Published in final edited form as:

Cell Mol Life Sci. 2015 July ; 72(14): 2645–2655. doi:10.1007/s00018-015-1892-4.

Regulation of intestinal IgA responses

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Abstract

The intestine harbors enormous numbers of commensal bacteria and is under frequent attack from food-borne pathogens and toxins. A properly regulated immune response is critical for homeostatic maintenance of commensals and for protection against infection and toxins in the intestine. IgA isotype antibodies function specifically in mucosal sites such as the intestines to help maintain intestinal health by binding to and regulating commensal microbiota, pathogens and toxins. IgA antibodies are produced by intestinal IgA antibody-secreting plasma cells generated in gut-associated lymphoid tissues from naïve B cells in response to stimulations of the intestinal bacteria and components. Research on generation, migration, and maintenance of IgA-secreting cells is important in our effort to understand the biology of IgA responses and to help better design vaccines against intestinal infections.

Keywords

IgA antibody; IgA antibody-secreting plasma cells; Follicular T helper cells; Migration and localization; Chemokine receptor; IgA memory

Introduction

As a site for food absorption, the intestines host an enormous number of commensal bacteria constituting the indigenous microbiota and are also a major portal of entry for pathogens. Balanced adaptive immune responses in the intestine are essential for the homeostatic maintenance of the intestinal environment and protection against infection. Immunoglobulin A (IgA) antibodies are a major functional component of the humoral branch of the adaptive immune system, specifically at mucosal sites such as the intestines. The antibodies are predominantly produced by IgA antibody-secreting plasma cells (IgA-ASCs) localized in the intestinal lamina propria (LP) as dimers linked by the joining (J) chain which contributes to the binding of dimeric IgA to the polymeric immunoglobulin receptor (pIgR) for transportation through intestinal epithelial cells and secretion into the intestinal lumen [1]. Expression of the J chain in IgA-ASCs is preferentially induced in the gut-associated lymphoid tissue (GALT) environments although underlying mechanisms are not well understood. In the intestinal lumen, secretory IgA (SIgA) antibodies bind to the surface of commensal bacteria to maintain their homeostatic existence with intestinal tissues [2-4].

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SIgA antibodies also bind to orally born pathogens and toxins to block them from attaching to and affecting intestinal tissues through a non-inflammatory process commonly known as “immune exclusion” [5-8]. Additionally, SIgA antibodies help facilitate the sampling of intestinal environments by dendritic cells (DCs) in the subepithelial dome region of the Peyer’s patches (PPs). This is accomplished by retro-transporting antigens from the intestinal lumen into the intestinal tissue through the M cells of the follicle-associated epithelium covering the PPs. IgA antibodies within the intestinal epithelial tissues can also intercept invading pathogens and excrete them from the tissues.

Due to the diverse functions of IgA antibodies in intestinal homeostasis and protection against pathogenic infections, major efforts are underway to understand the generation, distribution and maintenance of IgA-ASCs in intestinal tissues. It has been established that intestinal IgA-ASCs are derived from IgA⁺ plasmablasts generated from naïve B cells predominantly in GALT. PPs are considered to be the major site of the IgA⁺ plasmablast cell generation in response to intestinal antigen stimulations (Fig. 1). In addition, IgA⁺ plasmablasts can be generated in isolated lymphoid follicles (ILFs), small dynamic B cell-rich lymphoid structures scattered abundantly in both the small and large intestines of humans and mice [9]. Unlike PPs that are formed during the embryonic stage, ILFs are predominantly formed postnatally in response to the colonization of commensal bacteria in the intestines, suggesting their involvement in regulating bacterial homeostasis as an additional site of IgA⁺ plasmablast development [10, 11]. Consistent with this notion, mice lacking PPs displayed the enhanced generation of IgA⁺ plasmablasts in the ILFs [12, 13]. It was recently reported that the caecal patch (CPs), a lymphoid tissue in the appendix, is also a site for the generation of murine colonic IgA⁺ secreting cells [14]. IgA⁺ plasmablasts can also be generated in situ in the intestinal lamina propria (LP) of mice, particularly in the absence of PPs and ILFs [15]. Whether the in situ generation of IgA⁺ cells in intestinal LP occurs in normal physiological conditions is still under debate [16]. At most, the generation of IgA⁺ cells in the intestinal LP may contribute to a small fraction of the IgA pool under physiological conditions. In addition, there are reports that IgA⁺ cells could be generated in mesenteric lymph nodes (MLNs) and the peritoneal cavity of mice [17-20]. The importance of these IgA⁺ cell generation pathways in contribution to the total IgA pool under homeostatic conditions is also under debate [2]. It is likely that different IgA⁺ cell generation processes operate in concert to ensure proper IgA production in the intestines to maintain intestinal homeostasis and protect against infection.

During differentiation from naïve B cells in GALT, the resultant IgA⁺ plasmablasts are imprinted to upregulate gut-homing molecules, which direct their migration into the intestinal LP where the IgA⁺ plasmablasts mature further into IgA-ASCs [21, 22]. In the LP, mature IgA-ASCs can survive for a long period of time, providing a local source of SIgA antibodies [23, 24]. It was suggested that the intestines provide a permissive or supporting environment for the long-term survival of IgA-ASCs. Since the intestinal environment supports long-term maintenance of IgA-ASCs in absence of obvious antigen stimulation [23, 24], it is possible that intestine-resident IgA⁺ plasma and memory B cells are critically involved in memory protection against pathogenic infections.

In this article, we will review our current understanding of cellular and molecular mechanisms regulating the generation, migration and distribution of IgA⁺ cells, including both plasma cells and memory B cells, with special emphasis on the advances made in recent years. Most of those advances are from studies with mouse models unless otherwise indicated.

T cell-dependent differentiation of IgA⁺ cells

Most IgA⁺ plasmablasts are generated in the germinal centers of PPs with the help of T cells. Germinal centers are sites in PPs and other secondary lymphoid organs, where antigen-activated B cells proliferate, undergo class switch recombination (CSR) in the immunoglobulin heavy chain (IgH) locus from original IgM to IgA or another isotype, and undergo the somatic hypermutation (SHM) in antigen-recognizing regions of the IgH genes to generate plasmablasts and memory B cells with high-affinity antibodies-producing capacities [25]. T cells play several roles in both the formation of germinal centers and the generation of IgA⁺ cells. T cells, through the production of cytokines and cell-cell interaction, promote B cell proliferation. In addition, T cells help induction of the expression of activation-induced cytidine deaminase (AID) in B cells, which is the critical enzyme for the execution of CSR and hypermutation. In the absence of T cells, germinal centers failed to form, impairing the generation of IgA cells. Furthermore, IgA⁺ cells generated in a T cell-independent fashion have restricted antigen specificities with no or low frequencies of somatic hypermutation.

Helper T cells involved in the regulation of IgA⁺ cell development

Specialized populations of T cells are involved in the promotion of antibody isotype switching. Among them, follicular T help (Tfh) cells are an important helper T cell subset found in the B cell follicles of germinal centers of PPs and other secondary lymphoid organs such as lymph nodes and spleens (reviewed recently in [26]). Tfh cells constitutively express the chemokine receptor CXCR5, important for their localization into the B cell follicles. The expression of CXCR5 has been used to distinguish Tfh cells from other T cell subsets. Tfh cells express cytokines such as IL-21 and IL-4, important in promoting the IgA isotype switch (Fig. 1). Until recently, the developmental origins of Tfh cells and their association with other helper T cell subsets were not fully understood. In addition, how Tfh cells of PPs and other different secondary lymphoid organs are involved in the generation of IgA- versus other isotype antibody-producing cells is not clear.

Recently, several reports found that T helper 17 (Th17) cells play an important role in the generation of IgA-producing cells and one of them found that Th17 cells could give rise to Tfh cells. Th17 cells, through the production of IL-17, are important in maintaining intestinal immune homeostasis. Under homeostatic conditions Th17 cells are preferentially found in the intestines where their development depends on stimulation from commensal bacteria, particularly segmented filamentous bacteria (SFB), a member of Clostridiales [27, 28]. A recent report found that transferred Th17 cells in recipient mice preferentially home to the intestines and associated lymphoid organs such as PPs, where they acquired a Tfh phenotype, which includes the upregulation of the cell surface molecules CXCR5 and programmed cell death-1 (PD-1), transcription factor BCL6 and the cytokine IL-21 [29].

The acquisition of a Tfh phenotype was associated with the downregulation of Ror γ t, IL-17A and other molecules characteristic of Th17 cells [29].

Furthermore, the Th17-derived Tfh cells induce germinal center formation in PPs and promote the generation of IgA⁺ cells. Mice deficient in Th17 cells were impaired in an antigen-specific intestinal IgA response to immunization of cholera toxin, a model protein antigen commonly used to induce a T cell-dependent IgA response [29]. Consistent with this report, another study found that repletion of mice deficient of T cells with commensal antigen-specific Th17 cells increased the production of intestinal IgA antibodies specific to that antigen [30]. Furthermore, IL-17R^{-/-} mice had lower levels of intestinal IgA antibodies than wild type mice and displayed systemic anti-microflora antibody responses, likely due to increased infiltration of commensal bacteria into tissues [30]. Interestingly, microbiota-specific Th17 cells are also important for the induction of pIgR expression on intestinal epithelial cells, suggesting Th17 cells are involved in regulating both production and transportation of IgA antibodies for intestinal homeostatic maintenance [30].

Foxp3⁺ T cells are another important T cell population in the generation of IgA⁺ T cells [31, 32]. An earlier study found that a Foxp3-expressing helper T cell subset was able to acquire the Tfh cell phenotype in PPs [31]. When Foxp3⁺ T cells of spleens or lymph nodes were transferred into T cell-deficient mice, they could induce germinal center formation and increase the generation of IgA⁺ cells in the PPs and intestinal LP [31]. Correlating with this, in PPs of recipient mice, transferred T cells downregulated Foxp3 and upregulated the expression of CXCR5, ICOS, BCL6 and IL-21, all of which are features of Tfh cells. However, another study found that transferred Foxp3⁺ T cells could not home to the intestinal LP and associated lymphoid organs and were unable to differentiate into Tfh cells or support the generation of IgA-producing cells [29]. Therefore, whether Foxp3⁺ T cell-derived T cells in PPs are bona fide Tfh cells is not clear. The differentiation of Foxp3⁺ T cells into Tfh-like cells has been also reported in the spleen and other lymphoid organs [33, 34]. Like Foxp3⁺ T cell-derived Tfh cells in PPs, Foxp3⁺ T cell-derived Tfh-like cells in the spleen also downregulated the expression of Foxp3 and upregulated expression of molecules associated with Tfh cells, including CXCR5 and PD-1 and BCL6 [33]. However, the Foxp3⁺ T cell-derived Tfh-like T cells concurrently expressed the gene encoding Blimp-1, which distinguishes them from Tfh cells [33]. Since BCL6 induces expression of CXCR5 on Treg cells, it was concluded that Foxp3⁺ cell-derived Tfh-like T cells represented a different subset of T cells, named follicular regulatory T (Tfr) cells. Tfr cells suppress T cell proliferation and reduce Tfh and germinal center B cell numbers in spleens. A lack of CXCR5⁺ Tfr cells leads to greater germinal center reactions in spleens: increased numbers of germinal center B cells, affinity maturation of antibodies and differentiation of plasma cells [33, 34]. However, it was recently suggested that Foxp3⁺ Tfr cells function in germinal centers of PPs to promote somatic mutation and affinity maturation of IgA⁺ cells [2] (Fig. 1). Mechanisms underlying the seemingly opposite functions of Tfr cells in PPs and spleens require further investigation.

Molecular factors mediating T cell regulation of IgA⁺ cell development

Multiple soluble and cell surface molecules expressed by Tfh cells regulate the generation of IgA⁺ plasmablast cells in PPs. Among them, PD-1, an immune inhibitory receptor, was recently reported to regulate the generation and selection of IgA-producing cells in the intestine [3] (Fig. 1). PD-1-knockout mice had increased numbers of Tfh cells with altered phenotypes, which in turn resulted in increased numbers of germinal centers and IgA⁺ cells in PPs. However, PD-1-deficient Tfh cells were impaired in their ability to support the maintenance of IgA-ASCs in the intestinal LP. Furthermore, IgA antibodies generated in PD-1-knockout mice have a reduced capacity to bind commensal bacteria and maintain their homeostasis in the intestine. PD-1 expressed by Tfh cells in lymphoid organs other than GALT were also found to be important in the selection and survival of long-lived plasma cells secreting different isotype antibodies [35]. In the germinal centers of spleens, PD-1 knockout mice had more cell death and less Tfh cell cytokine production than wild type mice. However, remaining plasma cells generate antibodies with greater affinity for antigens. What distinguish the differential effects of PD-1 on the generation of plasma cells secreting antibodies of IgA and other isotypes is not clear.

Cytokine transforming growth factor β 1 (TGF- β 1), IL-4, IL-10 and IL-21 are all involved in the generation of IgA-ASCs (Fig. 1). IL-21 produced by Tfh cells is critically required while sources other than T cells are sufficient to generate other cytokines that promote the IgA⁺ cell generation [36, 37]. IL-21 produced by Tfh cells coordinates with TGF- β 1 to promote the generation of IgA⁺ plasmablast cells. TGF- β 1 promotes naive B cell proliferation and differentiation and suppresses IL-21-induced IgG class switching in favor of IgA⁺ cell generation [36, 37]. Reciprocally, IL-21, through interaction with the IL-21R on B cells, inhibited the TGF- β 1-induced IgG2b production to promote the selective generation of IgA⁺ plasmablasts [37]. However, IL-21 had little effect on the IgA generation by itself and did not alter TGF- β 1-induced IgA synthesis, suggesting a synergistic effect of IL-21 and TGF- β 1 in promoting the generation of IgA-producing cells.

T cell-independent differentiation of IgA-ASCs

While T cell-dependent differentiation of IgA⁺ plasmablast cells is essential for the generation of high affinity IgA antibodies, mice deficient of T cells have relatively normal levels of intestinal IgA-ASCs and antibodies, indicating that there exist T cell-independent IgA⁺ cell differentiation processes [17]. Past studies have found that PPs, ILFs, MLNs and CPs can all support the T cell-independent generation of IgA-ASCs [14, 15, 38]. The intestinal LP was also suggested to support the T cell-independent generation of IgA-ASCs, particularly of B cells derived from the peritoneal cavity in mice [15].

There is increasing evidence that ILFs are an important alternative site for the generation of IgA-ASCs in a T cell-independent fashion (Fig. 1). Unlike PPs, ILFs are generated postnatally in response to the stimulation of commensal bacterial colonization from cryptopatches, which are deposition sites of lymphoid inducer cells. In mice that lack the T-cell dependent generation of IgA-ASCs there was accelerated maturation of ILFs from cryptopatches and increased numbers of IgA⁺ cells in ILFs [12, 13]. In addition, we recently found that in the chemokine receptor CCR10 knockout (CCR10^{-/-}) mice where the

migration of CCR10^{-/-} IgA⁺ plasmablast cells into intestinal LP was impaired, there were compensatory increases in the numbers of ILFs and IgA⁺ cells in the ILFs [39].

ILFs were also found abundantly in human intestines [40] and could potentially function in the same fashion as the mouse ILFs in support of the generation of IgA-ASCs. However, since no defined cryptopatches were found in human intestines, the correlation of human and mouse ILFs has not been fully established. It was argued that human ILFs are derived from similar cryptopatch structures that are generated in the early postnatal stage but that such structures have already matured into ILFs in adult human subjects analyzed for ILFs and cryptopatches. A recent study staining for CCR6⁺lin⁻c-kit⁺ lymphocytes identified clusters of lymphocytes in the human intestine that resemble murine cryptopatches [41]. Furthermore, in humanized mice, the development of human GALT structures, including ILFs, could originate in mouse cryptopatches [42]. Isotype switching to IgA also occurred in these GALT structures, providing strong evidence that human and mouse ILFs share the same developmental processes for the generation of IgA⁺ cells.

Like the T cell-dependent generation of IgA⁺ plasmablasts, T cell independent generation of IgA⁺ plasmablasts is regulated by a similar set of cytokines, including TGF- β 1, IL-4 and IL-10. However, the cellular sources of these cytokines in the absence of T cells are not fully understood. Plasmacytoid dendritic cells (pDCs) were recently identified as a major source of a proliferation-inducing ligand (APRIL), B-cell activating factor (BAFF), IL-6 and IL-10 in promoting T cell-independent IgA CSR and the generation of IgA-ASCs in mice [38] (Fig. 1). In addition, a subset of newly identified innate lymphoid cells (ILC), ILC2 (also called neucytes or natural helper cells), were recently reported to produce large amounts of cytokines IL-4, IL5, IL6 and IL13 and enhance IgA production in mice [43] (Fig. 1). Furthermore, membrane lymphotoxin b expressed by ROR γ ⁺ ILCs was reported to promote inducible nitric oxide synthase (iNOS) expression on CD11c⁺ DCs, which in turn induces the T cell-independent IgA responses [44].

Regulation of migration of IgA⁺ plasmablasts into small and large intestines

Major gut-homing molecules involved in the migration of IgA⁺ plasmablasts

The same process that generates the IgA⁺ plasmablast cells in GALT also imprints their preferential gut-homing potentials through the induction of a unique combination of chemokine receptors and adhesion molecules. CCR9 and CCR10 are two major mucosa-specific chemokine receptors upregulated on IgA⁺ plasmablasts during their generation [39, 44-50] (Fig. 1). However, there are also notable differences in the expression patterns of CCR9 and CCR10 on IgA⁺ cells generated in different GALT and their ligands in the small and large intestines. For example, while IgA⁺ cells generated in PPs and CPs both express CCR9, IgA⁺ cells of CPs express significantly higher levels of CCR10 than those of PPs [14]. In addition, CCR10 remains expressed on all IgA-ASCs after their maturation from IgA⁺ plasmablasts in the intestines, while CCR9 is downregulated on mature IgA-ASCs [45-47, 51]. Furthermore, CCL28, the mucosal ligand of CCR10, is expressed in both small and large intestines while CCL25, the ligand for CCR9, is predominantly expressed in the small intestines [45-47, 51-53].

Consistent with their expression patterns, CCR9 and its ligand CCL25 play an important role in the homing of IgA⁺ plasmablasts into the small intestine [46, 47]. The functional importance of CCR10 and its ligand CCL28 in the intestinal IgA response was not clear until recently. In an earlier study, anti-CCL28 antibody blockage impaired the intestinal IgA response to oral immunization of cholera toxin (CT) [49]. However, anti-CCL28 antibody treatment did not affect intestinal IgA response to rotavirus infection [54], and the homeostatic IgA antibody levels in the intestines of CCR10-knockout mice were normal [55], suggesting that CCR10 and CCL28 are not required for intestinal IgA production in response to stimulations from commensal bacteria or pathogens. This might be partially due to redundant roles of CCR10 and CCR9 [54], at least in the small intestine. However, considering the unique expression patterns of CCR10 and CCL28, CCR10 likely has other functions that are distinct from those of CCR9.

Using a novel strain of CCR10-knockout/EGFP-knockin mice generated in our laboratory [56], we recently discovered several critical roles of CCR10 in intestinal IgA response and homeostasis [39]. We found that in CCR10-knockout/EGFP-knockin mice, enhanced generation of IgA⁺ cells in ILFs compensated for the defective migration of CCR10-deficient IgA⁺ cells into the intestine [39]. However, the compensatorily generated IgA-ASCs are qualitatively different from IgA-ASCs of wild type mice, likely because they are generated in a T cell-independent process [13, 39]. Correlating with this, CCR10-knockout/EGFP-knockin mice have increased amounts of commensal bacteria in the large intestine [39]. In addition, these mice have a profoundly defective intestinal IgA memory response to pathogen infection due to the impaired maintenance of long-lived IgA-ASCs and IgA⁺ memory B cells in the intestines [39].

In addition to the two chemokine receptors, the adhesion molecule $\alpha 4\beta 7$ integrin is expressed on intestine-homing IgA⁺ cells. Through the interaction with its ligand mucosal addressin cell adhesion molecule-1 (MAdCAM-1) expressed on postcapillary venules of the small and large intestines, $\alpha 4\beta 7$ helps the homing of IgA⁺ plasmablasts to the intestines [57] (Fig. 1).

Regulation of gut-homing molecules on IgA⁺ cells

DCs and T cells of the GALT imprint intestine-homing properties on newly generated IgA⁺ plasmablasts by secreting specific factors [58]. Among those factors, retinoic acid (RA), a metabolite of Vitamin A, is capable of inducing the expression of $\alpha 4\beta 7$ and CCR9 on IgA⁺ plasmablasts [58] (Fig. 1). Consistent with this, RA, when included with antigens in the skin immunization, can trigger the generation of gut-homing IgA⁺ plasmablasts in the skin-draining lymph nodes and enhance levels of antigen-specific IgA antibodies in the intestinal lumen of immunized mice [59]. RA is generated from vitamin A by retinal dehydrogenases, which are highly expressed by DCs of PPs and MLNs but not some other lymphoid organs such as spleens or inguinal peripheral lymphoid nodes [58, 60-62].

DCs of GALT were also involved in promoting CCR10 expression on IgA⁺ plasmablasts. In an *in vitro* study of human B cells, RA increased the percentage of CCR10⁺ IgA⁺ cells generated from naïve B cells in the presence of IL-21 [36]. In addition, 1,25-Dihydroxyvitamin D3, the active metabolite of Vitamin D, dramatically increased the

proportion of CD19⁺IgD⁻CD38⁺ cells expressing high levels of CCR10 that were generated from naïve human B cells [63]. It was suggested that 1,25-Dihydroxyvitamin D3 activates Vitamin D receptors, which then bind to a Vitamin D response element in the promoter region of the human CCR10 gene for inducible expression of CCR10. Interestingly, DCs of the CPs induce a higher CCR10 expression on IgA⁺ cells than DCs of the PPs do in mice while they induced similar CCR9 expression [14]. Whether the differential capacities of DCs of CPs and PPs in the induction of CCR10 expression are due to their differential expression of RA and 1,25-Dihydroxyvitamin D3 is not known. In addition, Vitamin D did not induce the CCR10 expression on mouse IgA⁺ cells *in vitro* [63, 64]. While an explanation for this is that the promoter region of the mouse CCR10 gene does not contain a Vitamin D response element [63, 64], whether Vitamin D plays a role in the expression of CCR10 *in vivo* has yet to be elucidated.

Several cytokines derived from Tfh cells were identified to induce the expression of intestine-homing molecules on IgA⁺ plasmablasts. *In vitro* studies found TGF-β1 and IL-21, two cytokines important in the generation of IgA⁺ plasmablasts, also downregulated CXCR5 and upregulated CCR10 on human IgA⁺ plasmablasts, suggesting their role in enabling exit of IgA⁺ plasmablasts from germinal centers and migration into the intestinal mucosa [36]. Whether the ability to induce gut-homing properties of IgA⁺ plasmablasts is unique to Tfh cells of GALT is not clear.

Differential regulation of migration and responses of IgA⁺ plasmablasts in the small and large intestines

Considering the differential expression of chemokines by the small and large intestines, regulation of the migration and localization of IgA⁺ plasmablasts into the small and large intestines is likely different. Our analysis of mice found that a significant percentage of IgA⁺ cells of the small intestines co-expressed CCR9 and CCR10 while IgA⁺ cells of the large intestines express only CCR10 (unpublished observations)(Fig. 1). In addition, compared to wild type mice, CCR10-knockout mice had more severely impaired migration of IgA⁺ cells into the large intestines than into the small intestines [39]. Appendectomy reduced IgA⁺ cells in the large but not the small intestines, likely because IgA⁺ cells generated in the CPs of the appendix predominantly contribute to the large intestine [14].

Types of antigens and their routes of stimulations are also important factors in regulating IgA responses in the small and large intestines. It was recently reported that while the inoculation of germ-free (GF) mice with *Bacteroides acidifaciens* or *Lactobacillus johnsonii* induced same levels of IgA production in the small intestine, the *Bacteroides acidifaciens*-associated mice had significantly higher levels of IgA production in the large intestine [65]. In another study, SFB induced a lower frequency of IgA⁺ cells but they stimulated development of ILFs more efficiently than nonpathogenic *E. coli* [66]. These studies indicate that different microorganisms use different pathways to induce intestinal IgA responses. In addition, IgA⁺ plasma cells generated from different immunization routes could migrate into the small and large intestines using different homing molecules. Intra-rectal immunization with protein antigens induced generation of IgA⁺ plasmablasts capable of homing into both the small and large intestines [67]. However, migration into the small

intestines was found to be independent of CCR9/CCR10 and instead dependent on $\alpha 4\beta 7$. In contrast, IgA⁺ plasmablasts induced by intra-nasal immunization expressed low levels of $\alpha 4\beta 7$ and were usually excluded from the gut. However, intra-nasal immunization increased Ag-specific IgA⁺ cells in the small intestine of $\beta 7$ -knockout mice, demonstrating that intestinal homing of IgA⁺ plasmablasts is a competitive process and that $\alpha 4\beta 7$ determines not only the intestinal localization of IgA⁺ plasmablasts generated in GALT but also the intestinal exclusion of lymphocytes primed in other inductive sites [67]. Further research is required to fully understand the molecular mechanisms underlying the regulation of differential expression of the small and large intestine homing molecules.

Maintenance of IgA-ASCs and establishment of IgA⁺ memory

Considering that immune reactivity at mucosal sites is critical for local control of pathogens and prevention of their spreading, understanding how IgA-ASCs and memory B cells are maintained in the intestine has significant implications in development of vaccines against many medically important pathogens that infect through the intestines. Recent studies have revealed that the maintenance of intestinal IgA-ASCs is uniquely different from that of plasma cells producing other isotypes of antibodies [23, 68], and have begun to shed light on mechanisms of long-term intestinal IgA-ASCs maintenance and IgA memory.

IgA-ASCs are long-lived in the intestinal environment

Recent studies in both mice and humans found that IgA-ASCs could survive in intestinal tissues for extended periods, even in the absence of antigen stimulations. In one study, the temporary colonization of germ-free mice with a strain of *E. coli* that were unable to replicate resulted in the generation of bacterium-specific IgA-ASCs that last for months without sign of receding after bacteria clearance. This suggests that IgA-ASCs generated against the bacteria are long-lived cells [23]. However, when immunized mice were re-colonized with different strains of bacteria, newly generated IgA-ASCs gradually became dominant, indicating that the maintenance of the IgA-ASCs repertoire in the intestines is modulated by the current status of commensal bacteria. Therefore, while IgA-ASCs are capable of long-term survival, their presence in the intestine would be restricted from the competition of newly formed IgA-ASCs in response to the current commensal stimulation. In an *ex vivo* culture study of human small intestines, IgA-secreting CD27⁺CD138⁺ plasma cells and their production of IgA antibodies could be sustained for more than four weeks [24]. One recent sequence analysis study of IgA variable heavy chain genes from human ileal biopsies found the occurrence of many clonally related sequences, suggesting a local expansion of IgA precursor cells, most likely IgA⁺ plasmablasts [69]. A similar murine IgA sequence study also found evidence for expanded clones of IgA-ASCs [70]. In addition, the IgA repertoire of an individual mouse was stable and could be recalled after a temporary depletion of IgA-ASCs from the intestine [70], indicative of functional IgA memory. However, the origin of this memory is not clear. Whether IgA memory towards pathogenic infection is regulated in the same way as the memory to commensal bacteria is unclear as well.

IgA⁺ memory B cells in intestinal IgA response

In addition to long-lived IgA-ASCs, IgA⁺ memory B cells are important in memory maintenance and response. Whether IgA memory B cells represent a major component of intestinal memory during pathogen infection remains a debated topic. In mouse studies using oral immunization of cholera toxin to induce intestinal IgA responses, it was found that the IgA memory response to cholera toxin re-challenge was mediated by memory B cells but not by long-lived IgA-ASCs [71-73]. A similar argument has been proposed to describe the IgA memory response to pathogen infections [39, 74]. Memory B cells also contribute significantly to IgA memory responses in humans immunized with an oral cholera vaccine [75]. However, it was recently reported that repeated immunization with the same strain of commensal bacteria only had an additive effect on the generation of IgA-ASCs, which argue against IgA memory response from these types of infections [23]. There are several possible explanations to account for this difference. First, immunization with commensal bacteria is different from that of protein antigens or pathogens. Second, the presence of previously formed IgA-ASCs and antibodies specific to the commensal bacteria is sufficient to suppress their invasion so that no strong memory IgA response is evoked. This would not be the case if a pathogenic infection is reintroduced. Third, it could be argued that a T cell-independent IgA response might be predominantly promoted by immunization with the bacteria, while oral immunization with T cell dependent antigens would induce more efficient memory B cell development [39, 76].

Molecular factors involved in the maintenance of intestinal IgA memory

Considering that IgA-ASCs and memory B cells have distinct properties than other types of plasma and memory B cells, molecular factors involved in their intestinal maintenance and regulation are likely different. Besides molecules commonly involved in the survival and proliferation of plasma and memory cells, there are also several unique requirements for efficient maintenance of IgA⁺ ASCs and memory B cells in the intestines. Among the common survival factors, a proliferation-inducing ligand (APRIL) and its receptor B cell maturation antigen (BCMA) are involved in the maintenance of IgA-ASCs and plasma cells of other isotypes [24, 77-79]. In ex vivo culture studies of human intestinal sections, IgA antibody production and plasma cell survival were also found to depend on IL-6 [24]. However, these factors do not explain unique intestinal maintenance requirements of IgA-ASCs or IgA⁺ memory B cells.

Our lab recently identified CCR10 as an important regulator of the intestinal maintenance of IgA-ASCs and memory B cells [39]. In CCR10-knockout mice infected with the intestinal bacterial pathogen *Citrobacter rodentium*, pathogen-specific long-lived IgA-producing plasma cells and IgA⁺ memory B cells were not properly maintained in the intestines, and the IgA memory response to pathogen reinfection was severely impaired. Additionally, we found that while IgA⁺ memory B cells of spleens did not express CCR10, IgA⁺ memory B cells isolated from the intestine did, suggesting that CCR10, through interaction with its mucosal ligand CCL28, may be directly involved in their maintenance in the intestine. Relating to this, earlier studies of murine rotavirus infection found that rotavirus-specific memory B cells expressed $\alpha 4\beta 7$, a molecule important for the efficient migration of memory B cells into the intestines [74]. IgA-ASCs were recently reported to express a functional

membrane B cell receptor (BCR) capable of inducing calcium mobilization and phosphorylation of extracellular signal-regulated kinase 1/2 and serine/threonine kinase AKT important for the survival of IgA-ASCs, suggesting that the maintenance of IgA memory is also modulated by specific antigens [80].

Acknowledgments

The work was partly supported by a grant from National Institute of Health (NIH) and a institutional funds of the Pennsylvania State University (to Na Xiong). We thank Micha Davila for editorial help.

Abbreviations

APRIL	A proliferation-inducing ligand
AID	Activation-induced cytidine deaminase
BAFF	B cell activating factor
BCMA	B cell maturation antigen
BCR	B cell receptor
CP	Caecal patch
CT	cholera toxin
CSR	Class switch recombination
DC	Dendritic cell
Tfh	follicular T help
Tfr	follicular regulatory T
GALT	Gut-associated lymphoid tissue
GF	germ-free
IgA	Immunoglobulin A
IgA-ASC	IgA antibody-secreting plasma cell
IgH	immunoglobulin heavy chain
ILC	Innate lymphoid cell
ILF	Isolated lymphoid follicle
LP	Lamina propria
LTi	Lymphoid tissue inducer
MLN	mesenteric lymph node
PD-1	programmed cell death-1
pDC	plasmacytoid dendritic cell
pIgR	polymeric immunoglobulin receptor
PP	Peyer's patch

RAG	Recombinase-activating gene
RA	retinoic acid
TGF-β1	transforming growth factor β 1
TH17	T helper 17
Treg	Regulatory T
SIgA	Secretory IgA
SFB	Segmented filamentous bacteria
SHM	Somatic hypermutation
TCR	T cell receptor

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Future perspective

Studies of past years have made significant progress towards understanding roles of IgA antibodies in regulation of intestinal homeostasis of commensals and the immune system. In addition, cellular and molecular factors involved in the differentiation and localization of IgA-producing cells in the intestinal mucosa are also better elucidated. However, many important questions remain to be addressed. Much of the current understanding of regulation of IgA responses focuses on their generation and maintenance under homeostatic conditions in relationship with stimulations of commensal bacteria. Whether IgA responses against intestinal pathogen infection under inflammatory conditions are regulated in a similar fashion is not well understood. In addition, while the intestinal mucosa were found to be able to support the long-term survival of IgA-ASCs, how the continuous stimulation from commensal bacteria and food-born antigens affects the presence of long-term maintenance of IgA-ASCs against intestinal pathogens needs to be investigated further. Are there any anatomic niches in the intestine and associated lymphoid organs for long-lived IgA-ASCs and memory B cells? What are cellular and molecular mechanisms for the establishment and maintenance of IgA memory in the intestine against pathogens infections. Considering important roles of IgA antibodies in prevention of mucosal infection, understanding these questions will help to design better vaccination strategies against pathogen infections.

Figure 1.

Schematic illustration of major cell types and molecular factors involved in regulation and generation of IgA⁺ cells and their migration and maintenance in the GALT and intestines. PP: Peyer's patch, ILF: Isolated lymphoid follicle, CP: Caecal patch, CSR: Class switch recombination, SHM: Somatic hypermutation, DC: Dendritic cell, ILC2: Type 2 innate lymphoid cell, Tfh: follicular T help (cell), Tfr: follicular regulatory T (cell), RA: retinoic acid, PD-1: programmed cell death-1 (molecule).