ANNUAL REVIEWS

Annual Review of Immunology

Production and Function of Immunoglobulin A

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Annu. Rev. Immunol. 2021. 39:695–718

First published as a Review in Advance on March 1, 2021

The *Annual Review of Immunology* is online at immunol.annualreviews.org

https://doi.org/10.1146/annurev-immunol-102119-074236

Keywords

immunoglobulin A, microbiota, Peyer's patch, mucosal immunology, germinal center, transforming growth factor beta

Abstract

Among antibodies, IgA is unique because it has evolved to be secreted onto mucosal surfaces. The structure of IgA and the associated secretory component allow IgA to survive the highly proteolytic environment of mucosal surfaces but also substantially limit IgA's ability to activate effector functions on immune cells. Despite these characteristics, IgA is critical for both preventing enteric infections and shaping the local microbiome. IgA's function is determined by a distinct antigen-binding repertoire, composed of antibodies with a variety of specificities, from permissive polyspecificity to cross-reactivity to exquisite specificity to a single epitope, which act together to regulate intestinal bacteria. Development of the unique function and specificities of IgA is shaped by local cues provided by the gut-associated lymphoid tissue, driven by the constantly changing environment of the intestine and microbiota.

INTRODUCTION

IgA is an antibody that has evolved to be secreted onto barrier surfaces. In humans, IgA is made in prodigious amounts and secreted at a rate of over one gram per day. After secretion, IgA is important for the prevention of invasion by pathogens but also for shaping the composition, gene expression, and metabolism of the local microbiome. In turn, the local environmental factors of the intestine and gut-associated lymphoid tissue (GALT) shape the specificity, development, and differentiation of IgA-producing B cells, contributing to IgA's distinct functionality. Here we review the structure, function, and production of IgA in the intestine.

IgA—AN ANTIBODY THAT HAS EVOLVED TO BE SECRETED

Of the major antibody types (A, D, E, G, and M), IgA is uniquely suited to secretion and function at mucosal surfaces (1). IgA is composed of four immunoglobulin domains and comes in three main forms, monomeric, dimeric, and secretory. Dimerization and secretion of IgA are facilitated by covalent attachment to the J chain, which occurs within plasma cells. The J chain binds to a tailpiece peptide extension of both IgA and IgM and is critical for the transcytosis of both, as it provides the bridge between these antibodies and the polymeric immunoglobulin receptor (PIGR) (2). Accordingly, monomeric IgA is not efficiently secreted and is substantially enriched in the serum (3). Binding of the J chain and IgA or IgM to PIGR activates uptake and transcytosis by epithelial cells, but IgA and IgM differ substantially in how they bind PIGR. IgM binds transiently, and IgA is covalently linked via disulfide bonds (4). Thus, upon arrival at mucosal surfaces, IgA is not released from PIGR binding, but rather an unknown protease cleaves PIGR and it remains bound to IgA as secretory factor (SF), thus creating secretory IgA (sIgA) (1). Binding of IgA by SF is believed to have important functional consequences. First, it is believed to impart increased resistance to proteolytic degradation in the gastrointestinal tract, which is critical, given the high concentration of proteases in the lumen. Thus, even though PIGR binds IgM and IgA with the same efficiency, IgA is much more abundant at mucosal surfaces because IgM is more susceptible to proteolysis and IgM-producing cells are less common at mucosal sites (1, 5). SF binding of IgA is also believed to sterically inhibit the binding of IgA to two Fc receptors, FcαμR and FcαR1 (also known as CD89 and not found in rodents). Indeed, PIGR binds to the same moieties on the constant heavy chain domain 3 (CH3) of IgA that bind FcαμR, and it thus hinders binding (6). However, FcαR1 can bind to four separate sites on the CH3 domain of IgA, and none of these sites is obscured by SF. Instead, two of these sites are sterically blocked by the J chain. Thus any effect of SF in reducing binding to FcαR1 is more likely mediated by a conformational change in IgA's quaternary structure (7). IgA is also poor at activating complement proteins for deposition on microbial membranes because, independent of SF binding, the Fc portion of IgA is not bound by the C1 convertase complex (8). Complement proteins are expressed by the intestinal epithelium, and neutrophils and monocytes do extravasate into the intestinal lumen. So the failure of IgA to activate complement upon binding to intestinal bacteria is likely an effort to protect the microbiome from complement-mediated elimination.

The function of IgA is also highly sensitive to glycosylation. sIgA is heavily glycosylated on all three components: *N*-glycans decorate SF and the J chain while the hinge region is arrayed with *O*-glycans. For monomeric IgA, glycosylation is critical for binding by the Fc receptors: FcαμR and FcαR1 but also potentially Fc receptor–like 4 (9), asialoglycoprotein receptor (10), CD71, and β-1,4-galactosyltransferase (11). *N*-Glycosylation also affects the uptake of bacteria from the intestine by microfold (M) cells in Peyer's patches (PPs) (12). In most primates there are two IgA isotypes generated through gene duplication (IgA1 and IgA2). Despite significant sequence similarity, they differ in their ability to activate effector mechanisms through Fc receptor

binding and antigen binding (3). These differences are likely due to a unique polypeptide hinge region in IgA1 that provides both additional sites for *O*-glycosylation and additional flexibility with regard to binding repetitive surface antigens (13–15). Critically, the pathogenesis of IgA nephropathy depends upon the creation of an autoantigen through aberrant galactose-deficient IgA1 *O*-glycosylation, leading to immune complex deposition in the kidneys (16).

Thus, activation of myeloid cells by binding to Fc receptors is believed to be primarily the domain of monomeric IgA in the circulation and is highly responsive to shifts in glycosylation. Conversely, sIgA seems to have been uniquely shaped by evolution to bind to its targets but not induce their lysis or phagocytosis or activate local immune cells.

SECRETORY IgA CONTROLS ENTERIC INFECTIONS

The task of IgA is more complex than that of other isotypes, as it must both protect against mucosal pathogens and also contribute to the function of a healthy microbiota. Despite the lack of effector functions, sIgA can mediate potent antipathogen effects (**Figure 1**). IgA is necessary for protection against both reovirus and rotavirus infections in animal models, and levels of neutralizing IgA correlate well with protection provided by oral rotavirus vaccines (17–20). sIgA may also be important for protection against poliovirus by vaccination (21). While the oral polio vaccine (OPV) induces potent amounts of mucoprotective IgA (22), the inactivated polio vaccine (IPV) does not.

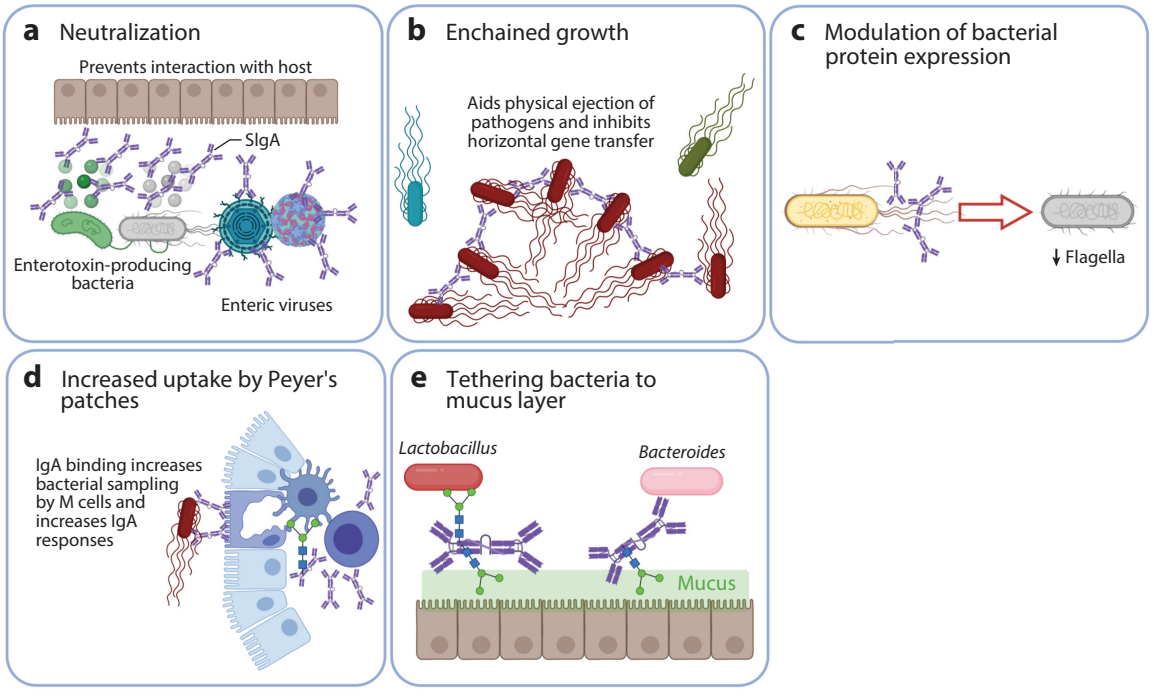

Figure 1

The many mechanisms by which IgA blocks infection and modulates the microbiota. Secretory IgA (sIgA) only poorly activates Fc receptors and does not activate complement. sIgA has other mechanisms. (*a*) Neutralization of viral and bacterial pathogens and toxins. (*b*) sIgA drives enchained growth, linking proliferating bacteria to create agglutinated clumps that assist in removal and limit opportunities for horizontal gene transfer. (*c*) Binding and modulating surface proteins on bacteria can affect their function and expression. (*d*) sIgA increases uptake by M cells on the surface of Peyer's patches. (*e*) Glycans on sIgA can tether bacteria to mucus. Figure adapted from image created with BioRender.com.

However, challenge of IPV-vaccinated children with the oral vaccine leads to the rapid development of neutralizing sIgA, and an IPV booster can activate OPV-induced memory IgA^+ B cells, so a single dose of OPV may provide long-lasting IgA-mediated protection (23, 24). IgA-producing B cells can also be found outside the intestine, in the oral and nasal mucosae. Differentiation and class switch to IgA can occur in nasal-associated lymphoid tissue, and sIgA may be important in protection against respiratory viruses, such as respiratory syncytial virus, and as a target for vaccines (25, 26). IgA is also protective against enteric bacterial pathogens. For example, sIgA is important in preventing diarrhea induced by enterotoxins, such as cholera toxin and the heat-labile toxin of enterotoxigenic *Escherichia coli*, as it can bind the toxins' enzymatically active sites and prevent their association with the intestinal epithelium (27, 28). IgA is also important in protection against invasive pathogenic bacteria, such as *Salmonella* (29). For many years, how IgA mediates protection against intestinal bacteria without activating cytolytic or phagocytic mechanisms was unclear, but it was believed to require the agglutination of bacteria, mediated by the tetravalent nature of the sIgA molecule (30). However, the volume of the intestine in relation to the numbers of bacteria early in an infection made it hard to understand how agglutination might function, since the likelihood that a given sIgA molecule would be able to agglutinate two bacteria that came into physical contact is quite low. Recently, Slack and colleagues (31) have shown that agglutination of infectious bacteria may be driven by a process termed enchained growth. The notion of enchained growth presumes that instead of relying upon the chaotic motion of individual bacteria, sIgA tethers proliferating bacteria, which leads to punctate accumulations. Enchained bacteria are presumably easier for the intestine to remove via peristalsis and have the added benefit of limiting the ability of different bacterial clones to meet and exchange genetic material (31). IgA binding of bacteria can also increase uptake by PPs through receptor-mediated transcytosis, which leads to increased antibody titers and higher-affinity responses (32, 33). Antibody binding can also change intestinal bacteria at the transcriptional level, where binding a specific surface molecule can lead to altered expression of the targeted protein (34). IgA-mediated effects on protein expression are important, since the most likely targets for IgA [lipopolysaccharide (LPS), flagella, and fimbriae] are critical mediators of association with the intestinal epithelium and thus bacterium-driven inflammation (35). Taken together, these findings indicate that there are multiple mechanisms for IgA to control infection in the intestine.

SECRETORY IgA SHAPES THE INTESTINAL MICROBIOME

B cells proliferate so rapidly after activation that if the immune response did not contract, all tissue sites would soon be filled with only these cells. This is a problem particularly in the intestine, where germinal center (GC) reactions within the PPs are constant and the burden of new IgA-producing B cells is large (36, 37). Therefore, in the intestine there is an additional process of attrition where it seems that established antibody-producing B cells can be pushed out by newly developed clones. Given the ubiquity of gastrointestinal pathogens in the environment, it seems counterintuitive that mucosal IgA-based immunity would wane. However, we should consider that the evolution of sIgA was likely shaped by both enteric pathogens and more symbiotic relationships with the resident microbiota. Within the microbiota, it is often the case that two individual bacteria are closely related but enact completely separate colonization programs. For example, enterotoxigenic *E. coli* is very closely related to totally benign commensal isolates of the same species (38). Therefore, it is possible that attrition of intestinal plasma cells enables flexibility in response to bacterial antigens that might be expressed by diverse bacteria with a wide variety of threat levels for the host. Indeed, Macpherson and colleagues (39) discovered that reversible colonization of germ-free mice with an auxotrophic *E. coli* strain that cannot grow in the intestine revealed

the short life of IgA-producing cells induced by intestinal bacteria (39). Upon colonization with a complex microbiome, IgA-producing B cells induced by the auxotrophic *E. coli* were rapidly replaced, revealing a potential ceiling on the intestinal B cell population that has not been observed for other types of lymphocytes (39). The mechanism for this attrition is still not well described, but one hypothesis that fits the data is that there is constant competition for the survival signals, and the new clones, which are more numerous, are more likely to survive the winnowing process. It will be important for the design of oral vaccines to determine whether this property of IgA plasma cell replacement can be counteracted to deliver longer-lasting mucosal protection.

In addition to protecting against enteric infection, IgA is important for the composition of the microbiota. Observations of both IgA-deficient mouse models and humans with mutations that affect IgA production support the notion that IgA deficiency is associated with a less diverse microbiome and increased levels of facultative anaerobes such as *Enterobacteriaceae* and *Enterococcaceae* (40, 41). The microbiome is a modifier of many diseases, particularly those associated with inflammation and immunity, and accordingly IgA-deficient patients have significantly increased incidence of type 1 diabetes, celiac disease, and inflammatory bowel disease (42). One likely mechanism for these effects is that IgA binding modulates the most aggressive members of the microbiota by reducing flagellin and fimbriae expression to limit their growth and ability to induce inflammation (35). In addition, inflammation favors the outgrowth of facultative anaerobes that contain modules to survive and even thrive in the face of the host immune response, while benign obligate anaerobes often respond poorly to inflammatory conditions (43–45). Therefore, IgA supports diversity by directly limiting the growth of facultative anaerobes while concurrently inhibiting conditions that might support their outgrowth. As support for the idea that IgA predominantly binds to bacteria that are intrinsically inflammatory, adoptive transfer into gnotobiotic mice of IgA-bound bacteria, from both patients with inflammatory bowel disease and children with environmental enteric dysfunction, is sufficient to predispose to worse disease outcomes in the mice (46, 47). Conversely, some types of bacteria seem to benefit from the enchaining effect of IgA. sIgA can adhere to mucus via glycan side chains (48, 49). Some bacteria can also bind to these glycans, potentially using them as a carbon source and allowing them access to the mucus layer and different metabolites as a potential food source (50). Fascinatingly, malnourishment reduces the glycan binding characteristics of *Lactobacillus* bacteria, and it is possible that these organisms are using IgA binding to enter the mucosal layer of nutrient-rich environments and limit expulsion. Alternatively, binding of glycans on IgA can lead to transcriptional changes in *Bacteroides thetaiotaomicron* that modulate bacterial metabolism and growth (51). Other bacteria, such as *Bacteroides fragilis*, can also gain an advantage from being bound by IgA, which assists in their colonization of the intestine (52). How these bacteria benefit from IgA without being inhibited by it at the same time is somewhat of a mystery because other mucus-resident members of the microbiota (for example, segmented filamentous bacteria) are definitely regulated by IgA (53, 54). One possible explanation lies in the fact that various bacteria differ with regard to their induction of IgA-independent immune mechanisms, such as antimicrobial peptides. For example, segmented filamentous bacteria induce IL-17 and IL-22 production by T cells and thereby increased levels of antimicrobial peptide production by the epithelium (55–57), whereas *Bacteroides* spp. and *Lactobacillus* spp. are more closely associated with the induction of regulatory T cells (Tregs) (58, 59).

MATERNAL IgA PROTECTS THE INFANT FROM INFECTION AND GUIDES THE DEVELOPING MICROBIOME

One indication of the importance of IgA in preventing enteric infection and regulating the microbiome is that substantial amounts of sIgA are secreted into breast milk by mammary gland B cells

to protect the neonatal intestine. sIgA is the most common antibody in breast milk, accounting for 90–95% of all secreted antibodies (60). In the mammary gland, most IgA is transported by PIGR. Importantly, however, some IgA is secreted by a separate process at this site, as $PIGR^{-/-}$ mice have small amounts of IgA in their breast milk (61, 62). Maternal IgA is important because most children only begin to make IgA of their own three to four weeks after delivery (63, 64). During pregnancy, IgA^+ memory cells are drawn from the intestine and PPs to travel through the body and accumulate in the mammary gland, as directed by CCL28 (65–67). Thus, it is reasonable to conclude that secretion of IgA into milk is an attempt to protect the infant against those microorganisms that have caused the strongest responses in the mother's intestine. Indeed, the vaccination of cows to produce protective breast milk–derived antibodies is common practice in veterinary medicine (68). However, the efficacy of maternal antibody responses is limited, because if a pathogen succeeds in infecting the infant, the maternal immune response does not scale with the growth of the pathogen.

Breast milk–derived IgA is also critical to the development of the microbiome in infants (61, 62). The first bacteria that colonize the infant are vaginal and skin bacteria present at delivery, but in almost all cases, they are soon supplanted by ubiquitous facultative anaerobes, such as *Enterobacteriaceae*, that play an important role in deoxygenating the intestine (69, 70). During healthy development, *Enterobacteriaceae* are soon replaced by obligate anaerobes, such as *Bifidobacteriaceae*, which metabolize milk oligosaccharides, followed by *Clostridiaceae*. While the mechanism remains unclear, IgA is important in regulating this process. In animal models and human infants, deficiency in maternal IgA leads to larger and longer-lived *Enterobacteriaceae* colonization (62, 71). Blocking *Enterobacteriaceae* and supporting the colonization of obligate anaerobes can also have acute benefits for the infant. Necrotizing enterocolitis is a disease of predominantly low-birthweight, preterm infants that is characterized by overgrowth of the intestine with *Enterobacteriaceae* and *Enterococcaceae* and subsequent immune-driven inflammatory damage to the small intestine (72). The disease develops much more commonly in children fed infant formula (which lacks IgA altogether), but not all infants fed breast milk are protected, indicating that there may be heterogeneity in the protective effect of different breast milk samples (63, 73). Interestingly, in human milk–fed infants that do develop necrotizing enterocolitis, an increase in *Enterobacteriaceae* that are unbound by IgA and a reduction in total anaerobic bacteria precede symptomatic disease (63).

In mouse models, IgA-dependent shifts in the microbiome can extend into adulthood, and one could hypothesize that IgA in breast milk modulating the microbiota is an important factor in the known long-term benefits of breastfeeding (61). Breastfeeding is also associated with increased cognitive function later in life and reduces the incidence of diabetes and inflammatory bowel disease (74). While there is a variety of breast milk–derived factors that could contribute to these effects, maternal IgA shaping of the microbiome and therefore the infant immune response is one of the most intriguing. One purpose of maternal antibodies may be to direct the development of infant intestine-resident T cell responses, which if dysregulated could contribute to lifelong complications via the formation of memory cells (75, 76). For example, in mice, early maternal IgA is critical for developing a set point for the population of colonic Rorγt-expressing $Foxp3^+$ Tregs, which themselves reciprocally repress the accumulation of both lamina propria and mammary gland IgA-producing B cells (67). Thus, the amount of IgA-producing B cells and Rorγt-expressing $Foxp3^+$ Tregs is controlled by the mother. This is relevant for chronic diseases, as establishing a population of Rorγt-expressing $Foxp3^+$ Tregs is important to protect from overly exuberant immune responses later in life and these cells are particularly potent at blocking the type 2 immune responses that characterize allergy and asthma (77, 78).

In addition to binding and regulating the most invasive and inflammatory bacteria of the intestine, IgA binds many other taxa; some benefit, and others are substantially inhibited. It is clear that

there is a complex and robust give-and-take between IgA and the microbiome in the intestine. We likely understand only a fraction of the pathways that bacteria have evolved to evade and manipulate IgA production, binding, and function. Discovering them and synthesizing these findings are a major goal and will require us to more deeply understand how IgA-producing B cells develop.

IgA CROSS-REACTIVITY IN THE CONTEXT OF THE MICROBIOTA

The function of antibodies, including IgA, is dependent upon unique antigen specificities that direct their binding. Determining the specificities of IgA is complicated, because the intestinal microbiome is composed of hundreds of constantly shifting bacterial strains that express both shared and unique antigens. Thus, the level of specificity, or conversely cross-reactivity, of a given IgA is dependent upon what organisms and antibodies are present in the moment. Recently, high-purity sorting and 16S ribosomal RNA sequencing of IgA-coated commensals coupled with the generation of monoclonal antibodies derived from single intestinal B cells allowed for the investigation of IgA specificity (46, 79). These studies suggest that some intestine-resident IgA clones are able to bind many different bacterial species (80). This feature has been described as cross-species reactivity (81) where an antibody can bind to a shared epitope (such as LPS) and polyspecificity where the antibody is permissive to binding a variety of seemingly unrelated structures (82). The general model for antibody production in response to infection calls for gradually increased affinity for a restricted epitope via clonal selection and affinity maturation. Given this general progression toward higher-affinity antigen binding by B cells, how is cross-reactivity or polyspecificity maintained in the chronically stimulated environment of the GALT? Two inclusive hypotheses have been proposed. The first suggests that germ-line B cell receptor (BCR) sequences have evolved to bind commensal bacteria in the germ-line configuration, and therefore intestinal B cell responses do not require affinity maturation (79, 82). The second proposes that the initial B cell stimulation that drives IgA production is antigen independent and that new IgA responses can be stochastic and not geared toward specific epitopes. Further study is needed to distinguish these hypotheses. Critically, the descriptions of polyspecific IgA clones that bind multiple structures have been drawn from laboratory mice. However, laboratory mice live in closely controlled environments, so the importance of polyspecific clones in hosts who are exposed to intermittent infection and constantly shifting microbiomes and diets is less clear. For example, mice that cannot undergo affinity maturation have significantly shifted microbiomes (83), suggesting that polyspecific antibodies are not able to maintain host-microbiome homeostasis in all contexts. Perhaps in the wild, polyspecific clones act as an early barrier by binding moderately to all structures but are soon supplanted by IgA clones specific to the most immunogenic microorganisms. Indeed, intestinal IgA^+ plasma cells are highly mutated in both humans and mice (83, 84), and somatic hypermutation of IgA plasma cells increases with age (66, 80, 85, 86). As discussed above in the section titled Secretory IgA Shapes the Intestinal Microbiome, newer cells might oust older ones by attrition, allowing the IgA repertoire of the intestine to be flexible to the changing landscape of microbiome-derived antigens (38).

B CELL RECEPTOR–INDEPENDENT INDUCTION OF IgA PRODUCTION

The BCR is formed by a rearrangement of gene segments that is driven by the *Rag1/2* genes and called VDJ recombination (87). The random nature of the process allows for the generation of unique antibody variable regions and therefore the potential recognition of an extraordinary number of foreign antigens, but it also makes possible self-specific B cells that could cause

autoimmunity. The exquisite specificity of B cells, which improves over time via clonal selection and affinity maturation, is one of the cardinal attributes of the immune system, and thus the large population of polyspecific IgA-producing B cells in the intestine must be produced via mechanisms that only operate in the GALT. Indeed, B cells in the GALT can be activated without stimulation through the BCR. This is best illustrated by experiments with mice where all B cells and T cells could respond only to model proteins derived from chicken eggs, which indicated that B cell activation and GC formation in the GALT were independent of BCR engagement and T cell help (88, 89). Moreover, B cell clones can be expanded in the absence of antigen in PP GCs (90). While these findings derive from extremely simplified immunological systems, they highlight possible pathways to induce IgA in the absence of cognate antigen–BCR interaction. How, then, are B cells activated independently of the BCR? One potential mechanism involves signals derived from Toll-like receptors (TLRs). In the gut, the proximity of B cells and the dense microbiota raises the possibility that direct stimulation of TLRs by bacterial products induces an IgA response (91). For example LPS, the bacterium-derived ligand of TLR4, is routinely used to stimulate mouse B cells to initiate class-switch recombination (CSR), including class switch to IgA (92). In addition, high levels of PolyI:C, flagellin, and CpG DNA, respectively the ligands for TLR3, TLR5, and TLR9, can also drive CSR to IgA in human cells. However, human B cells are less prone to TLR stimulation than mouse B cells (93), since naive human B cells express low levels of TLRs, and expression of TLRs is induced by BCR and cytokine stimulation (94). These findings suggest that TLR signaling alone might not robustly induce IgA responses independently of BCR activation in naive B cells, but instead TLR signals might be more important in sequential CSR in previously activated memory B cells. In accord with this notion, TLR signaling on B cells is not necessary for IgA production. For example, TLR9$^{-/-}$ mice have an unaltered IgA response (95); TLR3-deficient patients make normal amounts of IgA to the rotavirus vaccine (96); and TLR5$^{-/-}$ mice have an enhanced IgA response, due to an overgrowth of inflammatory flagellated intestinal bacteria (35). Thus, while TLR stimulation of B cells certainly plays a role in B cell activation and GC reactions (97), dissecting the role of TLR signaling in the B cell IgA response is complex due to the widespread expression of TLRs on other immune (98) and nonimmune cells (99) and the difficulty in designing experiments where other signals, such as help signals from T cells, cannot compensate.

The necessity for TLR ligands from the microbiota to induce GCs and IgA also seems to differ according to the secondary lymphoid organ (SLO) (100), with GCs in PPs being more reliant on commensals for their formation than those in mesenteric lymph nodes (mLNs). Germ-free mice, which have severely reduced PP GCs at steady state, can form GCs after oral immunization, indicating that tonic TLR signaling by microbiome-derived molecules is not an absolute requirement for PP GC formation (101, 102). However, the GC response in immunized, germ-free mice is skewed toward IgG1, highlighting the importance of the microbiome in IgA production. In contrast, abrogating BCR signaling in B cells severely reduces GC development in PPs (103). Thus, while TLR signals at very high levels are capable of activating B cells independently of the BCR, in most physiological contexts they likely augment canonical BCR-based activation.

There is a well-described example of an IgA-producing B cell where TLR stimulation is absolutely critical: B1 B cells (104). B1 lymphocytes are a specialized, innate-like subset of B cells that dominate antibody production in fetal and neonatal mice. In rodents the subtype of B1a cells has been implicated as a source of microbiota-reactive, class-switched IgG and IgA antibodies, which are important for intestinal homeostasis, especially early in life (76, 105–107). Interestingly, IgM-expressing B1 cells require activating TLR signals linked to their specificity (108). Specifically, microbiome-specific B1 cells require signals from TLRs that respond to bacterial molecules (TLR2 and TLR4), while self-specific B1 cells require signals from nucleic acid–sensing TLRs.

Notably, B1 cells are T-independent, perhaps providing an explanation for the reliance on TLR signaling.

T CELL–INDEPENDENT IgA PRODUCTION

Upon BCR stimulation, B cells move to the border of the T and B cell zones (T-B border), where they interact with activated $CD4^+$ T cells that share specificity to a peptide antigen physically linked to the molecule inducing the B cell response. Interactions with cytokines and receptors (109) expressed by $CD4^+$ T cells provide B cells survival and differentiation signals that are critical for the formation of GC responses, CSR, and somatic hypermutation. The T cells that provide help have generally differentiated into a distinct state specialized for traffic to the B cell zone and provision of help signals and are referred to as follicular helper T (Tfh) cells (110). T-B cognate interaction is central to the GC response and all its attendant results, including CSR, somatic hypermutation, and formation of long-lived plasma and memory B cells. While this general model works well for SLOs that do not drain the gastrointestinal tract, the differentiation of IgA-producing B cells in the GALT can be mediated by both T cell–dependent and –independent mechanisms (111).

If B cells were to be stimulated in a BCR-independent fashion (as discussed in the previous section), they would not be able to support cognate B cell–T cell interaction, leading to a T cell–independent IgA response. However, even when B cells are stimulated through the BCR, they can drive IgA responses without T cell help. For example, TLR ligands are also often repetitive surface components of bacteria (LPS, lipoteichoic acid) that are capable of activating B cells outside of T cell help via high valency, with assistance from robust TLR stimulation. Accordingly, T cell–deficient ($TCR\beta\delta^{-/-}$) mice possess a microbiota-reactive IgA repertoire largely overlapping the one observed in T cell–sufficient mice, including mutated variable regions (79, 112). However, affinity maturation requires T cell help and by definition does not occur in the polyspecific clones that make up the majority of IgA-producing B cells in laboratory mice. Therefore, it is still unclear what part of the IgA response is T cell–independent outside of defined laboratory settings, and to what degree the balance between T-dependent and -independent IgA^+ B cell populations is a function of the host's experience with enteric infection and the microbiome.

T CELL–DEPENDENT IgA PRODUCTION

Recently, two groups investigating GC dynamics in the GALT (100, 113) concluded that even with chronic exposure to signals from the microbiota (i.e., TLR ligands), both clonal selection and affinity maturation occur readily in PPs. Since both of these processes require T cell–B cell cognate interaction, these findings suggest that, while GCs can be induced and supported independently of BCR signaling and T cell help under certain circumstances, the canonical BCR/T cell help–driven mechanism dominates PP GC formation and the induction of IgA-producing B cells at steady state. Therefore, while an IgA response can be mounted in the absence of T cells, a fully mature, functional IgA repertoire likely requires T cell help (80, 86).

The role of T cells and GC formation in IgA production has been teased apart by several groups using genetic and adoptive transfer approaches. The intestine-resident T cell response is dominated by $Foxp3^+CD4^+$ Tregs and IL-17-producing T helper 17 (Th17) cells, but neither of these differentiation states is well suited to helping B cells. Importantly, $CD4^+$ T cell differentiation is plastic, and adoptive transfer of Tregs or Th17 cells into T cell–deficient hosts results in their subsequent differentiation into Tfh cells that drive GC and IgA responses (114–118). During GC reactions, $CD4^+$ T cells provide a series of surface molecules that interact with

corresponding ligands or receptors on GC B cells to drive their selection. For example, CD40L-CD40 interaction is critical for GC formation: $CD40L^{-/-}$ mice lack GCs in the spleen and LNs (119). While $CD40^{-/-}$ and $CD40L^{-/-}$ mice have normal levels of steady-state IgA, their IgA responses to oral antigens are impaired (120) and they lack peanut-specific IgA in an oral allergy model (121). Conversely, and illuminating the importance of T cell help for the IgA response in nonlaboratory settings, patients with mutations in CD40L have lower levels of both total and antigen-specific IgA and hyperproduction of IgM. Notably, CD40L-deficient patients also have phenotypic abnormalities and a reduced number of follicular dendritic cells (FDCs) that lead to abnormal B cell follicle development (122), a feature not reported of $CD40L^{-/-}$ mice that might contribute to the different phenotypes. In contrast to the role of CD40 and CD40L, not all the molecules that have been shown to affect the GC response affect IgA production. For example, ICOS-ICOSL interaction is required for GC formation (123, 124), and $ICOS^{-/-}$ mice do not possess PP GCs but do make IgA, albeit at reduced levels (125). Tfh cells also express PD1, and $PD1^{-/-}$ mice are unable to promote a fully functional IgA response but have relatively unaffected GCs in the PPs (126). Importantly, the presence of T cell–dependent, affinity-matured IgA is critical for controlling oral pathogens, but it has implications for the microbiota as well: Both T cell help and somatic mutations are important for maintaining diversity within the microbiota (83, 126).

THE MOLECULAR MECHANISM OF IgA CLASS-SWITCH RECOMBINATION

In accord with the unique mucosal functionality of IgA, the activation and differentiation of IgA-producing B cells differ substantially from those of IgG- and IgM-producing B cells that respond to systemic infection. Cues taken from the local mucosal environment and the microbiota are critical to the development of IgA-producing B cells.

Initially, all mature B cells express the immunoglobulin heavy-chain constant region μ, which corresponds to IgM. The acquisition of IgA heavy chain expression is controlled by CSR that replaces the IgM constant region (C_μ) with that of IgA (C_α) to express IgA, a process that requires the enzyme activation-induced deaminase (AID). AID induces double-strand breaks at a switch region and requires transcription of the germ-line transcript (GT) by upstream intronic promoters (I_μ, I_α, etc.) that are specifically regulated by cytokine-responsive promoter elements (127). In line with the absolute requirement for AID for CSR, mice lacking AID failed to produce IgA and all immunoglobulin classes except IgM (128). AID is usually absent in resting naive B cells but is rapidly upregulated by BCR signaling in the context of T cell help and proliferation (129, 130). Cytokines produced by Th cells can influence the process of CSR by directing transcription to the relevant switch regions. When completed, CSR loops out all the C_H genes upstream of C_α segments and gives rise to a BCR where the upstream variable (VDJ) region is joined directly in-frame to C_α. The excised circular DNA fragment contains the active Iα promoter and therefore can still undergo transcription that produces α circle transcripts (αCTs). While cells that have finished IgA CSR can be easily detected by amplifying Iμ and C_α, which are now juxtaposed, identifying cells actively undergoing IgA CSR is more problematic. In contrast to GTs, AID, or circular DNAs, αCTs can be detected before the appearance of the IgA BCR on the surface, and their short half-life makes them ideal to identify cells actively undergoing IgA CSR (131). Simultaneous analysis of both αGT and αCT allows for the identification of the direct requirements for IgA CSR, outside of signals involved in other processes, such as trafficking and survival. Without these molecular (αGT and αCT) measures of IgA CSR activity, confounding results on the cellular and molecular requirements for IgA CSR are possible. One example is retinoic acid (RA), a vitamin A metabolite

derived from the diet and produced by intestine-derived dendritic cells (DCs) that plays a critical role in controlling IgA secretion in vivo (132). Despite the apparent effect of RA in an in vitro IgA CSR assay, it is now appreciated that RA receptor signaling in B cells does not directly control CSR to IgA but instead drives expression of integrin $\alpha_4\beta_7$ and CCR9 to allow IgA^+ cells to migrate to the intestinal lamina propria (133). RA also influences IgA response indirectly via potent effects on T cell and DC differentiation (134–137).

In contrast, the cytokine transforming growth factor beta (TGF-β) has emerged as an essential cytokine for IgA CSR. Seminal experiments on B cell lines identified TGF-β as an inducer of αGT (138, 139). TGF-β deficiency (140) and TGF-β receptor II (TGFβRII) deficiency on B cells (141, 142) virtually eliminated the production of IgA. Control of TGFβRII is therefore critical for assuring proper IgA CSR. For instance, nitric oxide (NO), which is generated by inducible NO synthase (iNOS) in intestinal DCs, can increase IgA CSR in PPs by upregulating TGFβRII on B cells (143). The clathrin light chain also regulates IgA CSR by controlling TGFβRII signaling by endocytosis (144). TGF-β signaling can also cooperate with IL-21 to promote IgA CSR and upregulation of mucosal homing receptors (118).

As TGF-β is critical to the development of IgA-producing B cells, the mechanism of its delivery to activated B cells has been a focus of study. After translation, TGF-β is not readily available for binding by the TGFβR, as it is made in a membrane-bound, latent form that requires cleavage-dependent activation (145). Two main mechanisms have been proposed for TGF-β activation: one involving matrix metalloproteases expressed by FDCs (146) and the other requiring $\alpha_v\beta_8$ integrins expressed by DCs and in some instances Tregs (147). While production of TGF-β by FDCs present in the GC would dovetail well with the notion that CSR occurs primarily in GCs, recent results have redefined temporally and anatomically the components of IgA CSR, suggesting that IgA CSR initiates before GC entry (103). Elegant imaging analysis and conditional knockout of TGFβRII on either all B cells or GC B cells alone revealed that TGFβR signaling takes place outside of the GC and that abolishing TGF-β signaling in GC B cells has no effect on IgA CSR (142). However, while IgA CSR is initiated outside GCs, it is possible that TGF-β generated by FDCs plays a role in IgA CSR of reactivated memory B cells. Tregs express $\alpha_v\beta_8$ (148), but the effect of Tregs on IgA CSR is complicated. While Tregs play a role in IgA CSR under certain conditions, mice with Tregs deficient for either c-Maf or IL-10 lack expression of $\alpha_v\beta_8$ but have increased IgA production (149, 150). This suggests that the ability of Tregs to activate TGF-β is less important than the necessity to maintain immune homeostasis. Similarly, the origin of latent TGF-β required for IgA CSR is unclear: B cells can themselves be a source of TGF-β (151, 152), although other cells, including Tregs (153, 154), T follicular regulatory cells (Tregs that traffic to GCs) (155), FDCs (146), and DCs (156), can also express this cytokine. Studies using conditional knockout technologies to reduce TGF-β production and/or TGF-β activation by $\alpha_v\beta_8$ in specific cell subsets are needed to map the cellular contribution to IgA CSR.

In addition to TGF-β, two other cytokines, a proliferation-inducing ligand (APRIL) and B cell–activating factor (BAFF), have been described as acting directly on surface receptors expressed on B cells to induce AID and IgA CSR. Several lines of evidence support the role of APRIL in controlling IgA induction: APRIL-deficient mice and humans lacking the APRIL receptor transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) present with defective IgA production (157, 158). The involvement of BAFF is less clear: While BAFF can induce IgA CSR (159, 160), patients and animals with deletion of the BAFF receptor or BAFF (161) have normal levels of IgA (161–163). APRIL and BAFF can be produced by intestinal innate immune cells and epithelial cells (159, 164–166). Experiments with conditional deletion of these molecules and the corresponding receptors analyzing the presence of αCT and αGT need to be performed to dissect their role in IgA CSR.

ANATOMY OF IgA CLASS-SWITCH RECOMBINATION

IgA is predominantly produced by B cells that inhabit mucosal surfaces. The phenotype of IgA^+ B cells is in part determined by the local environment within which they are activated, the GALT and other mucosal-associated lymphoid tissue. Here we focus on those different tissue sites (**Figure 2**).

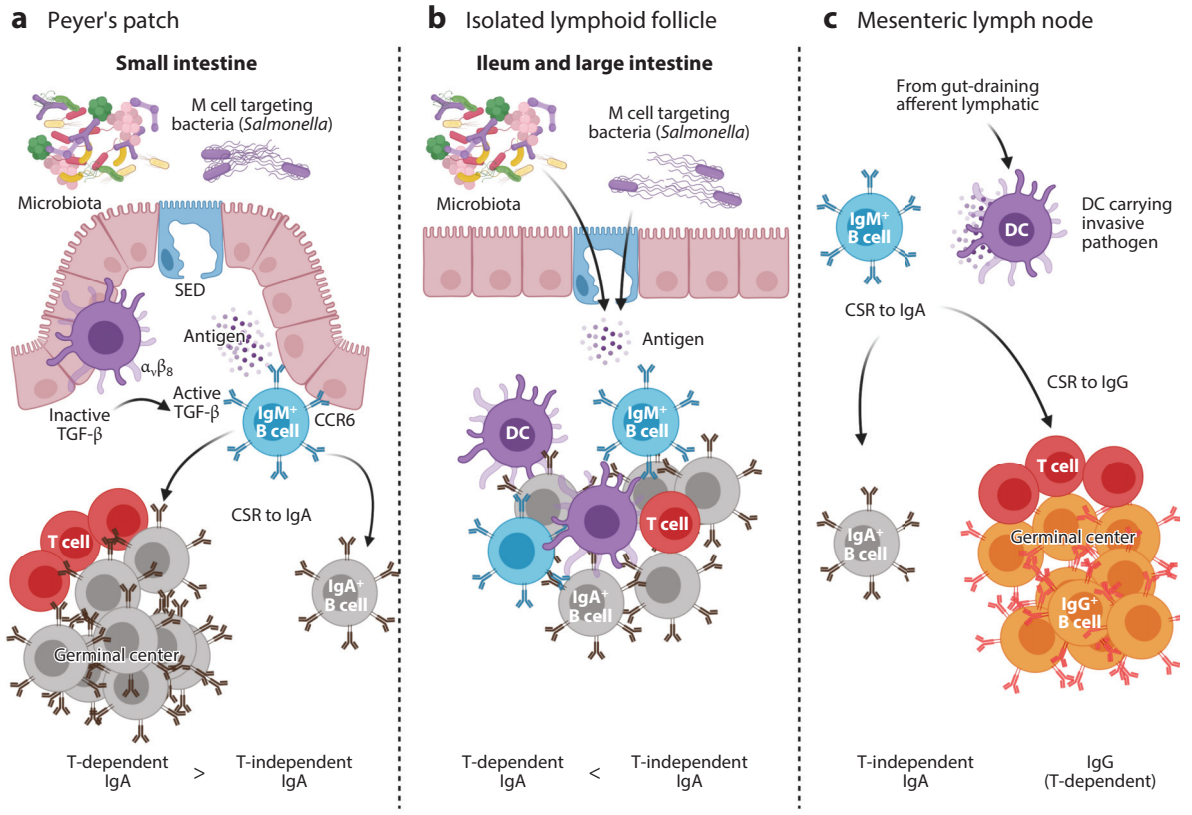

Figure 2

IgA responses in anatomically distinct GALTs. (*a*) Commensals and enteric pathogens gain entry into PPs through M cells and stimulate antigen-specific naive B cells to upregulate CCR6. CCR6 mediates B cell migration to the SED, where integrin $\alpha_v\beta_8$–dependent DC activation of latent TGF-β1 provides the signal for IgA CSR. Upon initiation of IgA CSR, B cells either differentiate into IgA^+ PCs independently of T cells or receive T cell help to enter GC reactions. IgA^+ GC B cells slowly undergo somatic hypermutation to increase IgA affinity and give rise to somatically mutated PCs that eventually home to the lamina propria. Overall, the IgA response in PPs is a predominantly T cell–dependent process. (*b*) In ILFs, B cells respond to commensal and enteric pathogens entering through M cells and undergo IgA CSR in a largely T cell–independent fashion. The function of ILF-derived IgA when intestinal lymphoid organs are present is still under investigation. (*c*) Lamina propria DCs that migrate to the lymph nodes through afferent lymphatics can carry invasive pathogens or commensals and stimulate B cell responses in mLNs. T cell–dependent responses in mLNs are geared toward IgG1 production, which can prevent bacteria from reaching the bloodstream. Conversely, the IgA response in mLNs is mainly generated in a T cell–independent way, possibly against selected commensals that gain access to the lamina propria and are eventually transported to the lymph nodes. The environmental cues, immune cell subsets, and bacterial species required for instructing the generation of different antibody isotypes in mLNs have not been fully characterized. Abbreviations: CSR, class-switch recombination; DC, dendritic cell; GALT, gut-associated lymphoid tissue; GC, germinal center; ILF, isolated lymphoid follicle; mLN, mesenteric lymph node; PC, plasma cell; PP, Peyer's patch; SED, subepithelial dome. Figure adapted from images created with BioRender.com.

Peyer's Patches

Seminal work by Craig & Cebra (167) identified PPs as a source of IgA precursor cells that can restore IgA levels when adoptively transferred into irradiated rabbits. PPs are SLOs that develop in utero on the antimesenteric site of the small intestine. PP anatomy is strikingly different from that of other SLOs, as PPs lack afferent lymphatics and a subcapsular sinus and therefore do not drain tissue antigens. Instead PPs rely on antigen entry through specialized epithelial M cells (168) that are found on the follicle-associated epithelium. While M cells express receptors for binding bacterial molecules such as GP2 and PGRP (169, 170), the mechanism by which antigens are acquired and transferred into PPs is not clear. Loss of M cells due to Sox8 deficiency impaired PP formation, GC formation, and IgA production (171). PPs also contain areas that are not present in other lymph nodes, such as the DC-rich subepithelial dome (SED), located at the top of the B cell follicle, just beneath the M cell (36).

Under homeostatic conditions, IgA CSR is largely restricted to PPs, with roughly 50% of GC B cells in PPs being IgA^+, while IgA-producing B cells make up a much smaller fraction of mLN B cells (112, 142). As discussed, CSR to IgA in PPs is initiated outside the GC, as upon antigen engagement B cells upregulate CCR6 and move to the SED, following the CCR6 ligand CCL20, which is produced by epithelial cells in the follicle-associated epithelium. AID^+ cells can be identified in the SED (103, 126), and both αCT and αGT can be found in pre-GC cells (103, 172). CCR6-mediated migration to the SED is required for IgA CSR because it facilitates B cell interaction with $\alpha_v\beta_8$-expressing DCs, which are likely responsible for TGF-β activation and IgA CSR (103). In accord with this hypothesis, measurement of SMAD phosphorylation in the PPs revealed that TGF-β signaling occurs in the SED (142). Together, these data conclusively identify the SED as the initial site of IgA CSR in PPs, occurring through the production of TGF-β. Interestingly, CSR to other antibody isotypes outside of GCs has now been confirmed, which makes sense because expression of the cytokines that direct CSR does not necessarily coincide with differentiation to the Tfh state and traffic to the GCs (173).

Mesenteric Lymph Nodes

The various mLNs that drain different areas of the small and large intestine (174, 175) are critical to the activation of T cells by intestinal antigens, but their relative contribution to IgA production seems to be limited, and probably enriched for T cell–independent B cell responses (176, 177). In the absence of PPs (178), mLNs can coordinate IgA induction (179), and experiments measuring the response to novel bacterial colonization of germ-free mice have highlighted a role for mLNs in IgA responses (180). However, B cell responses in the mLNs are much more likely to induce IgG production than those in PPs, which makes sense given that the presence of microorganisms at this site could imply infection, making the mLNs the last firewall before bacteremia (76, 181). Thus, mLN-driven IgA responses might be dependent upon the relatively rare occurrence of bacteria and viruses escaping the lamina propria to enter the mLN, while intestine-resident organisms of the microbiota are commonly sampled by M cells to preferentially drive PP responses.

IgA Induction Outside Secondary Lymphoid Organs

While the majority of IgA production is believed to occur in the PPs and, to a lesser extent, in the mLNs, bone marrow transplantation into mice born without PPs or mLNs revealed that IgA production is possible outside these tissues (182, 183). In these experiments, SLO-independent IgA induction was mapped to isolated lymphoid follicles (ILFs), clusters of B cells that are surrounded by DCs and a small number of T cells and located in the terminal ileum and colon. In contrast to

SLO formation, ILFs develop after birth and require peptidoglycan recognition through NOD1 for their maturation (184). It is unclear what the physiologic contribution of ILFs to the IgA response is when PPs and mLNs are present, but they might play a dominant role during early life or in response to pathogens that bypass the small intestine entirely and infect only the colon (185). In addition to ILFs, tertiary lymphoid follicles can be generated in instances of chronic inflammation (186) and contribute to IgA in response to both the microbiota and enteric infection (187, 188).

IgA CSR in the lamina propria, outside of any lymphoid tissue, is more controversial. While AID expression has been observed (183, 189) and αGT and αCT have been detected in lamina propria B cells (190), these findings have not been consistently reproduced (172, 191), suggesting that direct IgA CSR in the lamina propria might be sensitive to external cues such as genetic background, intestinal microbiome, and age.

ROLE OF THE INTESTINAL MICROBIOME IN DRIVING IgA PRODUCTION

It is clear that IgA production occurs most commonly in the GALT. The most salient aspect of the intestinal environment is the dense and diverse microbiome, which provides enormous enzymatic capacity to the host and is the source of a multitude of important metabolites. Unsurprisingly, signals derived from the microbiome are required for the development of IgA-producing B cells, and secretion of IgA is almost undetectable in germ-free mice (192). Unfortunately, the signals derived from the microbiota that directly regulate B cells are mostly unknown. As discussed, TLR ligands can activate CSR to IgA, and certainly these ligands are enriched in the GALT, but they do not exclusively support IgA production. The production of short-chain fatty acids (SCFAs) by the enzymatic digestion of fiber is a unique function of the intestinal microbiome, and SCFAs are sensed by multiple immune and stromal cell types as biomarkers for the proper function of the microbiota (193). One SCFA, acetate, promotes the production of IgA via binding to its receptor, GPR43 (194). However, the effect of acetate is not direct, and GPR43 activation induces RALDH transcription and RA production in DCs to increase IgA^+ B cell trafficking to the lamina propria. SCFAs are also crucial to support TGF-β transcription by at least two mechanisms. First, SCFA signaling in enterocytes promotes the production of TGF-β (195), though whether this effects B cell CSR in the PP is unclear. Second, SCFAs support the differentiation and homeostasis of Tregs (196, 197), which are potent producers of TGF-β known to affect IgA induction. Outside of SCFA function, high-level expression of $\alpha_v\beta_8$ is driven by TLR and RA signaling to DCs, and thus antibiotic treatment of mice reduces $\alpha_v\beta_8$ expression in the GALT (198).

In addition to signals derived from the metabolism of food, the microbiome also provides antigen for the activation of B cells. However, induction of IgA production is not a characteristic of all bacteria, with some organisms, such as segmented filamentous bacteria, dominating the IgA response (79). A key factor in the immunogenicity of an intestinal bacterium is adherence to the intestinal epithelium or location within the mucus layer, where presumably they can be sampled by host phagocytic cells or M cells. Intestine-adherent bacteria also induce the activation and differentiation of IL-17-producing T cells, which potently induce the expression of PIGR in the intestinal epithelium and substantially increase transepithelial transport of IgA (199, 200). In addition, whether any given strain of bacteria can induce IgA is a complex question and may be the determined by interactions between different bacterial isolates within the microbiome. For example, various strains of *Bacteroides ovatus* differ in their ability to induce colonic IgA, but strains that are not IgA inducers inhibit IgA induction by other related strains (201). How different members of the microbiota modify the host to benefit themselves and disadvantage their competitors will

be a fascinating area for future research, and the answer may lead to a deeper understanding of the pathways via which the microbiome can activate or regulate intestinal B cells.

CONCLUSION

The last few years have seen an explosion of new knowledge of the structure of IgA, how it works, and how it is formed. While mucosal immunology and IgA were once seen as a specialized system with modest applicability, it is now clear that this view should be reversed, since most of the work done by the immune system is to regulate interactions with colonizing microorganisms to prevent host invasion and inflammation. In that vein, a critical unanswered question is how IgA is shaping the microbiome both at the level of individual bacterial isolates and as a functioning consortium. Furthermore, how does the specificity of IgA affect the resultant response in microorganisms, and are polyspecific antibodies that bind to a variety of surface epitopes producing the same effects as focused T-dependent IgAs targeting surface proteins?

Vaccines have never been more important, and IgA, given its modest inflammatory profile, could be an important protective mediator. However, there is much we still need to know about how to best activate IgA-producing B cells to be effective. Given the apparent attrition of IgA-producing B cells in the intestine, it is important to more deeply understand how IgA-producing plasma cells are formed in GCs so we can boost their number in the intestine. Finally, while our review has focused almost entirely on the intestine, there are other sites where IgA secretion is common, most notably the mouth and nose, where IgA-producing B cells are found within the salivary gland and nasal mucosa. How to direct IgA-producing B cells to these sites and what their affect is on local microorganisms are largely unknown and could be a fascinating area for future research.

DISCLOSURE STATEMENT

T.W.H. has submitted a patent on a device/methodology for determining the antibacterial specificity of human immunoglobulin A.

ACKNOWLEDGMENTS

We would like to thank J. Muppidi and D. Wesemann for critical reading and comments and the Hand and Reboldi laboratories for helpful discussions. This work was supported by the National Institutes of Health (R01 DK120697 to T.W.H.). We apologize that not all studies in this growing field could be discussed and cited.

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Annual Review of Immunology

Volume 39, 2021

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