**Abstract**

Secretory immunoglobulin A (SIgA) is intimately involved in the transfer of maternal immunity to the newborn breastfed infant. Recent research demonstrates the significance of SIgA in the initial development of the newborn’s microbiota and in the establishment of a tolerogenic immunologic disposition towards nonpathogenic organisms and environmental antigens. SIgA has long been known to prevent pathogen binding to the host epithelium through immune exclusion involving numerous mechanisms. This process primarily involves T-cell-dependent, somatically hypermutated monoclonal antibodies with high specificity towards pathogen surface antigens, and the success of the immune response is dependent upon the specific antigen recognition. Whereas this role is important, there is an alternate, dual role for SIgA in the health of the host – protection and promotion of commensal colonization and maintenance of homeostatic immunity. This latter role is primarily dependent upon N- and O-glycan moieties lining the secretory component and heavy chain of the SIgA dimer, with interactions independent of immunoglobulin specificity. These SIgA molecules are nonspecific polyclonal antibodies generated from plasma cells activated by dendritic cell sampling of luminal contents in the absence of inflammation. Breast milk is the primary supply of such polyclonal polyreactive SIgA in the initial stages of neonatal colonization, and it provides vital pathogen resistance while promoting colonization of commensal microbiota.
**Secretory IgA Production**

In the mucosa, the majority of the immunoglobulin repertoire consists of somatically hypermutated IgA proteins, and over 25% are polyreactive to microbial-associated molecular patterns and other common antigens [1]. In addition, mucosal IgA production maintains a very high constant level in the absence of infection, up to 5 g/kg/day in an adult intestine [2], but it can be increased in an inflammatory environment. Recent studies using photoconversion to track Peyer’s patch B cells showed marked small intestinal Peyer’s patch germinal center clonal exchange of memory B cells during a 3-day period [3], indicating considerable trafficking of memory B cells throughout mucosal surfaces in the gastrointestinal tract. In the mucosa, there seems to be a high level of low-affinity, diversified germinal-center B-cell responses to microbial antigens that are required to maintain intestinal homeostasis.

In addition to these T-cell-dependent responses which account for about 75% of IgA induction in the mucosa, B cells can be activated via T-independent processes. These may involve pattern recognition receptor signaling, as most IgA generated in mice deficient in T cells or with deficiencies in somatic hypermutation but without class switching produce primarily microbial-pattern (low-affinity) antibodies [4]. Interestingly, in these studies, the microbiota expanded into a dysbiotic state, with higher levels of Proteobacteria, suggesting the need for T-cell-dependent IgA responses to maintain intestinal homeostasis.

Mature B cells are released into the circulation as plasmablasts and home back to the site of mucosal induction to secrete dimeric IgA. SIgA is derived upon transcytosis of dimeric IgA through mucosal epithelial cells bound to the polymeric immunoglobulin receptor (pIgR) on the basolateral membrane, and the subsequent apical cleavage of the immunoglobulin-bound secretory component (SC), which releases free SIgA into the lumen.

**Immune Exclusion**

Once in the lumen, there are a number of mechanisms by which SIgA functions and more are being discovered. Traditionally, it was thought that SIgA acts through agglutination and neutralization, excluding pathogens from binding to epithelial cells via Fab-dependent, high-affinity interactions to pathogen surface antigens. Although neutralization is supported by imaging of viral pathogens [5], agglutination has come into question recently as the concentration of an infectious agent is likely too low to provide enough localized pathogen to create immune complexes with SIgA. A recent study proposed a concept of “enchained
growth” whereby bacterial pathogens coated with SIgA exhibit incomplete binary fission and remain attached after replication [6]. In fact, this creates similar immune complexes as agglutination, preventing bacterial adhesion to epithelial cells and reducing pathology. In addition to these mechanisms, a novel function for Fab-dependent SIgA has been identified in work with the recombinant monoclonal SIgA, Sal4, directed at the acetylated O5 antigen of Salmonella enterica serovar Typhimurium (ST) [7]. In these studies, researchers found that Sal4 binding altered bacterial membrane integrity, compromising the cell energy gradient. As proton motif force is needed to power the bacterial flagella, motility was inhibited, as was ATP production and overall metabolic processes. While it is still uncertain how exactly the binding of SIgA to LPS alters the membrane potential, it likely involves cross-linking of several O-antigens, as the use of a recombinant immunoglobulin single chain did not create this effect.

**Glycan-Mediated Binding**

Although the Fab-mediated interactions of SIgA are vital for reducing pathogenic infections in the mucosa, as mice deficient in class-switch recombination and somatic hypermutation (AID−/−) [4] who are unable to create high-affinity immunoglobulins demonstrate heightened susceptibility to infection, antigen-independent, glycan-mediated interactions are also important in the function of this molecule. SIgA is highly decorated with N-linked glycans: 2 sites on each heavy chain, 8 in total, plus 7 on the SC, 1 on the J-chain, and an additional 2 on the hinge region on IgA2 (Fig. 1a) [8]. Many studies have now demonstrated that these glycans play a pivotal role in pathogen clearance, antigen presentation via M cells, and commensal homeostasis. For example, enteropathogenic Escherichia coli binds terminal sialic acid residues on the glycoprotein with intimin adhesion proteins [9], and Clostridium difficile toxin A neutralization is mediated by glycans [10]. Rochereau et al. [11] showed that sialic acid residues on IgA2 N-glycans (Fig. 1b) are required to bind on the apical surface of M cells via dectin-1 for reverse transcytosis and subsequent antigen presentation in the subepithelial dome. In addition, subepithelial dome dendritic cells require SIgA glycans for binding these immune complexes via DC-SIGN, a C-type lectin receptor [12].

Not only are these glycans on SIgA necessary for mucosal response to pathogens, more recent studies have also shown the utility of these glycans in binding to commensal bacteria, promoting immune tolerance, and maintaining homeostatic regulation. The first evidence of glycan-mediated commensal binding was shown utilizing a SIgA specific for Shigella flexneri LPS and 3 commensal bacte-
Bifidobacterium lactis, Lactobacillus rhamnosus, and E. coli Nissle 1917 [13].

Using fluorescence microscopy, researchers visualized associations of this non-specific SIgA to all bacteria, but when pretreated with glycosyl hydrolase (N-glycosidase F) to remove the N-linked glycans, SIgA was not able to bind to the 2 gram-positive bacteria, though it remained bound to the gram-negative one. Although further studies are needed to confirm the glycan dependency of commensals to SIgA, research from our lab and others are supporting the antigen-independent association of SIgA with commensal bacteria. Nakajima et al. [14] validated nonspecific SIgA binding using OTII mice. In their study, ovalbumin (OVA)-specific T cells were transferred into T-cell-deficient (CD3−/−) mice who were then provided with OVA antigen daily to induce an OVA-specific humoral response. Flow cytometry of fecal bacteria revealed complexes of commensal-SIgA-OVA, compared to commensal-SIgA complexes in wild-type counterparts, which was recapitulated in vitro with hybridoma-derived OVA-specific IgA but not IgG. Our own studies utilizing flow cytometry and fluorescence microscopy have shown a concentration-dependent interaction of both hetero-

**Fig. 1.** Schematic representing the SIgA molecule (a), an example of the glycan isoforms (b), and some of the functions of the glycans and the antigen-binding region (c) on the molecule.
geneously pooled milk SIgA (Fig. 2) and recombinant monoclonal SIgA with select commensal bacteria and enteropathogens [unpubl. data].

The utility of the nonspecific SIgA association with commensals is still unclear, though these studies and others have given some indication. Work using Caco-2 colonocytes exposed to the commensal bacteria *Lactobacillus* and *Bifidobacterium* demonstrated a 3.4-fold increase in the binding of bacteria to these cells, a reduction in NF-κB-induced proinflammatory cytokine production, and induction of the tight junction binding protein occludin when the bacteria were first associated with SIgA [15]. Our research group has recapitulated these studies and also found a significant reduction in the neutrophil recruiting IL-8 cytokine when SIgA is first associated to the commensals prior to binding in vitro on colonocytes. In addition, our studies have found that nonspecific SIgA association with commensal bacteria improves colonization in BALBc mice when the complex is provided orally [unpubl. data]. Gnotobiotic mice deficient in pIgR (pIgR−/−) are unable to produce SC-coated IgA, but serum and luminal IgA levels remain unchanged. These mice demonstrated a heightened translocation of commensal bacteria to the mesenteric lymph nodes, and an induction of a systemic immune response when introduced to commensal intestinal flora from specific pathogen-free mice. The systemic IgG response to colonization with specific pathogen-free microbio-

![Flow-cytometric scatter plot showing increased pooled milk SIgA associated with a *Bifidobacterium* species with increased concentration of SIgA (percent circled). Syto9 is a DNA chelator indicating live bacteria, and A650 is anti-human-IgA with Alexa 647 fluorophore. Concentration is per 10⁷ CFU bacteria.](image)
ta was not seen in wild-type counterpart mice, demonstrating functional immune compartmentalization in the presence of SIgA [16]. Murine studies show that SIgA coating of commensal bacteria promotes colonization in the outer mucosal layer of the colon [17], preventing the stimulation of intraepithelial lymphocytes and epithelial pattern recognition receptors, and thereby reducing the induction of innate inflammatory responses to nonpathogenic bacteria. In the OTII-CD3−/− mouse work, researchers found that Fab-independent association of SIgA with *Bacteroides thetaiotaomicron* altered the expression of several genes involved in carbohydrate utilization and in exopolysaccharide production [14]. This was also seen in a study by Donaldson et al. [18] investigating gene expression changes with *Bacteroides fragilis* upon nonspecific SIgA association, where they found a change in exopolysaccharide production that led to colonization of a different ecological niche in the small intestine. Our research group has shown a significant reduction in enteropathogenic invasion both in vitro in human colonocytes and in vivo in BALBc mice when SIgA-associated commensals are introduced into the cells or animal prior to pathogen challenge, but not when either the commensal or SIgA alone are provided [unpubl. data].

Corthesy and Phalipon [19] demonstrated that in the respiratory tract, tissue localization was dependent on the glycosylation of the SIgA molecule. Upon intranasal challenge with *S. flexneri*, the glycosylated SIgA molecule was viewed in close association with the mucosa, and dissemination of the pathogen was confined to the nasal cavity, but deglycosylation of SIgA led to pathogenic colonization in the deep lung alveoli. Gibbins et al. [20] demonstrated an association of SIgA with salivary mucin proteins that then bind with other epithelial cell-produced mucin proteins, indicating a mucin-mucin-driven SIgA interaction. While research supports the association of glycosylated SIgA with the outer mucosal layer in the large intestine, Rogier et al. [17] showed through fluorescent microscopy that the mucin MUC2 protein in the innermost mucosal lining of the gut epithelium is important not in binding to SIgA, but in excluding this complex from interacting with the epithelia. The exact mechanisms of SIgA interacting with the loose outer mucosa remain unclear, but examinations of other mucosal sites within mammals demonstrate the importance of the carbohydrate moieties in maintaining associations with both microorganisms and the mucosa.

**Breast Milk Secretory IgA**

The gut of the neonate, upon parturition, contains a number of innate immune barriers, an underdeveloped gut-associated lymphoid tissue, and naïve adaptive immunity, which can take up to 10 days to become stimulated [21]. These con-
ditions can lead to an imbalanced inflammatory response without proper guidance from the mother’s passive immune defenses in breast milk that aid in establishing a regulated environment during the initial onslaught of microbial colonization. SIgA is the primary mucosal antibody found in the highest abundance over other immunoglobulins in milk, with concentrations up to 15 mg/mL in colostrum and ~1 mg/ml in mature milk [22], providing the breastfed infant 0.5–1 g/day. The origin of milk antibodies has been evaluated through many studies. Although antibody-secreting plasma cells can be detected in excreted milk [23], the production of SIgA and SIgM likely arises from secreting plasma cells in the basolateral region of the mammary gland to allow for transcytosis through the epithelium and release of SC-bound immunoglobulins. The induction site for these mammary-homing lymphocytes is predominantly the gut-associated lymphoid tissue, with migration through the established enteromammary pathway [24]; early work in this field showed that radioactively labeled IgA-secreting mesenteric lymphocytes were found in the mammary gland of lactating murine dams 3–10 times higher in number than lymphocytes of peripheral lymph node origin [25]. In addition, antigen-specific SIgA can be detected in milk following oral introduction of the antigen in lactating mothers [26].

Antibodies secreted in breast milk have been shown experimentally in rodents and epidemiologically in humans to prevent pathogenic infection in the infant gastrointestinal tract through targeted pathogen-specific SIgA both in infancy and extended through childhood [26]. Harris et al. [27] showed that C57BL/6 mouse pups were protected against the intestinal parasite *Heligmosoides polygyrus* when fostered on milk rich in pathogen-specific immunoglobulins (IgA and IgG), but not when the milk contained no *H. polygyrus*-specific immunoglobulins. Studies of breastfed human infants infected with the intestinal pathogen *Campylobacter jejuni* showed that milk containing antiflagellin or anti-outer membrane IgA antibodies [28] is protective against symptomatic *C. jejuni* infections. These studies and others have led to the exploration of a therapeutic SIgA approach: vaccinating mothers to promote protective SIgA antibodies in breast milk [29], a strategy warranting further investigation.

**Milk Secretory IgA and Infant Commensal Colonization**

Whereas the protective role of pathogen-targeted secretory milk antibodies is well established, recent studies have emerged focusing on the functional consequences of milk SIgA in association with commensals in the development of the newborn microbiota. Analyses of the milk antibody repertoire show significant...
amounts of polyclonal autoreactive antibodies with low levels of somatic hypermutation evident [30]. Multiple studies have observed cross-reactivity of both antigen-targeted monoclonal antibodies and polyreactive autoantibodies to commensal microbiota of the small intestine [1, 14, 18]. Thus, there is a high likelihood of the ability for the diverse polyclonal antibody repertoire of breast milk to bind with low affinity to bacteria found both within the mammary gland and in the gut of the breastfed infant. Whereas to date methodology has not been developed to differentiate the maternally versus endogenously derived SIgA in the SIgA-coated fraction of the infant gut microbiota, studies utilizing BugFACS reveal distinct microbiota coated with SIgA under homeostatic conditions [31] or in malnourished Malawian infants [32]. Consistent with IgA-seq studies in adults [33], malnourished infants had a higher relative abundance of Enterobacteriaceae associated with intestinal inflammation, which represented a significant portion of the SIgA-associated taxa. Conversely, healthy infants showed consistently high SIgA association in members of the genera Akkermansia and Clostridium, regardless of dietary intake or age (1–24 months). Likely, these observed variations in IgA-coated fractions in the infant gut are a reflection of the differences in IgA repertoire development under varying disease conditions in both the infant mucosa and also in the breast milk environment.

A recent study investigated the impact of breast milk SIgA on the development of necrotizing enterocolitis (NEC), a disease with complex etiology and high mortality afflicting preterm infants born under 33 weeks of gestation [34]. The study found that NEC development was correlated with a loss of IgA association to Enterobacteriaceae – through unclear mechanisms. The significance of IgA was confirmed in a NEC mouse model where suckling mouse pups were fostered on dams deficient in total immunoglobulins (Rag−/−) or IgA (Igha−/−). These mice developed NEC at the same rate as formula-fed pups, whereas control pups with milk sufficient in IgA had low rates of NEC development. Although milk has a plethora of immune components and oligosaccharides able to modify the microbiota and contribute to fortifying the immune system of the young infant, this study supports a crucial role for IgA in the preterm infant.

Rogier et al. [35] sought to understand the difference in the function of maternally derived SIgA versus actively secreted endogenous IgA in the newborn gut. In their murine study, they found that the microbiome of adult mice, whose nursing mothers were deficient in pIgR and, therefore, provided no maternal SIgA during the suckling period (although milk IgA levels were unchanged), differed in key taxa from those who were exposed to SIgA during suckling. Specifically, they found a more pronounced presence of Proteobacteria of the family Pasteurellaceae, and Firmicutes of the family Lachnospiraceae. Analysis of the RNA component of intestinal epithelial cells of the same
two mouse groups after challenge with epithelial-disrupting dextran sulfate sodium (DSS) showed two major groups of genes varying in expression level between the mice. Notably, many genes involved in cell repair were seen to be linked to DSS exposure and were up-regulated in adult mice with suckling exposure to SIgA, and a cluster of genes important in cell metabolism and growth that were not associated with DSS exposure were also up-regulated in the same group of mice. These reports, although based on a murine model, indicate the importance of early exposure to maternally derived SIgA to the long-term health of the child. Further research is needed to elucidate the mechanisms behind these results.

In addition to these functions, SC may be important for protection against proteolytic degradation of the IgA antibody itself and perhaps the bacteria they coat, as evidenced by the presence of intact SIgA in the stool of exclusively breastfed infants [36] and in the 2011 study by Mathias and Corthésy [13], who showed that SC-bound, but not unbound, IgA resisted degradation in vitro. This protein-coating model may be one mechanism by which breast milk commensals survive intestinal digestion in order to colonize the gut of the infant. Indeed, our research group has found increased viability of commensal bacterial following in vitro intestinal digestion when the bacteria are first associated to pooled milk SIgA [unpubl. data].

**Conclusion**

SIgA plays important roles in the developing infant gut, both in pathogen defense and commensal homeostasis. Although breast milk provides a plethora of biologically active immune components and microbial-modifying factors, studies involving a reductionist approach to investigate individual bioactive molecules have helped elucidate the importance of the heavily glycosylated SIgA both in infancy and into adulthood. Studies are needed to determine the functional consequence of a SIgA-commensal complex both within the milk milieu and in the neonate gut with regard to commensal colonization and regulation of the infant mucosal immune response. Continued investigation into this relationship may translate into improvements in infant health and nutrition.

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