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

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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 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.

Keywords: breastfeeding, gut microbiota, gene expression, intestinal epithelial cells, inflammatory bowel disease, polymeric immunoglobulin receptor, secretory IgA

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Breast Milk-Derived S IgA vs. Endogenous S IgA

Breast milk contains many bioactive molecules, including S IgA, 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 S IgA via plgR-mediated transport of locally synthesized IgA into the gut lumen. In human infants the timing of endogenous S IgA production varies widely, influenced by environmental factors such as microbial load in the intestine. Whereas endogenous production of S IgA has been demonstrated in infants in developing countries, it may take several years before the concentrations of intestinal S IgA 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 S IgA, while holding other factors in breast milk constant, we utilized mice with a targeted deletion in the PlgR gene (Fig. 1). PlgR−/− mice cannot transport S IgA into external secretions, including milk and intestinal fluids. By breeding PlgR+/− females with PlgR−/− males, and vice versa, we generated offspring that were or were not exposed to maternal S IgA in breast milk. The genotypes of the offspring of both crosses were equally distributed between PlgR+/− and PlgR−/−, the former of which could produce endogenous S IgA 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 PlgR+/− and PlgR−/− 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 PlgR−/− dams were totally devoid of IgA, whereas the offspring of PlgR+/− 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 S IgA. Fecal IgA levels began to increase at about 4 weeks of age in PlgR−/− offspring, regardless of maternal PlgR genotype, reflecting endogenous plgR-mediated transport of IgA across intestinal epithelial cells. By contrast, fecal IgA was undetectable in PlgR−/− 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 S IgA.

Breast Milk-Derived S IgA Strengthens the Intestinal Barrier

Epidemiological studies have provided support for the concept that S IgA 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). There is also evidence that early exposure to breast milk-derived S IgA may reduce the incidence of allergic diseases later in life. However, the benefits of breast milk-derived S IgA remain controversial, largely due to the lack of standardization of epidemiological studies with regard to duration of breastfeeding, concentrations of S IgA and other anti-inflammatory factors in breast milk, and effects of other variables such as diet and sanitation. While the predominant protective mechanism of S IgA 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 S IgA 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 S IgA in breast milk resulted in increased translocation of aerobic bacteria from the neonatal gut into draining mesenteric lymph nodes, regardless of the PlgR 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.
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<sup>−/−</sup> offspring of Pigr<sup>−/−</sup> and Pigr<sup>−/+</sup> 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 (phyllum Proteobacteria). Interestingly, increased abundance of Pasteurellaceae and Lachnospiraceae has been observed in the gut microbiota of pediatric patients with inflammatory bowel disease (IBD),<sup>23</sup> and increased abundance of Comamonadaceae has been observed in the gut microbiota of adult IBD patients with chronic pouchitis.<sup>24</sup> 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 microbes 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...
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 plgR-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 plgR, 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 measure 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. 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.

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