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Secretory IgA: (Cross)linking Microbes, Maternal and Infant Health through Human Milk

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Corresponding Author:	Meghan B Azad CANADA
First Author:	Katherine Donald
Order of Authors:	Katherine Donald
	Charisse Petersen
	Stuart E. Turvey
	Meghan B Azad
	B. Brett Finlay
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Secretory IgA: (Cross)linking Microbes, Maternal and Infant Health through Human Milk

Katherine Donald*[1,2], Charisse Petersen*[3,4], Stuart E. Turvey[3,4], B. Brett Finlay[1,2,5,6], and Meghan B. Azad[6,7,8]

1. Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada
2. Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada
3. Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada
4. British Columbia Children's Hospital, Vancouver, BC, Canada
5. Department of Biochemistry, University of British Columbia, Vancouver, BC, Canada
6. Department of Molecular Biology, University of British Columbia, Vancouver, BC, Canada
7. Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB, Canada
8. Manitoba Interdisciplinary Lactation Centre (MILC), Children's Hospital Research Institute of Manitoba, Winnipeg, MB, Canada

* These authors contributed equally

Corresponding Author: Meghan B. Azad

501G-715 McDermot Ave. | Winnipeg, MB Canada | R3E 3P4

+1 (204) 975-7754 | meghan.azad@umanitoba.ca | @MeghanAzad

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SUMMARY

Secretory Immunoglobulin A (SIgA) in human milk plays a central role in complex maternal-infant interactions that influence long term health outcomes. Governed by genetics and maternal microbial ~~experience~~exposure, human milk SIgA shapes both the microbiota and immune system of the infant. Historically, SIgA-microbe interactions have been challenging to unravel due to their dynamic and personalized nature, particularly during early life. Recent advances have helped to clarify how SIgA acts beyond simple pathogen clearance to help guide and constrain a healthy microbiota, promote tolerance, and influence immune system development. In this review, we highlight these new findings in the context of the critical early-life window and propose outstanding areas of study that will be key to harnessing the benefits of SIgA to support healthy immune development in infancy.

KEYWORDS

immunoglobulin A, SIgA, microbiome, breastfeeding, human milk, breast milk, immunity, infection, infancy

INTRODUCTION

Human milk is a complex mixture of nutrients, immunomodulatory factors, maternal cells, and microbes that function collectively to support healthy host-microbe interactions during infant development. As the prevalence of childhood allergic and metabolic disorders increased over the latter twentieth century, and the role of microbiota in these ~~pathologies is increasingly recognized~~pathologies gained increasing attention (Petersen and Turvey, 2020), it ~~has become~~became abundantly clear that the maternal-milk-infant relationship is critical to promoting healthy development on a population level (Bode et al., 2020). However, instead of revealing a clear and common mechanism driving this beneficial interaction, recent studies and technological advances have instead highlighted the diverse and personalized aspects of the ~~mother-milk-infant triad~~maternal milk (Munblit et al., 2017; Nuzzi et al., 2021). Furthermore, this complexity is multilayered. In addition to maternal factors (e.g. genetics, parity, diet, allergies) which contribute to differences in milk composition, many components change over the course of lactation and each component seems capable of influencing infant development via interconnecting mechanistic pathways (Munblit et al., 2017). Thus, the dynamic nature of human milk makes it important but challenging to understand.

The first 3 months of life represent an important period of immune development during which microbial encounters can shape life-long immune health (Arrieta et al., 2015; Boutin et al., 2020; Al Nabhani and Eberl, 2020). Despite its intricacies, human milk is clearly a vital factor in microbiota establishment and microbiota-mediated immune imprinting during this critical early-life window. To aid early colonization, human milk seeds the infant microbiota with a select consortium of microbes (Fehr et al., 2020; Moossavi et al., 2018) along with human milk oligosaccharides (HMOs), which act as a nutrient source to establish this ~~a~~ niche (Bode, 2012). The microbial breakdown of these HMOs results in secondary metabolites that can then support

healthy gut and immune cell development (Zuurveld et al., 2020). Human milk components also directly prime immune cells towards tolerant responses via cytokines (e.g. TGF-beta, IL-10), lactoferrin, and short-chain fatty acids that alter cellular differentiation and gene expression (Dawod and Marshall, 2019; Gridneva et al., 2021). Immune cell tolerance can be measured on a specific antigenic level as well as in relation to broader microbiota compositional fluctuations that occur early in life, as was evidenced in a mouse model demonstrating a rapid immune reaction towards the microbiota once animals were weaned (Al Nabhani et al., 2019; Verhasselt et al., 2008). Importantly, antibiotic exposure during this weaning-associated immune reaction can lead to aberrations in immune development (Al Nabhani et al., 2019), highlighting the importance of microbial colonization in early-life. While each of these mechanisms and milk components are broadly understood, their individual makeup and influence vary significantly among mother-infant dyads. For example, HMO and milk microbe compositions are highly personalized (Azad et al., 2018; Moossavi and Azad, 2020), and ranges of cytokine, immunoglobulin, and fatty acid levels are highly variable (Munblit et al., 2017).

While many infant-mother interactions within and beyond human milk steer early-life development through multiple mechanisms, one of the most abundant human milk proteins, secretory immunoglobulin A (SIgA), has only recently been appreciated for its ability to regulate both microbial colonization and infant immune responses. IgA's impact on health has been historically restricted to infection defense via pathogen clearance; however, its function within the infant gut has now been broadened to include promoting colonization of beneficial microbes, maintaining microbial diversity, acting as a nutrient source, and regulating microbial genetic expression to modify metabolic outputs and virulence (Huus et al., 2021) **(FIGURE 1)**. In this way, the effects of SIgA~~SIgA's effects~~ are not limited to the microbiota, but also influence immune cell development resulting in tolerant host-microbe interactions within the gut. In this review, we will

highlight the complexities of human milk SIgA and its role in establishing healthy host-microbe interactions in the infant gut.

SECRETORY IMMUNOGLOBULIN A: A KEY PLAYER IN THE GUT ENVIRONMENT

SIgA is the most abundant antibody in the human body~~,~~ and comprises two IgA monomers linked by a J chain and secreted via polymeric Ig receptor (pIgR). Upon translocation across the epithelium, pIgR donates a secretory component (SC) to the IgA dimer. The presence of SC differentiates secreted SIgA, found in human milk and at other mucosal sites, from dimeric IgA found in the serum. Unlike other antibodies, SIgA comes in two forms: T-cell dependent SIgA, which is monoclonal and forms specific, high affinity interactions, and T-cell independent SIgA that is poly-clonal and binds with less affinity (Bunker and Bendelac, 2018; Huus et al., 2021). SIgA can interact with bacteria in the gut through traditional Fab interactions, in the case of high-affinity IgA, or through non-specific Fc interactions, or even through binding to the SC or associated glycan structures (Huus et al., 2021).

SIgA levels and targeting patterns vary tremendously between people and in response to environmental factors and infection. In healthy individuals, 10-50% of the gut microbiota is bound by SIgA, and any one species may be targeted in one individual but not another (Palm et al., 2014; Sterlin et al., 2020). Host genetics and environmental factors contribute to this variation, and distinct patterns in IgA-targeting within populations can be identified. In order to study IgA-targeting patterns in feces and other mucosal secretions, the technique IgA-SEQ has been developed **(FIGURE 2)**. This technique involves staining IgA-bound bacteria and then using fluorescence-associated cell sorting to sort bacteria into IgA+ and IgA- fractions. Cells in each fraction are then sequenced using 16S~~s~~ or shotgun sequencing and an IgA index, which represents the relative proportion of bacteria targeted by IgA, is calculated for each strain

(Jackson et al., 2021; Kau et al., 2015; Palm et al., 2014). Altered SIgA responses to the gut microbiota have been linked to several disease states, including inflammatory bowel disease (IBD), allergic disease, multiple sclerosis, metabolic disorders, and others (Huus et al., 2020a; Kukkonen et al., 2010; Palm et al., 2014; Pröbstel et al., 2020). In the case of ~~inflammatory bowel disease (~~IBD~~)~~, increased overall coating of the gut microbiota, along with preferential coating of pathogenic bacteria in the gut, are associated with disease (Palm et al., 2014). Host nutrition and geographic location can also heavily influence IgA responses to the gut microbiota (Huus et al., 2020b). Whether these alterations represent~~s~~ a response to a disease-associated dysbiotic microbiota or an altered immune state which drives dysbiosis is not known, but the study of IgA-targeting patterns may provide a clearer diagnostic marker than microbiota sequencing alone. While gut microbiota composition can provide insight into host health, the identification of IgA-targeted and un-targeted species adds a layer of information about how the host is interacting with the microbes present.

The effects of antibody binding on bacteria and on the host are varied and complex **(FIGURE 1)**. SIgA has been shown to act in the traditional antibody role of neutralizing and clearing pathogens. One mechanism for this clearance is agglutination, which involves SIgA-mediated clumping of bacteria. In the case of fast-growing bacterial cells, this clumping facilitates movement of the bacteria through and out of the intestine (Hoces et al., 2020). SIgA can also prevent translocation of pathogenic bacteria across the epithelium (Randal Bollinger et al., 2003). Interestingly, SIgA also promotes the establishment of symbionts in the gut through supporting microbial adherence to epithelium and biofilm formation (Orndorff et al., 2004). Indeed, members of the microbiota have evolved to utilize SIgA, including *Bacteroides fragilis* which actively alters surface antigen expression to increase SIgA binding and enhance its colonization, as well as members of *Lachnospiraceae* which utilize superantigens to stimulate and bind SIgA (Bunker et al., 2019; Donaldson et al., 2018). Glycan structures on SIgA, which ~~also~~ vary greatly between

individuals, can also provide a carbon source for growing bacteria (Cao et al., 2014; Huang et al., 2015).

Multiple studies of individual SIgA-targeted strains have shown that antibody-binding can also alter microbial gene expression. *Bacteroides thetaiotamicron,* a well-studied example of this, upregulates a gene cassette involved in inter-bacterial symbiosis in the gut in response to SIgA binding (Nakajima et al., 2018). The transfer of hybridoma "backpacks" containing SIgA specific to *B. thetaiotamicron* was used to confirm the pro-colonization effects of SIgA on this microbe *in vivo*. Genes involved in virulence can also be down-regulated in response to binding, facilitating host-microbe homeostasis in the gut (Bunker and Bendelac, 2018; Peterson et al., 2007; Tran et al., 2019). The mode of SIgA-binding to a bacterium may also play a role in its effect. In one study, a monoclonal SIgA (W27) isolated from the mouse gut was found to bind to multiple microbes with varying strength (Okai et al., 2016). W27 inhibited growth of bacteria expressing the epitope targeted by the antibody Fab portion, while having no effect on bound bacteria which did not express the specific epitope. Antibody binding to these bacteria occurred presumably through Fab-independent interactions, indicating that stronger, Fab-dependent binding to bacteria may have a more dramatic hinderance effect on growth. Upon oral administration of W27, mice showed reduced susceptibility to colitis and an altered gut microbiota, supporting a potential therapeutic role for SIgA in the gut. In a study of host-microbe interactions associated with malnutrition, binding though antibody glycan structures seemed to promote adherence of beneficial bacteria in the gut (Huus et al., 2020b). Thus, with its broad array of binding modes, targets, and functions, SIgA is a powerful and multifaceted modulator of the gut microbiota.

HUMAN MILK SIGA: THE LINK BETWEEN THE MATERNAL AND INFANT GUT

SIgA is abundant at all mucosal sites, including the mammary gland. SIgA is the dominant immunoglobulin in human milk, although IgM and IgG are also present. In colostrum, the milk produced in the first 5 days after birth, SIgA concentrations have been reported to range from 1.5 to 83.7 g/L (Mickleson and Moriarty, 1982). The levels decline after this period and continue to vary greatly between mothers (Mickleson and Moriarty, 1982; Rio-aige et al., 2021; Weaver et al., 1998). Many studies have quantified SIgA in human milk, but there is great heterogeneity in results due to differences in quantification method, sample handling, collection techniques, low sample size, and general heterogeneity in IgA levels between individuals. A recent meta-analysis of these studies concluded that SIgA is highest in colostrum, lower in transition milk (5-15 days post-gestation), and possibly more varied in mature milk (Rio-aige et al., 2021). The average SIgA concentrations were found to be 7.5 g/L in colostrum, and 1.6-2 g/L in transitional and mature milk. In contrast, IgM and IgG concentrations were primarily found to be lower than 1 g/L in milk at all post-gestation stages. Similar to intestinal SIgA, milk SIgA varies based on host genetics, environmental factors, and health. Several studies have shown stark differences in antibody levels between mothers from different geographic locations (Childs et al., 2017; Johansson et al., 2010; Munblit et al., 2015).

In preparation for lactation, IgA-secreting plasma cells expressing CCR10 are recruited to the mammary gland by the chemokine CCL28 beginning during late pregnancy (Wilson and Butcher, 2004). Epithelial cells within the milk ducts express pIgR, enabling secretion of the full SIgA molecule directly into the milk. Upon weaning, CCL28 signaling and plasma cell accumulation decline rapidly in the mammary gland (Niimi et al., 2018). Plasma cells throughout the body, including the gut, express CCR10; however, gut associated plasma cells have a higher propensity to home to mammary tissue. The gastrointestinal origins of breast tissue plasma cells

were demonstrated by a study in which radioactively labeled gut mesenteric lymph node or peripheral lymph node cells were transferred to pregnant or virgin mice (Roux et al., 1977). A higher level of radioactivity was recovered in the mammary glands of pregnant mice receiving gut labeled plasma cells, and these cells produced higher levels of IgA that persisted from late pregnancy throughout lactation in these mice. A more recent study used a photoconvertible reporter mouse model to show that plasma cells migrate from the intestine to the mammary gland during late pregnancy (Ramanan et al., 2020). Furthermore, a comparison of the IgA repertoire at different body sites in mice showed great similarity between milk SIgA and intestinal SIgA (Lindner et al., 2015). Thus, maternal microbial encounters in the gut governs the quality and quantity of SIgA within human milk to help shape the infant microbiota.

SIgA in early life

Infants are not equipped with a fully developed immune system at birth, making them highly susceptible to infection and immune dysregulation as they enter and interact with a microbe-rich world (Torow et al., 2017). The first 3 months of life in particular represents a critical window for immune development. Host-microbe interactions which occur during this time contribute to immune imprinting, affecting life-long health (Al Nabhani and Eberl, 2020). Certain events during this critical period have been linked to later development of allergic and autoimmune disease, and the ways in which early life host-microbe interactions influence immune development are still being uncovered (Boutin et al., 2020). Antibody responses are particularly lacking in the first several months of life. Adult antibody levels are not reached until after the first year of life, and serum IgA is extremely low or undetectable for the first 2 months of life (South et al., 1967; Weemaes et al., 2003), while fecal SIgA levels are high ~~in~~ (Hibel and Schiltz, 2016). It is currently thought that IgA+ B cells are not produced by the infant until at least 4 weeks of age (Rognum et

al., 1992). Thus, milk ingested by the infant is the primary source of SIgA during the vulnerable and vital early-life period. Milk SIgA plays many roles in the infant gut, including microbiota modulation, protection against pathogens, and dampening the infant immune response to the plethora of unfamiliar invaders (Atyeo and Alter, 2021). Defining the interactions between this maternal immune factor and the developing microbiota is thus an important step in understanding immune maturation.

Infants who do not receive human milk start producing SIgA in the intestine around 4 weeks of age (Neu and Walker, 2021). Breastfed infants show substantially increased SIgA levels in the feces for at least the first 3 months of life in comparison to formula-fed infants (Azad et al., 2012; Koutras and Vigorita, 1989). Interestingly, one study found that infant fecal SIgA levels at 6 months of age are higher in formula-fed infants compared to breastfed infants (Hibel and Schiltz, 2016). This may indicate premature SIgA production in response to the lack of maternal antibodies present, which aligns with the immune-dampening role of human milk immune factors in the infant gut (Hornef and Torow, 2020). It could also reflect compensation: Breastfed infants may not need to produce as much SIgA because the maternal SIgA repertoire present is more efficient at targeting and modulating the gut microbiota.

Human milk SIgA and infection

The role of –milk SIgA in infant health has been extensively studied in the context of infection and inflammation. Milk SIgA has been shown to target rotavirus, poliovirus, and other enteric pathogens previously encountered by the mother through infection or vaccination (Brandtzaeg, 2003; Patel et al., 2013; Wright et al., 2014). Influenza- and, more recently, SARS-CoV-2- specific IgA have also been found in the milk of immunized and recently recovered mothers (Nunes et al., 2017; Pace et al., 2021; Perl et al., 2021). These antibodies may protect

the infant against infection while the immune system is still developing. One interesting analysis of the pathogen targeting abilities of milk from women across geographical regions displayed region-specific patterns in targeting, supporting the idea that human milk is tailored to the infant based on the maternal environment (McGuire et al., 2021). However, non-specific SIgA interactions may confer protection as well, as studies of a Canadian birth cohort have shown that overall increased infant fecal SIgA levels are associated with breastfeeding and reduced *Clostridium difficile* colonization (Azad et al., 2012; Bridgman et al., 2016).

SIgA in preterm delivery and necrotizing enterocolitis (NEC)

~~Click or tap here to enter text.Click or tap here to enter text.Click or tap here to enter text.Click or tap here to enter text.~~Preterm delivery affects all arms of the immune system and puts infants at increased risk for infection and chronic inflammatory disease (Melville and Moss, 2013). Necrotizing enterocolitis (NEC) is a dangerous gastrointestinal disease that is common among premature infants (Neu and Walker, 2021). It involves an inflammatory response to the gut microbiota and is associated with reduced microbiota diversity and over-representation of *Enterobacteriaceae* (Pammi et al., 2017). It is unclear whether preterm milk has an altered SIgA composition. One study showed increased SIgA levels following preterm birth (Koenig et al., 2005), while others show no difference (Trend et al., 2016), although survival of secretory immunoglobulins in the digestive tract of preterm infants may be increased (Demers-Mathieu et al., 2018). It is clear that breastfeeding, and SIgA in human milk specifically, protects the preterm infant against infections and NEC ~~common among preterm infants~~ (Neu and Walker, 2021). However, the mother's own milk may not always be available to the preterm infant, depending on the gestational stage at delivery and maternal health status, and other factors. Many of these infants receive pasteurized human donor milk, which maintains partial immunological activity,

including from SIgA (Irazusta et al., 2020). One clinical trial from 1988 showed that administration of an oral immunoglobulin supplement containing IgA and IgG protects against NEC in preterm infants not receiving human milk (Eibb et al., 1988). IgA supplementation has not been widely pursued since this small study, perhaps on account of difficulty in IgA manufacturing and a lack of knowledge related to IgA specificity in the gut. However, recent characterization of IgA's multifaceted role support that this antibody should be further pursued for its potential therapeutic and preventative effects.~~may be a safer and more approachable avenue for gut microbiota modulation than probiotic supplementation in infants who are at risk for disease or unable to breastfeed.~~

~~Necrotizing enterocolitis (NEC) is a dangerous gastrointestinal disease that primarily affects premature infants (Neu and Walker, 2021). It involves an inflammatory response to the gut microbiota and is associated with reduced microbiota diversity and over representation of *Enterobacteriaceae* (Pammi et al., 2017). Breastfeeding reduces the risk of NEC (Gopalakrishna et al., 2019), and this is likely through the immune factors present in human milk which dampen the neonatal immune response. SIgA in milk has been shown to play a role in preventing NEC.~~More recent studies have clarified the roles of SIgA in preventing NEC. Infants who developed NEC showed reduced SIgA targeting of the gut microbiota compared to healthy, age-matched controls (Gopalakrishna et al., 2019). The changes associated with NEC seemed to be driven by a reduction in SIgA-targeting of *Enterobacteriaceae*, enabling their overgrowth in the gut. In comparison, IgG and IgM did not play a detectable role. Supporting this association, a mouse model of NEC in which offspring fed by IgA-deficient dams were more susceptible to disease (Gopalakrishna et al., 2019). Notably, the outcomes of the pups given SIgA deficient milk were indistinguishable from formula-fed pups. The effects of SIgA on NEC susceptibility and pathogenesis therefore supports the important role of this antibody in modulating the gut microbiota and preventing inflammation in early life.

HUMAN MILK ~~SIGA~~ SIgA AND THE DEVELOPING GUT MICROBIOTA

Human milk is the primary source of antibodies in the infant intestine, and SIgA is gaining more attention for its role in modulating the early life gut microbiota (**FIGURE 3**). It is clear that this antibody plays an important role in protecting the underdeveloped immune and mucosal tissue from pathogens and inflammation, but it likely also plays an even more complex role in gut microbiota development and host-microbe symbiosis. This is an exciting hypothesis that warrants further study, as changes in the gut microbiota during the first few months have been shown to affect life-long host health (Boutin et al., 2020; Nino et al., 2021). Studies of human milk SIgA may contribute to a better understanding of the "microflora hypothesis" which postulates that microbial exposure in infancy affect the developing immune system (Penders et al., 2007; Wold, 1998). Reduced diversity and a loss of specific taxa in the infant gut are associated with later development of several chronic diseases, but the reasons for microbiota differences between infants and the mechanisms driving microbial maintenance and adherence in the gut are not fully elucidated (Bisgaard et al., 2011; Nino et al., 2021; Petersen and Turvey, 2020). SIgA is a promising candidate factor in shaping a healthy gut microbiota, and human and animal studies are just beginning to uncover the interactions between milk SIgA and beneficial members of the gut microbiota. Other human milk components, such as HMOs, microbes and cytokines, have been studied for their effects on the infant gut, but SIgA is only starting~~beginning~~ to be appreciated for its contribution to breastmilk's role in early life microbiota development.

At 10 days of age, 80% of the gut microbiota is bound by SIgA in breastfed infants (Gopalakrishna et al., 2019). Targeting decreases slightly over the course of infancy, to 50% at 1 month and 30% at 12 months of age, but varies significantly between infants (Dzidic et al., 2017). *Bifidobacterium* and *Enterobacteriaceae* are enriched in IgA+ fractions obtained from infant feces and are both positively associated with fecal IgA levels (Janzon et al., 2019). *Bifidobacterium*

species are the most well-characterized members of the infant gut microbiota and are known to be favored by the prebiotic content of human milk (Henrick et al., 2021). Certain strains of Bifidobacteria also have numerous beneficial effects on infant development, and their abundance has been linked to increased immunoregulatory responses, protection against enteropathogens, and reduced allergic disease susceptibility (Fukuda et al., 2011; Henrick et al., 2021; Kalliomäki et al., 2001). While HMOs are utilized by some Bifidobacteria, human milk SIgA may also play a role in the establishment of Bifidobacterial communities in the infant gut (Chichlowski et al., 2012; Henrick et al., 2021). As described above, SIgA has been shown to promote colonization of certain commensals in the intestine, but a pro-colonization mechanism of SIgA-Bifidobacteria interactions has yet to be explored. Through clearance and inhibition of other species, SIgA might also indirectly provide a niche for Bifidobacteria and other beneficial species in the infant gut. This is supported by dysbiosis associated with both SIgA deficiency and formula feeding (Catanzaro et al., 2019; Gopalakrishna and Hand, 2020). Increased levels of *Enterobacteriaceae* are associated with formula-feeding (Kim and Yi, 2020), and a loss of IgA-targeting of this family was linked to overgrowth and development of NEC (Gopalakrishna et al., 2019). Future studies should focus in on individual bacterial taxa and the effect that maternal SIgA has on them in the gut.

In vivo mouse studies of milk SIgA support its role in shaping the gut microbiota and mucosal immunity early in life. In one informative study, a transgenic mouse lacking pIgR was used to isolate the influence of maternal milk SIgA from that of host endogenous SIgA produced by offspring. The pIgR-deficient mouse (pIgR(-/-)) is unable to secrete IgA across the mucosal epithelium, but produces normal levels of serum IgA, while pIgR(+/-) mice secrete normal levels of IgA into the intestine and mammary gland. After crossing a pIgR(-/-) female with a pIgR(+/-) male, Rogier et al. studied several alterations associated with maternal SIgA specifically (Rogier et al., 2014). All pups were nursed by their biological pIgR(-/-) mother, and half of them inherited the functional pIgR gene through the cross, but none of them received SIgA through milk. Pups

that did not receive SIgA through nursing displayed increased bacterial translocation across the epithelium, as demonstrated by increased culturable bacteria in harvested mesenteric lymph nodes. These mice also displayed distinct shifts in microbiota composition which lasted through adulthood, even after endogenous SIgA production in pIgR(+/-) offspring began. This indicates that early-life SIgA-microbe interactions have a lasting impact on the gut microbiota. A lack of milk SIgA was also associated with increased expression of genes related to intestinal inflammatory disease (Rogier et al., 2014). The breeding scheme in this study provided a wealth of information about milk SIgA in the neonatal gut and should be utilized in the future to look specifically at the role of milk SIgA in disease models and other phenotypes.

Human milk SIgA and milk microbes

SIgA interacts with the microbes found in human milk and may facilitate their maintenance in the infant gut. This is a new topic of interest and only a few publications include analysis of the targeting patterns of SIgA within human milk. Based on the limited available data, approximately 40% of the human milk microbiota is IgA-bound (Dzidic et al., 2020). *Bifidobacteria, Streptococcus,* and *Lactobacillus* are IgA-targeted in the maternal gut, milk, and infant intestine, suggesting that SIgA aids in the mother-to-infant transfer of microbes. A phylogenetic analysis of *Bifidobacterium longum* strains show identical strains in mother-infant dyads, suggesting direct vertical transmission of this species, though the role of SIgA in this transfer was not directly studied (Meyer et al., 2019). The mechanism for transmission of microbes from the maternal gut and environment to the infant is not well-understood (Wang et al., 2021), but SIgA may mediate transfer and maintenance of specific strains in the infant. IgA-SEQ has been developed and well-utilized in adult gut microbiota studies and can be used to further characterize the host-microbe interactions occurring within human milk **(FIGURE 2)**.

IgA-MICROBIOTA INTERACTIONS, THE DEVELOPING IMMUNE SYSTEM AND ALLERGIC DISEASE

In addition to providing passive immunity, breastfeeding has been shown to dampen immune responses early in life, preventing inflammation and overactive responses to unfamiliar microbes throughout the body. In this way, human milk can be thought of as the “training wheels” for the developing immune system. Upon weaning, the gut microbiota increases in diversity and shifts toward a more adult-like state. In mice, a rapid increase in various immune cell populations accompanies this shift (Dogra et al., 2021; Al Nabhani and Eberl, 2020). Perhaps counterintuitively, maintaining an “immature state” of intestinal host-microbe interactions through breastfeeding actually seems to benefit the infant, protecting against later development of chronic disease (Knoop et al., 2018). Studies suggest that immune system dampening in this early stage of life promotes a more balanced and regulatory immune response to the environment as the infant matures (Al Nabhani et al., 2019).

In vivo and epidemiological studies suggest that SIgA may play a role in the phenomenon of immune dampening. When pIgR+/- mice, which produce their own SIgA, are fed by pIgR-/- dams, which do not secrete IgA, they begin to produce endogenous SIgA prematurely and are more prone a mouse model of colitis (Harris et al., 2021). Mice fed by pIgR-/+ dams with normal levels of milk SIgA show increased expression of genes involved in maintenance of the epithelial layer in comparison to mice fed by pIgR-/- dams, suggesting that early life milk SIgA drives changes in the mucosal epithelium (Rogier et al., 2014). Maternally transferred IgA also mediates regulatory T cell homeostasis in the developing gut. Through cross-fostering of mouse pups by genetically different dams, Ramanan *et al.* showed that milk SIgA affects RORγt Treg cell proportions in the neonate, and that the set-point established during the early post-birth period

impacts life-long T cell levels (Ramanan et al., 2020). These effects persisted through multiple generations, supporting the long-term importance of human milk SIgA in early life. Furthermore, SIgA has also been demonstrated to coordinate with maternally acquired IgG to promote tolerant T cell responses within the gut (Koch et al., 2016). Thus, SIgA provides a mode of non-genetic inheritance from mother to infant which occurs exclusively during the early infancy window.

Human studies also suggest that milk IgA postpones infant immune plasma cell development. As mentioned previously, formula-fed infants have increased SIgA responses at 6 months of age in comparison to breastfed infants, potentially representing an overactive immune response to the gut microbiota in the absence of maternal SIgA's dampening effects (Hibel and Schiltz, 2016). At the same time, formula-fed infants display a more mature gut microbiota earlier than breastfed infants (Ma et al., 2020), and the relative contribution of SIgA has not been disentangled from the effects of differences in microbiota composition alone on host health. Gut microbiota diversity and IgA repertoire diversity are dependent on each other, as increases in one lead to increases in the other. It is likely that the early spike in diversity observed in the formula-fed infant gut drives the relative rise in SIgA levels, although whether the pre-mature activation of infant plasma cells affects the antibody efficiency or sensitivity is not known.

Human milk SIgA and its interactions with gut microbiota may also play a role in allergy susceptibility. Rates of allergic disease have increased markedly over the past 2-3 decades, and allergies have been linked to the "microflora hypothesis" described earlier (Boutin et al., 2020; Petersen and Turvey, 2020; Sbihi et al., 2019). Whether breastfeeding protects against allergies is not entirely clear, which may be due to the great heterogeneity that exists in human milk composition among mothers (Nuzzi et al., 2021; Oddy, 2017). Human milk SIgA levels and targeting patterns vary among mothers, and can be influenced by genetics and microbial experience. Despite the variability, fecal SIgA levels seem to be reduced in infants who develop

allergies (Kukkonen et al., 2010; Orivuori et al., 2014). Furthermore, at one month of age, when fecal SIgA is likely almost entirely of maternal origin, infants who go on to develop allergies show altered IgA-targeting of several intestinal taxa (Dzidic et al., 2017). Since human milk SIgA originates in the mother's intestine, differences in the SIgA repertoire are likely explained by differences in the gut microbiota among mothers. Therefore, as beneficial IgA-targeted species are identified, probiotic supplementation which drives intestinal IgA responses to these species in the mother could potentially be used to improve and diversify the IgA transmitted to the infant.

CONCLUSIONS AND FUTURE DIRECTIONS

Intestinal secretory IgA has been extensively studied for its role in modulating the microbiota, clearing pathogens, and impacting systemic host health. IgA-SEQ analyses have shed light on the great variation in targeting patterns between healthy individuals, as well as the specific alterations in IgA activity associated with several disease states. Human milk SIgA represents an exciting new domain for understanding the close interaction between the host and the gut microbiota during the critical and vulnerable period of infancy. So far, research has shown that human milk SIgA originates in the maternal intestine and varies greatly among mothers. Breastfeeding enables antibody-mediated protection of the infant against enteropathogens and ~~NEC, and~~NEC and can have multigenerational effects. Milk SIgA also influences the developing gut microbiota (e.g. via pathogen exclusion, aiding early colonizers, and niche promotion) and can thus impact life-long health and immunity, and a limited set of studies suggest that SIgA may target milk microbes, affecting their ability to colonize the infant gut.

While the origins of human milk SIgA and its ability to clear pathogens are well-established, its capacity to promote beneficial taxa and drive immune and microbiota maturation are still far from understood. Future studies of the human milk 'immunoglobulin-ome' should seek

to not only quantify SIgA, but to identify its targets within the milk itself and upon delivery to the infant gut. It is clear that SIgA specificity may be more important than the overall amount of SIgA present (Dzidic et al., 2017; Palm et al., 2014). Further, *in vivo* studies using mouse models of IgA-deficiency and cross-fostering schemes should be used to understand the role of maternal SIgA, in addition to endogenously produced IgA, in various chronic diseases and inflammatory phenotypes. *In vitro* studies used to study IgA-mediated biofilm formation, adherence, and changes in gene expression can serve as models in the study of milk SIgA interactions with microbes important in the infant gut, such as species within the families *Bifidobacteriaceae* and *Enterobacteriaceae*. Human milk is rich in immune and nutritional components adapted to the infant's needs, but it differs greatly in composition among women and across populations. Given the life-long impact of early-life gut microbiota development patterns, understanding the contribution of milk SIgA to gut homeostasis in the infant is critical. Future research in this field may guide improvements in infant feeding practices and products, maternal probiotic supplementation, and preventative health interventions.

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courses on breast milk and the infant microbiome produced by Microbiome Courses. She serves in a volunteer capacity for the International Society for Research on Human Milk and Lactation and as a member of the National Academy of Sciences, Engineering and Medicine Committee on Scanning New Evidence on the Nutrient Content of Human Milk. She has consulted for DSM Nutritional Products and serves on the Malaika Vx Scientific Advisory Board. Work in BBF.'s lab is supported by a Canadian Institutes for Health Research (CIHR) Foundation Grant. BBF. is also a Canadian Institute For Advanced Research (CIFAR) Senior Fellow. SET holds the Aubrey J Tingle Professorship in Pediatric Immunology and the Tier 1 Canada Research Chair in Pediatric Precision Health. Support for his lab is provided by Canadian Institutes of Health Research, the Allergy, Genes and Environment Network of Centres of Excellence, and Genome Canada/Genome BC. KD is supported by the Four Year Fellowship Tuition Award, President's Academic Excellence Initiative PhD Award, and International Tuition Award at UBC.

FIGURES

Figure 1. The dual role of SIgA in the gut: maintenance and clearance of targeted microbes.

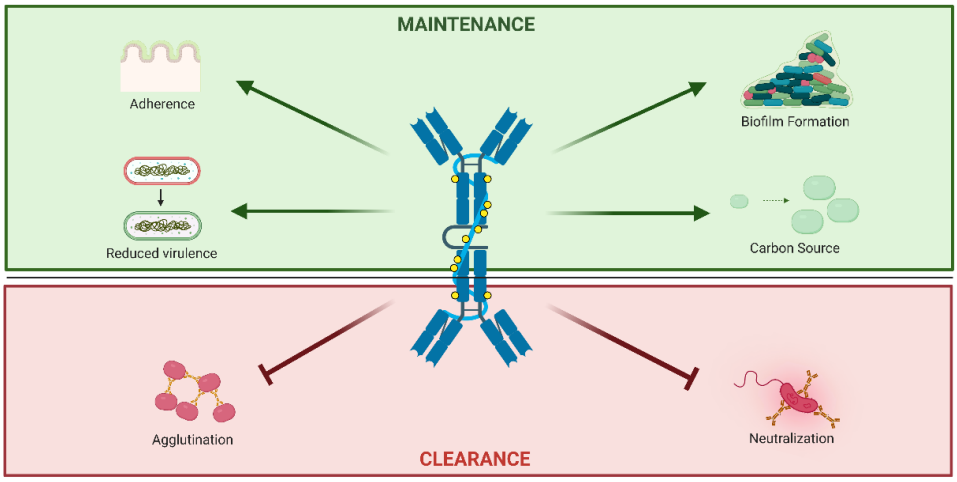

Figure 1. The dual role of SIgA in the gut: maintenance and clearance of targeted microbes. SIgA can carry out multiple functions upon binding to bacteria and other microbes in the gut. Dimeric IgA (dark blue) contains two IgA monomers linked by a J chain. The secretory component (light blue; SC) protects the SIgA against degradation and provides additional points for attachment to targets. Glycans (yellow) decorate the IgA dimer and the secretory component. SIgA can bind to targets through traditional Fab-dependent binding, or through binding to the Fc region, glycans, or SC. Upon binding to its target, SIgA can aid in bacterial adherence to the epithelium, promote biofilm formation, provide a carbon source, and alter gene expression to reduce bacterial virulence. These mechanisms of action may aid in maintaining certain targeted species within the gut. SIgA binding can also neutralize pathogens, preventing their translocation and immune activation, and agglutinate fast-growing pathogens to promote their movement through the gastrointestinal tract. Thus, SIgA is also able to promote the clearance of potentially detrimental taxa.

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Figure 2. IgA-SEQ and its application to human milk IgA

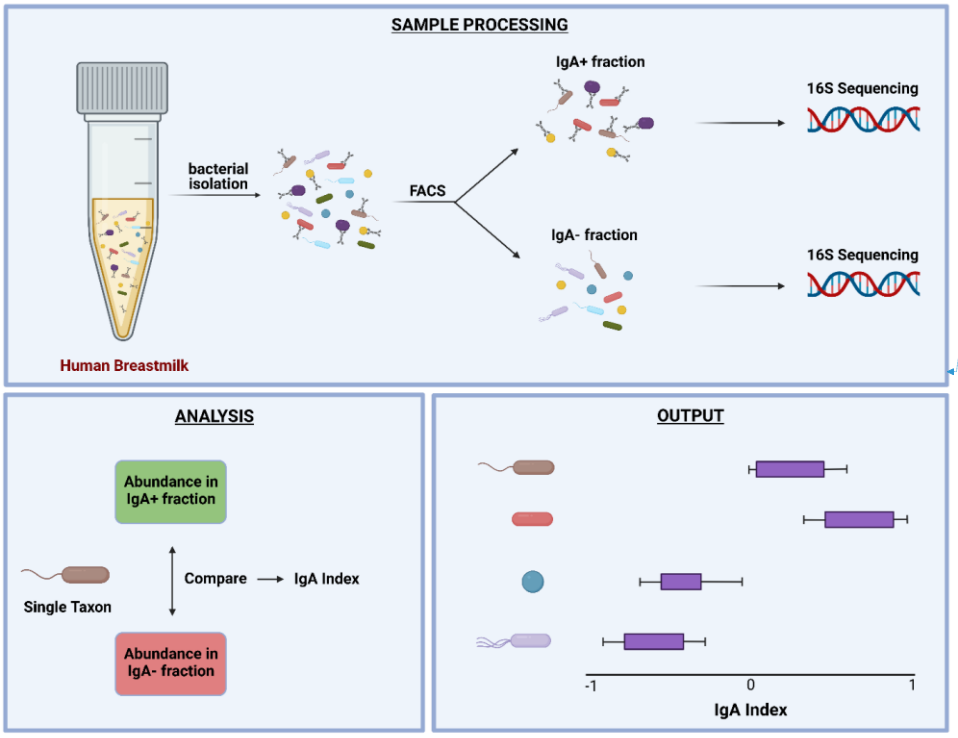

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Figure 2. IgA-SEQ and its application to human milk IgA. IgA-SEQ is a widely used technique in the study of IgA-targeting patterns in fecal samples. The~~is~~ schematic details the technique and its potential application in human ~~human~~ milk studies. A biological sample containing bacteria (feces, human ~~human~~ milk, etc.) is used. Bacteria are isolated from the sample via centrifugation and filtration techniques. Cells are then stained using a nucleic acid stain, to identify bacteria, and a fluorescent antibody which binds the SIgA molecule. Stained bacteria are then sorted into IgA+ and IgA- fractions using fluorescence-associated cell sorting. The two fractions are then characterized by next-generation sequencing. Analysis of sequencing data involves generating an IgA index, which represents the ratio of bacterial abundance between IgA+ and IgA- fractions, for each genus or taxon present. When analyzing fecal or human milk samples from a group of individuals, IgA-targeting can be summarized for each taxa separately, enabling comparison of IgA-targeting between microbes and identification of patterns in IgA-targeting common to the group and/or different between groups. The use of this technique on human ~~human~~ milk samples would aid in understanding of the roles and targeting patterns of SIgA.

Figure 3. Human milk SIgA and the infant gut microbiota

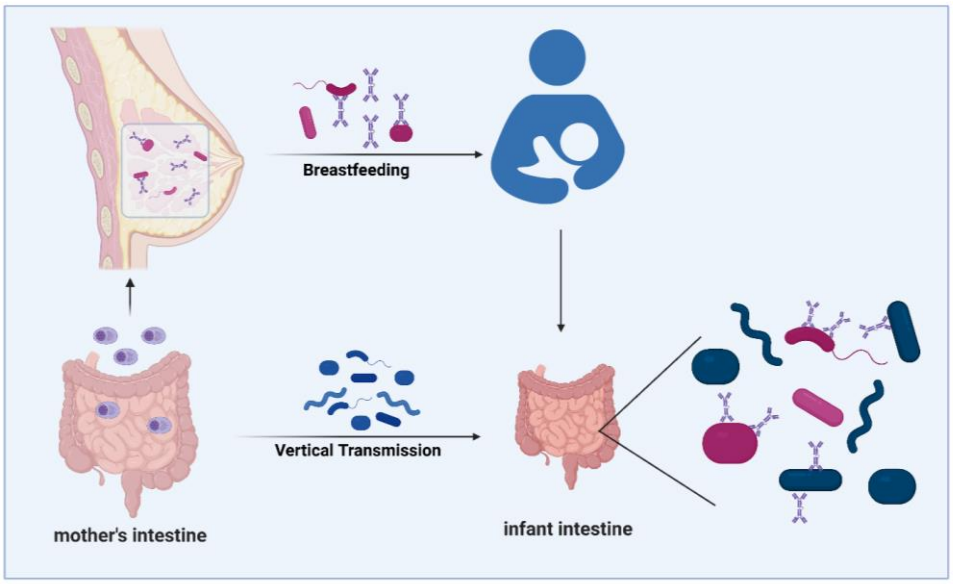

Figure 3. Human milk SIgA and the infant gut microbiota. Plasma cells originating in the maternal intestine migrate to the mammary gland through circulation during late pregnancy and lactation. These cells produce IgA antibodies which are secreted into the human milk. Breastmilk SIgA is transferred to the infant through breastmilk feeding, along with human milk microbes (shown in pink), some of which are SIgA-bound. During birth and early life, microbes from the maternal gut (shown in blue) are transmitted to the infant gut. SIgA, the maternal gut flora, and human milk microbes from the mother work together to establish and maintain the infant gut community.

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Figure 1

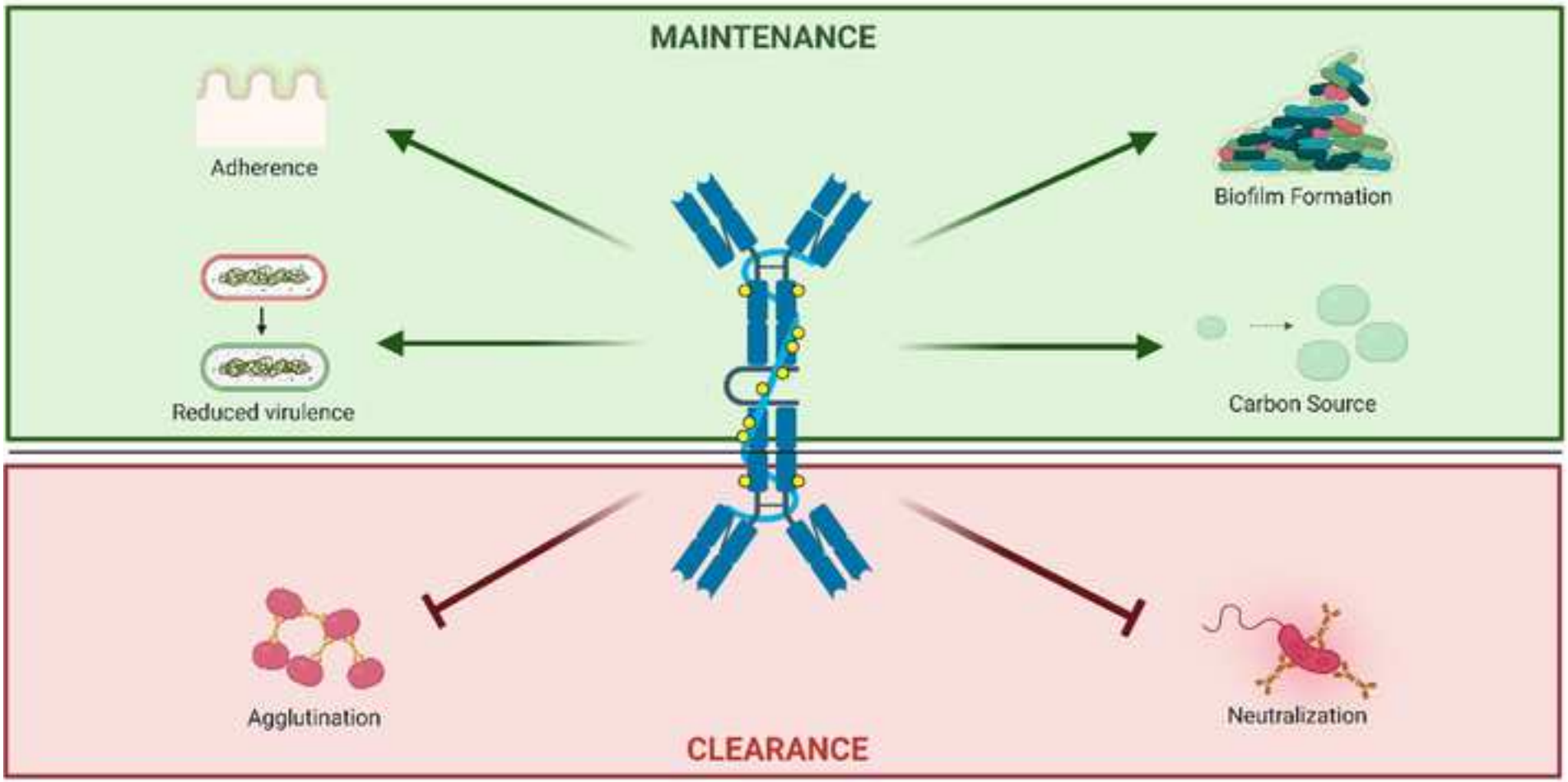

Figure 2

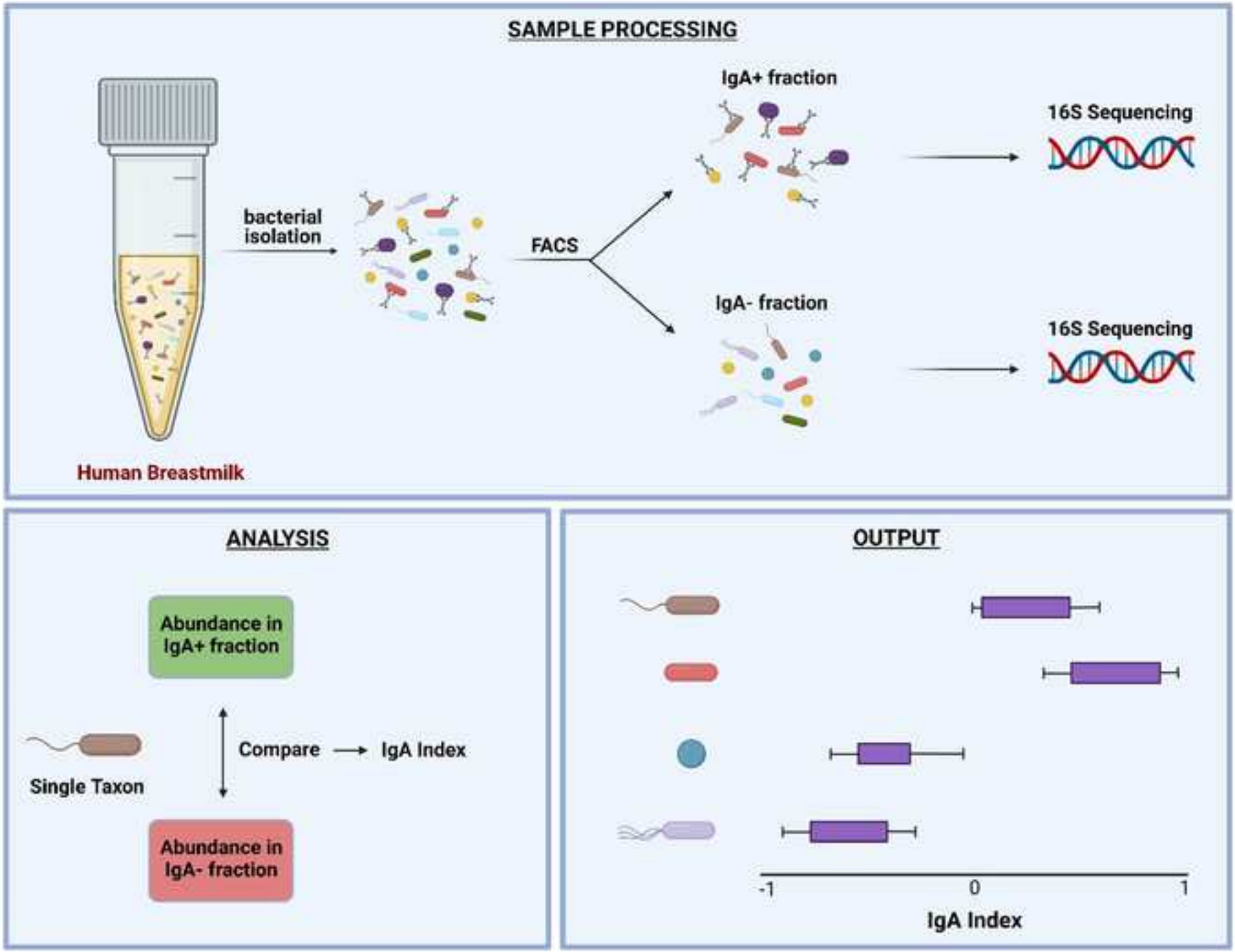

Figure 3