11] smoking during pregnancy,[11,17] and exposure to pets and barn animals.[11] As IgA cooperates with gut microbes to train the immune system, variation in commensal microbiota, at birth and throughout infancy,[1] may also account for observed differences in IgA between and within feeding groups. In fact, the production and diversification of intestinal IgA requires the presence of both gut microbiota and immune-modulatory proteins from breastmilk; gut microbiota composition is heavily influenced by early infant feeding.[18–21]

First-born infants in our study had higher fecal IgA levels than infants with siblings, whether they were breastfed or not. As firstborns were also more likely to be breastfed in our study, breastfeeding status explained some of the association between parity and fecal IgA levels, and vice versa. Our parity findings are consistent with two pregnancy programming observations in the literature. First, IgA levels in colostrum are also reported to be inversely related to parity.[5] Second, there is evidence for less rapid gut colonization of the firstborn in the Dutch KOALA cohort; colonization with lactobacilli and *Bacteriodes* species was less likely and that for the clostridial microbes was enhanced 1 month after birth in newborns with fewer older siblings.[22] It is conceivable that firstborns receive a greater bolus of IgA from breastmilk in anticipation of delayed gut microbial colonization. Consistent with several other examples of male susceptibility and female resilience to fetal exposures,[23] we also observed sex-specific associations between breastfeeding and fecal IgA. Lower fecal IgA levels were seen in non-breastfed male infants compared with breastfed male infants, whereas no variations by breastfeeding status were evident in female infants. As reported in a review paper in this issue, sex dimorphism in gut microbiota composition exists and may lead to greater stimulation of mucosal IgA in female infants to compensate for the absence of IgA delivery by breastmilk.[24]

A postnatal environment with a higher microbial burden has the capacity to promote gut microbial development, enhance colonization resistance to *Clostridium difficile* and stimulate IgA production in infants. In the current analysis, exposure to pets

Table 1. *Log immunoglobulin A (IgA) levels according to breastfeeding status, adjusted for pre and postnatal exposures*

$n = 46$	Unadjusted [β (95% CI)]	Model 1 adjusted for parity [β (95% CI)]	Model 2 adjusted for pets [β (95% CI)]	Model 3 adjusted for parity and pets [β (95% CI)]
Extent of breastfeeding[a]	0.37 (0.07, 0.68)	0.36 (0.06, 0.65)	0.34 (0.04, 0.65)	0.33 (0.03, 0.63)
Primiparous (yes/no)	0.48 (−0.03, 1.00)	0.45 (−0.04, 0.94)	–	0.44 (−0.05, 0.93)
Household pets (yes/no)	−0.40 (−0.92, 0.13)	–	−0.29 (−0.81, 0.22)	−0.27 (−0.77, 0.23)

CI, confidence interval.

Unstandardized β estimated by linear regression using log IgA. Fecal IgA and breastfeeding status measured at mean age of 4 months.

[a]Breastfeeding as an ordered categorical variable of no breastfeeding (0), partial breastfeeding (1) and exclusive breastfeeding (2).

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modified the association between breastfeeding and IgA to a greater extent than parity. Fecal IgA in breastfed infants was higher relative to non-breastfed infants only in households without pets, suggesting that exposure to pets induces IgA production in non-breastfed infants at comparable levels with those who are breastfed. Although these data should be interpreted with caution due to the small numbers in some groups, we and others have found that pets increase the bacterial diversity of house dust and that 4-month-old infants living with household pets possess a gut microbiota with greater species richness and diversity.[25]

The main limitation of the study is sample size; this would have affected the precision of our estimates of associations between breastfeeding and IgA. In addition, although we were able to adjust for a number of important factors we cannot rule out residual confounding by other unmeasured variables. Exposure to pre and postnatal antibiotics is one variable that will be important to include in future studies due to its significant impact on gut microbiota. There was up to 2 months variation in the age at which fecal samples were collected in infants that may have affected IgA concentration, although there was no evidence of this in our analysis. Finally, our assay measured total IgA, based on the assumption that IgA found in fecal samples represents sIgA.

Our study provides evidence that breastfeeding status is associated with successive increases to infant fecal IgA; exclusively formula-fed infants produce their own IgA but have lower total levels. Research suggests that delayed maturation of B cells to produce IgA in the gut of infants may contribute to the risk of developing atopy;[10–12] the potential impact of breastfeeding on the development of infant mucosal immunity may therefore prove important in later child health. Maternal parity, infant sex and household pets may alter associations between infant feeding and fecal IgA levels and thus, change risk of atopic disease. Our infant IgA findings also point to the potential benefits of partial breastfeeding over no breastfeeding.

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Conflicts of Interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation and the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the University of Alberta, University of British Columbia and University of Manitoba Human Research Ethics Boards.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S2040174415007862

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