Lessons from mother: Long-term impact of antibodies in breast milk on the gut microbiota and intestinal immune system of breastfed offspring

Eric W Rogier1,3, Aubrey L Frantz1,4, Maria EC Bruno1, Leia Wedlund1, Donald A Cohen1, Arnold J Stromberg2, and Charlotte S Kaetzel1,5
1Department of Microbiology; Immunology; and Molecular Genetics; University of Kentucky; Lexington, KY USA; 2Department of Statistics; University of Kentucky; Lexington, KY USA; 3Current address: Centers for Disease Control and Prevention; Division of Parasitic Diseases and Malaria; Malaria Branch, Atlanta, GA USA; 4Current address: Division of Liberal Arts & Life Sciences; University of North Texas at Dallas; Dallas, TX USA

Abstract

From birth to adulthood, the gut microbiota matures from a simple community dominated by a few major bacterial groups into a highly diverse ecosystem that provides both benefits and challenges to the host. Currently there is great interest in identifying environmental and host factors that shape the development of our gut microbiota. Breast milk is a rich source of maternal antibodies, which provide the first source of adaptive immunity in the newborn’s intestinal tract. In this addendum, we summarize our recent data demonstrating that maternal antibodies in breast milk promote long-term intestinal homeostasis in suckling mice by regulating the gut microbiota and host gene expression. We also discuss important unanswered questions, future directions for research in this field, and implications for human health and disease.

Introduction

As children we are often told, “Listen to your mother!” and (usually much later) we appreciate the wisdom of her advice. It turns out that in addition to regulating our behavior, our mothers play a key role in regulating our gut microbiota and our own responses to that diverse microbial community. During parturition, our previously sterile gastrointestinal tracts receive their first inoculation with microbes from our mothers. Interestingly, the composition of our initial gut microbiota is influenced substantially by the mode of delivery. A recent study of mothers and newborns revealed that the early microbiota of vaginally delivered infants resembled their mother’s vaginal microbiota, whereas the microbiota of infants delivered by Caesarian section was more similar to that found on the skin surface.1 Over time, our gut microbiota matures from a simple community dominated by a few major bacterial groups into a highly diverse adult microbiota (for a review, see ref. 2). The development of our gut microbiota is regulated by environmental factors, including diet, and by host factors associated with innate and adaptive immunity. There is a growing body of epidemiological evidence that the composition of the gut microbiota differs significantly between breast-fed and formula-fed infants.3-10 For breastfed infants, maternal antibodies provide the first source of antigen-specific immunity in the intestinal tract (for a review see ref. 11). The predominant type of antibody in breast milk is secretory (S)IgA, which is transported across mammary gland epithelial cells by the polymeric immunoglobulin receptor (pIgR).12 At the apical surface, proteolytic cleavage releases SlgA, in which IgA is covalently attached to secretory component (SC), the extracellular domain of pIgR. The SC moiety of SlgA protects SlgA from degradation by host and microbial proteases and provides additional innate immune functions. The mucosal epithelial chemokine CCL28 is up-regulated in the mammary gland during lactation, and plays a key role in attracting IgA+CCR10+ antibody-secreting cells that were primed in mucosal lymphoid tissues of the intestinal and...
Breast Milk-Derived SlgA vs. Endogenous SlgA

Breast milk contains many bioactive molecules, including SlgA, which could potentially regulate the composition of the gut microbiota and the host response to these microbes. As the intestinal immune system develops in the offspring, there is a slow transition to endogenous production of SlgA via plgR-mediated transport of locally synthesized IgA into the gut lumen. In human infants the timing of endogenous SlgA production varies widely, influenced by environmental factors such as microbial load in the intestine. Whereas endogenous production of SlgA has been demonstrated in infants in developing countries, it may take several years before the concentrations of intestinal SlgA achieve adult levels in developed countries where exposure to environmental and dietary microbes is lower. To develop a mouse model in which we could separate the effects of breast milk-derived and endogenous SlgA, while holding other factors in breast milk constant, we utilized mice with a targeted deletion in the Pigr gene (Fig. 1). Pigr<sup>-/-</sup> mice cannot transport SlgA into external secretions, including milk and intestinal fluids. By breeding Pigr<sup>+/+</sup> females with Pigr<sup>-/-</sup> males, and vice versa, we generated offspring that were or were not exposed to maternal SlgA in breast milk. The genotypes of the offspring of both crosses were equally distributed between Pigr<sup>+/+</sup> and Pigr<sup>-/-</sup>, the former of which could produce endogenous SlgA by transport across intestinal epithelial cells, and the latter of which could not. Surprisingly, we found high levels of IgA in the milk from both Pigr<sup>+/+</sup> and Pigr<sup>-/-</sup> dams, suggesting that IgA could enter the milk stream by paracellular leakage in the absence of plgR-mediated transport. While this SC-devoid IgA was found in the stomachs of suckling newborns, it did not survive transport through the protease-rich environment of the intestinal tract. We found that the colonic lumens of newborn offspring of Pigr<sup>-/-</sup> dams were totally devoid of IgA, whereas the offspring of Pigr<sup>+/+</sup> mice had abundant IgA in their colonic lumens and in their feces. At weaning, fecal IgA levels dropped to low levels in all mice, reflecting the loss of maternal SlgA. Fecal IgA levels began to increase at about 4 weeks of age in Pigr<sup>-/-</sup> offspring, regardless of maternal Pigr genotype, reflecting endogenous plgR-mediated transport of IgA across intestinal epithelial cells. By contrast, fecal IgA was undetectable in Pigr<sup>-/-</sup> offspring for the rest of their lives. Thus our model system allowed us to study 4 distinct groups of mice that differed only in the presence of maternal and/or endogenous SlgA.

Breast Milk-Derived SlgA Strengthens the Intestinal Barrier

Epidemiological studies have provided support for the concept that SlgA antibodies in breast milk provide protection against a wide range of bacterial, viral and parasitic infections in suckling infants (for a review see ref. 11). However, the benefits of breast milk-derived SlgA remain controversial, largely due to the lack of standardization of epidemiological studies with regard to duration of breastfeeding, concentrations of SlgA and other anti-inflammatory factors in breast milk, and effects of other variables such as diet and sanitation. While the predominant protective mechanism of SlgA in breast milk likely involves immune exclusion of pathogens and allergens at mucosal surfaces of the suckling infant, other protective mechanisms could include innate enhancement of the intestinal barrier and promotion of a healthy commensal microbiota that limits the growth of potential pathogens. We hypothesized that early exposure to maternal SlgA antibodies in breast milk would foster a mutualistic relationship with the developing microbiota, and could enhance intestinal barrier function. In our mouse model, we found that failure to receive SlgA in breast milk resulted in increased translocation of aerobic bacteria from the neonatal gut into draining mesenteric lymph nodes, regardless of the Pigr genotype of the offspring. A prominent species among the bacteria that breached the epithelial barrier was *Ochrobactrum anthropi*, which has been identified as an opportunistic pathogen in preterm infants and immunocompromised individuals. Although

![Breeding scheme for generating offspring mice exposed to breast milk-derived maternal SlgA and/or endogenous SlgA derived from intestinal epithelial transport of locally synthesized IgA.](Image)
significant numbers of bacteria were able to penetrate the intestinal barrier in the absence of breast milk-derived SlgA, there was no evidence of systemic infection. This finding was not surprising, given that the systemic immune systems of the neonatal mice were intact. However, it should be noted that our mice were maintained under specific pathogen-free conditions, housed in barrier cages with sterile bedding, and fed autoclaved food and water. Future experiments in which mice deprived of maternal SlgA are challenged with known pathogens could shed light on the protective effects of SlgA antibodies in breast milk. It will be particularly interesting to compare responses to pathogen challenges in the early post-weaning period vs. later in life.

**SlgA Antibodies in Breast Milk Regulate the Commensal Gut Microbiota**

There is substantial evidence from studies in humans and mice that intestinal SlgA regulates the composition and activity of the commensal microbiota (for a review, see ref. 11). Less well understood are the effects of breast milk-derived SlgA on the developing gut microbiota, and whether these effects persist into later life. To analyze the short- and long-term effects of maternal SlgA, while controlling for endogenous SlgA, we analyzed the composition of the fecal microbiota in Pigr−/− offspring of Pigr−/+ dams at weaning (3 weeks of age), and in the same mice when they reached adulthood (10 weeks of age). Not only did early exposure to breast milk-derived SlgA result in a significantly different microbiota at weaning, but these differences were magnified when the mice reached adulthood. Some bacterial taxa varied in abundance due to lack of exposure to maternal SlgA, whereas other taxa were uniquely present or absent in the gut microbiota. Gram-negative Proteobacteria of the family Pasteurellaceae and Gram-positive Firmicutes of the family Lachnospiraceae dominated the taxa that were upregulated in the absence of passive SlgA. By contrast, the majority of unique taxa that were present in mice deprived of maternal SlgA were members of the family Comamonadaceae (phylum Proteobacteria). Interestingly, increased abundance of Pasteurellaceae and Lachnospiraceae has been observed in the gut microbiota of pediatric patients with inflammatory bowel disease (IBD), and increased abundance of Comamonadaceae has been observed in the gut microbiota of adult IBD patients with chronic pouchitis.

Although caution must be exercised when comparing results from controlled animal experiments to human diseases, our findings are consistent with the concept that early exposure to SlgA in breast milk promotes the development of a healthy microbiota that may provide long-term protection against inflammatory diseases.

**Early Exposure to SlgA in Breast Milk Affects Intestinal Epithelial Gene Expression Later in Life**

Microbial-host mutualism in the gut is promoted by extensive cross-talk between the gut microbiota and epithelial cells at the luminal interface, largely mediated by host receptors that recognize microbe-associated-molecular patterns (for a review see ref. 25). Having observed that early exposure to breast milk-derived SlgA caused long-term alterations in the composition of the gut microbiota, we hypothesized that the epithelial cell response to this distinctive microbiota would be altered as well. To test this hypothesis, we analyzed global gene expression in epithelial cells isolated from the colons of adult mice in the 4 groups comprising our model system. We observed a unique (and presumably optimal) pattern of gene expression in mice that had received both maternal and endogenous SlgA, with specific changes resulting from the loss of either source of SlgA. When we challenged these mice in vivo with the epithelial-disrupting agent dextran sulfate sodium, we observed that altered patterns of epithelial gene expression were correlated with increased susceptibility to colonic inflammation. It can be postulated that signals from the epithelium (including but not limited to SlgA) regulate the composition and activity of the microbiota in such a manner as to discourage mutually destructive inflammatory responses. In this regard it was significant that some of the genes whose expression was regulated by SlgA were murine orthologs of genes that have been associated with IBD and other inflammatory diseases in humans, including FUT2, IRF1, PLA2G2A, SLC26A3, VDR and ZMIZ1. In summary, our recent findings suggest that host-microbial mutualism is promoted by a lifelong relationship between SlgA and gut microbes, beginning at birth with maternal SlgA antibodies provided in breast milk, and continuing throughout life with endogenous SlgA antibodies provided by plgR-mediated transport across intestinal epithelial cells. A key aspect of this relationship, which deserves further investigation, is the specificity and affinity of SlgA antibodies directed against gut microbes.

**Unanswered Questions, Future Directions for Research, and Therapeutic Opportunities**

While we believe that our recent findings provide compelling support for the benefits of breast milk-derived SlgA in promoting lifelong intestinal homeostasis, there remain many unanswered questions and exciting directions for future research. In order for this field to progress, it will be essential to elucidate the mechanisms through which SlgA regulates the gut microbiota and in turn, gene expression by host cells. Fig. 2 illustrates theoretical scenarios for the development of neonatal intestinal immunity in the presence and absence of breast milk-derived maternal SlgA. Our findings suggest that maternal SlgA regulates the composition of the gut microbiota in suckling infants, and that some microbes can penetrate the epithelial barrier in the absence of maternal SlgA. However, it is not known whether SlgA associates preferentially with selected members of the gut microbiota. If so, does SlgA promote or inhibit the proliferation of bacteria with which it is associated? We recently reported that SlgA is concentrated with gut microbes in the loose outer layer of colonic mucus in mice and humans, whereas the dense inner layer of colonic mucus, rich in antimicrobial
peptides, is relatively devoid of both SIgA and bacteria. One could envision mechanisms through which coating of the bacterial surface with SIgA could facilitate (or inhibit) the association of bacteria with colonic mucus, thus promoting (or blocking) biofilm formation. Methods for fluorescence-activated cell sorting of IgA-bound fecal bacteria have been developed, and this approach could be coupled with deep sequence analysis of the SIgA-bound and unbound bacterial cohorts. Similar approaches could be used to investigate how bound SIgA affects gene expression and metabolic activity of gut microbes. Understanding the transition from maternal to endogenous SIgA will require longitudinal studies in experimental animals and humans. A key question to be resolved is, how does early exposure to breast milk-derived SIgA exert

![A. Neonatal intestinal immunity in the presence of maternal SIgA](image)

Maternal SIgA regulates the gut microbiota

Healthy host-microbial mutualism promotes balanced pro- and anti-inflammatory gene expression in intestinal epithelial cells

![B. Neonatal intestinal immunity in the absence of maternal SIgA](image)

The gut microbiota is altered and becomes more invasive in the absence of maternal SIgA

Altered host-microbial interactions in the absence of maternal SIgA leads to increased pro-inflammatory gene expression in intestinal epithelial cells.

Figure 2. Hypothetical model of the role of breast milk-derived secretory (SIgA) in the development of intestinal immunity. (A) In the presence of maternal SIgA, gut microbes are concentrated in the loose outer layer of colonic mucus (shown here in light blue), while the dense inner layer of mucus (shown here in darker blue) is relatively devoid of both SIgA and microbes. Microbial products stimulate intestinal epithelial cells and promote a healthy balance between pro- and anti-inflammatory gene expression. (B) In the absence of maternal SIgA, the composition of the gut microbiota is altered, and some microbes penetrate the mucus and epithelial barriers to invade draining mesenteric lymph nodes. Intestinal dysbiosis can persist into adult life and result in imbalanced pro-inflammatory gene expression in intestinal epithelial cells.
long-term effects on host-microbial mutualism? There are a number of potential answers, which are not mutually exclusive and can be addressed with current technologies. Key among these involves the role of SlgA in the transition from the simple gut microbiota of newborn mammals to the complex microbiota of adults. An interesting, and testable, hypothesis is that early exposure to breast milk-derived SlgA shapes the subsequent endogenous IgA response to gut microbes. SlgA has been shown to play a role in the natural sensing of commensal bacteria by mouse Peyer’s patch dendritic cells, likely involving both antigen-specific and glycan-mediated recognition of microbes by SlgA. Thus maternal SlgA could “prime” the neonatal immune system for recognizing specific types of gut microbes, which could lead to the generation of microbespecific memory IgA. B cells as a lifelong source of endogenous SlgA. We know that, once established, the gut microbiota is resilient in the face of a wide range of environmental challenges, such as infections and antibiotics. What is the role of SlgA in this resilience? Perhaps SlgA-bound microbes are more resistant to perturbation and/or SlgA promotes the recovery of selected microbial communities. What is the role of host genetics in SlgA-epithelial-microbial mutualism? This question could readily be addressed by crossing pIgR-sufficient and -deficient mice with mice bearing other genetic mutations known to impact intestinal homeostasis. Given the technology to generate cell type-specific genetic mutations in mice, there are limitless opportunities to explore the host-microbial interface. For example, we recently reported that mice with an intestinal epithelial-specific deletion of the Myd88 gene, encoding a cytoplasmic receptor that interacts with microbial pattern recognition receptors, had reduced expression of pIgR, impaired transport of SlgA, an altered composition of the gut microbiota, and increased susceptibility to chemically-induced intestinal inflammation. Recent advances in genome-wide studies of polymorphisms in humans associated with increased risk for IBD and other inflammatory diseases should provide many opportunities for studying the impact of human genetics on SlgA expression and function.

In conjunction with mechanistic studies, there are many avenues through which the potential benefits of SlgA in infectious, allergic and inflammatory diseases could be explored. The protective role of SlgA as a constituent of breast milk in humans needs to be better defined, beginning with epidemiological studies that link the concentration and activity of SlgA in breast milk to its beneficial effects. An example of this approach is the PASTURE project (Protection against Allergy: STudy in Rural Environments), a large prospective birth cohort study conducted in Austria, Finland, France, Germany and Switzerland. In this study, levels of SlgA were analyzed in 610 breast milk samples collected 2 months after delivery. Multivariate logistic regression analysis revealed a significant inverse association between the total amount of SlgA that was ingested via breast milk during the first year of life and the development of atopic dermatitis. While this type of epidemiological study represents a step in the right direction for assessing the potential benefits of breast milk-derived SlgA, many questions remain to be answered. It will be important to assess the relationship between early exposure to SlgA and subsequent allergic (and inflammatory) diseases in other geographic settings (including urban areas) and in other demographic groups. Approaches could be developed to improve the quantity and quality of SlgA in breast milk, for example, nutritional supplementation and vaccination of pregnant women. These studies should be coupled with analyses of the gut microbiota at birth and throughout life to illuminate the role of host-microbial interactions in immune-mediated diseases. Advances in epidemiological studies should pave the way for therapeutic applications of human SlgA. An obvious starting point would be to analyze the concentration and activity of SlgA in donated human milk used to supplement the diets of newborn infants faced with challenges such as prematurity and infectious diseases. For example, the methods used for pasteurization of donor milk can dramatically affect its content of SlgA and other bioactive molecules (for a review, see ref. 32). SlgA purified from human colostrum and/or mature milk could represent a safe, effective and standardized therapy for infectious, allergic and inflammatory diseases in infants and possibly in children and adults. Development of SlgA-based therapeutic approaches should be informed by findings from mechanistic studies regarding the optimal timing and dosage of SlgA for specific diseases in targeted populations.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Funding
This work was supported by an NIH grant AI069027 (and an associated American Recovery and Reinvestment Act supplement), a Senior Research Award from the Crohn’s and Colitis Foundation of America (CCFA), and a grant from the Kentucky Bioinformatics Research Infrastructure Network to CSK; a Senior Research Award from the CCFA to DAC; and NIH grants NCATS UL1TR000117, NCRR 5P20RR016481-12 and NIGMS 8 P20 GM103436-12 to AJS.

References
3. Roeger LC, Costabile A, Holland DT, Hoyles L, McCartney AL. Examination of fecal Bifidobacterium populations in breast- and formula-fed infants during the first 18 months of life. Microbiology 2010; 156:3239-41; PMID:20864478; http://dx.doi.org/10.1099/mic.0.043224-0


12. Kaetzel CS, Bruno MEC, Kaelzer CS. Secretory IgA is concentrated in the outer layer of intestinal mucosal fold. J Pediatr Gastroenterol Nutr 2014; 58:525-34; PMID:24710995; http://dx.doi.org/10.1097/mpd.0b013e3182b43e97


18. Kaetzel CS. The polymeric immunoglobulin receptor. eLife 2013; http://dx.doi.org/10.1029/387804701509201502437


24. Kaetzel CS. The polymeric immunoglobulin receptor. eLife 2013; http://dx.doi.org/10.1029/387804701509201502437

25. Rogier EW, Franzl AL, Bruno MEC, Kaelzer CS. Secretory IgA is concentrated in the outer layer of intestinal mucosal fold. J Pediatr Gastroenterol Nutr 2014; 58:525-34; PMID:24710995; http://dx.doi.org/10.1097/mpd.0b013e3182b43e97

26. Rogier EW, Franzl AL, Bruno MEC, Kaelzer CS. Secretory IgA is concentrated in the outer layer of intestinal mucosal fold. J Pediatr Gastroenterol Nutr 2014; 58:525-34; PMID:24710995; http://dx.doi.org/10.1097/mpd.0b013e3182b43e97

27. van der Waaij LA, Limburg PC, Mesander G, van der WD. In vivo IgA coating of anaerobic bacteria in human faeces. Gut 1996; 38:348-54; PMID:8675085; http://dx.doi.org/10.1136/gut.38.3.348


32. Colazi TT. Donor human milk for preterm infants: what it is, what it can do, and what still needs to be learned. Clin Perinatol 2014; 41:457-50; PMID:24873842; http://dx.doi.org/10.1016/j.clop.2014.02.003