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Differentiation and homing of IgA-secreting cells

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Most antibody-secreting cells (ASCs) in mucosal tissues produce immunoglobulin A (IgA), the most abundant immunoglobulin in the body and the main class of antibody found in secretions. IgA-ASCs differentiate in the mucosal-associated lymphoid tissues and are usually considered as a homogeneous population of cells. However, IgA-ASCs that travel to the small intestine have unique characteristics in terms of their migratory requirements. These IgA-ASCs require the homing molecules $\alpha 4\beta 7$ and CCR9 to interact with their ligands, mucosal addressin cell adhesion molecule-1 and CCL25, which are constitutively expressed in the small intestine. Indeed, recent work has shown that IgA-ASCs specific for the small bowel are generated under different conditions as compared with IgA-ASCs in other mucosal compartments. Moreover, the mechanisms inducing IgA class switching may also vary according to the tissue where IgA-ASCs differentiate. Here we describe the mechanisms involved in the differentiation of IgA-ASCs in mucosal compartments, in particular those involved in the generation of gut-homing IgA-ASCs.

Immunoglobulin A (IgA) is the most abundant immunoglobulin isotype produced in the body and it is estimated that around 80% of all IgA-antibody-secreting cells (IgA-ASCs) reside in the gut mucosa.[1,2] The importance of secretory IgA is evidenced by the fact that mice deficient in the J-chain, which produce only monomeric IgA that cannot be secreted, show markedly decreased protection from intestinal toxins.[3] Similarly, mice that cannot secrete antibodies because they lack the polymeric Ig receptor (pIgR or secretory component) exhibit a higher susceptibility to primary *Salmonella* infections,[4] although they have normal recall responses.[5] However, other studies using mice deficient in IgA show that they can efficiently mount protective immune responses against respiratory and gastrointestinal mucosal pathogens.[6,7] Therefore, in spite of its massive production in mucosal tissues, the physiological significance of IgA is still being debated. Nonetheless, it has been proposed that, aside from its role in neutralizing toxins and protecting against pathogenic infections, IgA, owing to its lack of complement-activating capacity and its binding to the inhibitory FcαRI receptor, may play a role as a noninflammatory mechanism of "immune exclusion" in the gut.[8–10] In this way, IgA may help to prevent local inflammation and systemic immune responses triggered by innocuous antigens and/or commensal flora.[2,9,11] In fact, although isolated IgA deficiency in humans translates into only a mild and often asymptomatic primary immunodeficiency, it has been associated with autoimmune pathologies such as celiac disease.[12]

On the other hand, the migration of B cells and ASCs to the gut is critical for conferring protection against intestinal pathogens.[7,13–16] B cells that lack gut-homing capacity confer poor protection against intestinal rotavirus infection, whereas gut-homing B cells from IgA knockout mice are as protective as wild-type cells.[7] The latter example suggests that, at least for some infections, the capacity of ASCs to migrate to the gut may be more important than their capacity to secrete IgA in order to confer protective immune responses.

In this review, we will discuss our current understanding of the mechanisms imprinting tissue-specific migration onto activated B cells as well as their relationship and potential intersection with the complex mechanisms involved in the differentiation of IgA-ASCs in mucosal tissues.

HOMING OF NAIVE B CELLS

Naive B lymphocytes migrate to secondary lymphoid organs (lymph nodes, Peyer's patches (PPs), spleen), where they are activated by their cognate antigen.[17] Naive B cells use L-selectin (CD62L) to tether and roll on specialized postcapillary microvessels known as high endothelial venules (HEVs), which are found in peripheral lymph nodes (PLNs), mesenteric lymph nodes (MLNs), and PPs[18,19] (**Figure 1**). HEVs in PLNs and MLNs express L-selectin ligands, collectively known as peripheral node addressin.[20–22] In addition, the chemokines CCL21/SLC and CCL19/ELC are presented in HEVs of LN and PPs and they bind to CCR7 expressed on naive B cells,[23–26]

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Received 2 November 2007; accepted 21 November 2007; published online 23 January 2008.doi:10.1038/mi.2007.14

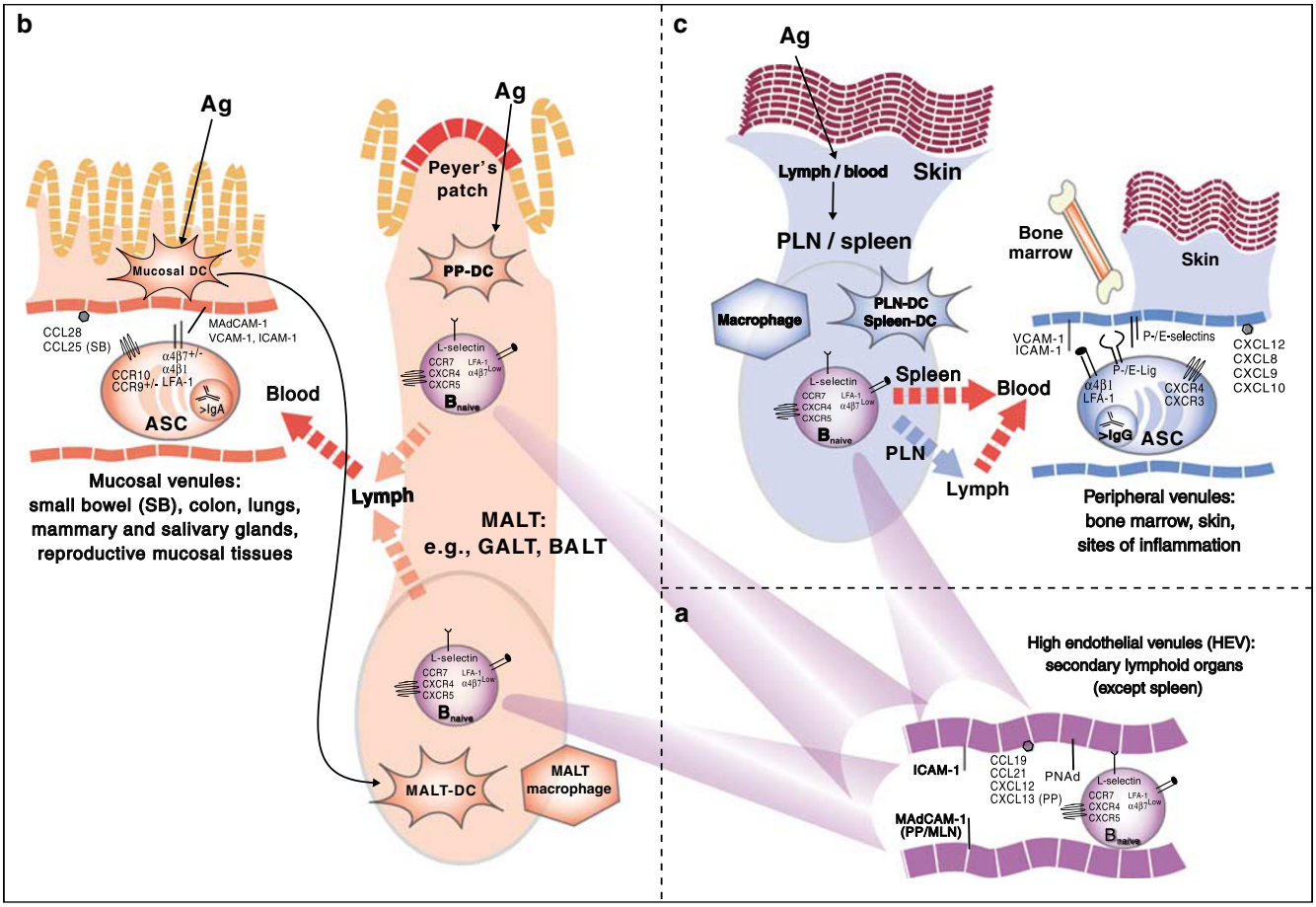

Figure 1 Homing of naive and antigen-experienced B cells. (**a**) Naive B cells access MLNs, PPs, other MALTs, and PLNs through HEVs. DCs and/or subcapsular macrophages interact with and present antigens (in their native conformation) to naive B cells *in vivo*. Also, B cells are likely to require DCs to differentiate efficiently into ASCs. (**b**) DCs in PPs and MLNs present orally administered antigens to B cells, inducing their differentiation into ASC-expressing the gut-homing receptors $\alpha 4\beta 7$ and CCR9. Furthermore, ASCs migrating to all mucosal compartments express CCR10, and the CCR10 ligand MEC/CCL28 is expressed in all mucosal tissues. In addition, B cells activated in PPs, MLNs, and other MALTs can become IgA-ASCs. (**c**) If B cells are activated in peripheral nonmucosal lymphoid tissues (such as PLNs or spleen), they do not upregulate gut-homing receptors or switch to IgA. Instead, they home to the bone marrow, skin, and sites of inflammation and switch to other immunoglobulin isotypes, such as IgG. Once B cells are activated in the lymphoid organs, the resulting B_{Mem} and ASCs leave the lymphoid tissues via lymphatics (except in the spleen where they go directly to the blood), reach the blood, and home to the respective peripheral tissues through postcapillary venules, which express the corresponding tissue-specific ligands (addressins). Ag, antigen; ASC, antibody-secreting cell; B_{Mem}, memory B cell; BALT, bronchus-associated lymphoid tissue; DC, dentritic cell; GALT, gut-associated lymphoid tissue; HEV, high endothelial venule; MALT, mucosal-associated lymphoid tissue; MLN, mesenteric lymph node; PLN, peripheral lymph node; PP, Peyer's patch.

triggering the activation of integrins LFA-1 and/or $\alpha 4\beta 7$ and lymphocyte arrest in HEVs.[18,19,27] Naive B cells can also use CXCR4 for integrin activation; the CXCR4 ligand CXCL12/SDF1a is presented in PLN-HEVs.[25] In addition, the CXCR5–CXCL13 pathway can also support naive B cell homing.[25,28]

In PPs, HEVs do not express peripheral node addressin in the lumen.[21] Instead, they express high levels of MAdCAM-1 (mucosal addressin cell adhesion molecule-1).[29] MAdCAM-1 in PPs can interact with both L-selectin and $\alpha 4\beta 7$.[30,31] The $\alpha 4\beta 7$ integrin is expressed at low levels in naive B cells but plays an important role in lymphocyte migration into PPs and MLNs.[18,27,32] MAdCAM-1 on PP-HEVs contributes to both rolling and sticking in these organs.[18,31,33] The firm arrest of naive B cells in PP HEV is mediated by LFA-1 and $\alpha 4\beta 7$, which are activated by CCL21-CCR7.[18,23] In addition, as in PLNs, naive B cells can also use CXCR4 and CXCR5 for integrin activation in PPs.[25]

High endothelial venules in MLNs express a mosaic of both peripheral node addressin[21] and MAdCAM-1,[29] thus supporting both the PLN-like and PP-like adhesion cascades for lymphocyte homing.[21]

HOMING OF MUCOSAL ANTIBODY-SECRETING CELLS

When conventional B cells (B2) are activated by T cell-dependent (TD) antigens, i.e., antigens that elicit concomitant "helper" CD4 T-cell responses (usually proteins), they become either ASCs with no proliferative capacity (plasma cells) or memory B cells (B_{Mem}), which can proliferate and give rise to ASCs (and more B_{Mem}) upon reactivation[34] (**Figure 2**). In addition, B cells responding to TD antigens participate in germinal center reactions, where they undergo affinity maturation and immuno-

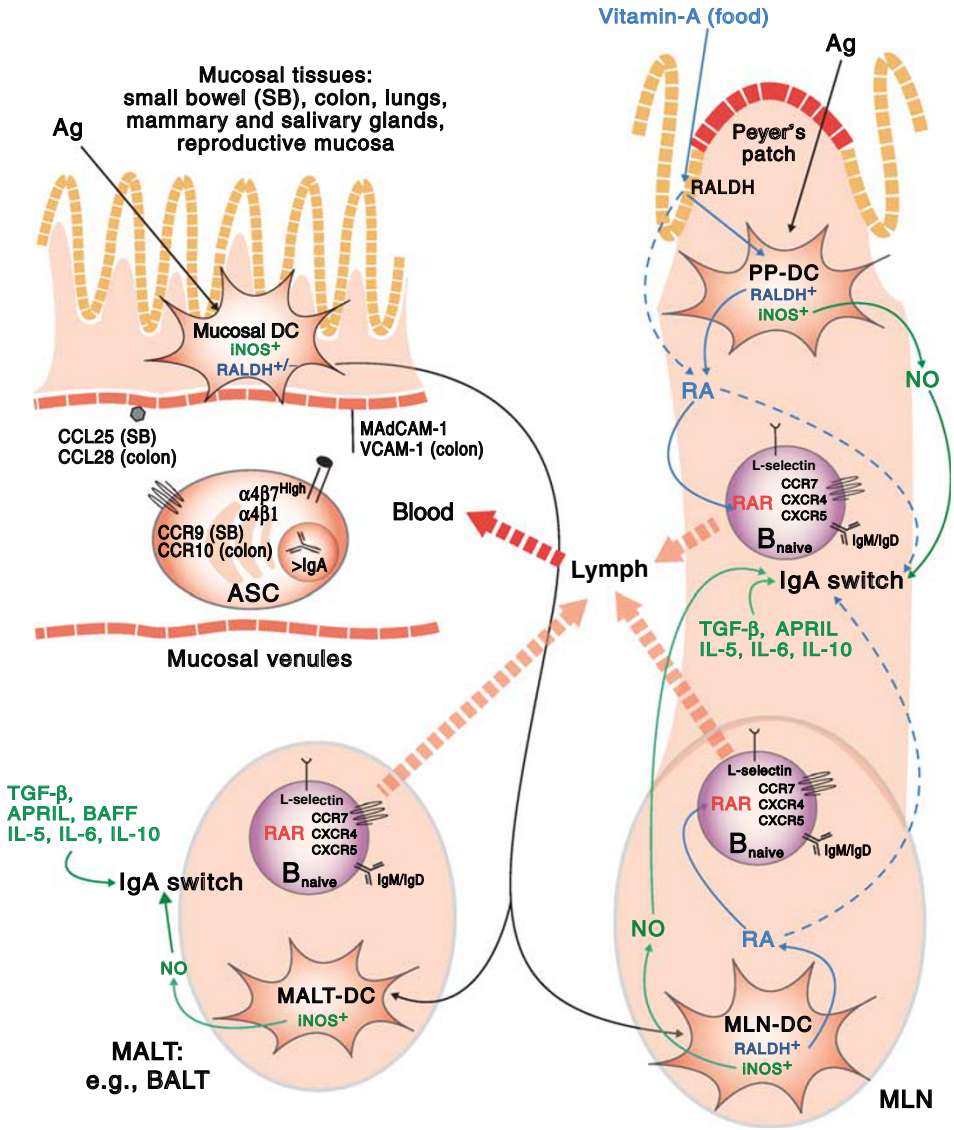

Figure 2 Activation of B cells in MALTs. DCs in peripheral mucosal tissues capture antigens and migrate to their respective draining lymph nodes (MALTs). Intestinal epithelial cells (IEC), as well as DCs in PPs and MLNs (GALT), express retinal dehydrogenase (RALDH) enzymes and therefore have the capacity to metabolize vitamin A from the diet into retinoic acid (RA). RA induces the expression of $\alpha 4\beta 7$ and CCR9 in ASCs and probably also in B_{Mem}, endowing them with the capacity to home to the small bowel. In addition, ASCs migrating to mucosal tissues express CCR10, and the CCR10 ligand MEC/CCL28 is expressed in all mucosal compartments. However, it is unknown where, when, and how CCR10 is upregulated on mucosal ASCs. In addition, B cells activated in the MALT microenvironment can switch to IgA-ASCs. IgA responses to TD antigens (e.g., proteins) require CD4 T-cell help and also TGF-β1. The cytokines IL-5, IL-6, and IL-10 have also been reported as important for IgA secretion, although they are probably not directly involved in IgA class switching. IgA responses to TI antigens type 1 (e.g., LPS) or type 2 (e.g., bacterial capsule polysaccharides) do not require CD40-CD40L but rely on the cytokine APRIL, which are produced by activated DCs and macrophages. It is noteworthy that the enzyme iNOS, which synthesizes NO, is specifically expressed in MALT-DCs and is critical for both TD and TI IgA responses. In addition, RA also plays a role in promoting the generation of IgA-ASCs in GALT. APRIL, a proliferation-inducing ligand; ASC, antibody-secreting cells; B_{Mem}, memory B cells; DC, dentritic cell; GALT, gut-associated lymphoid tissue; IEC, intestinal epithelial cell; LPS, lipopolysaccharide; MALT, mucosal-associated lymphoid tissue; NO, nitric oxide ; RA, retinoic acid; RALDH, retinal dehydrogenase; TD, thymus-dependent; TI, thymus-independent.

globulin class switching.[34] On the other hand, B cells can be activated by T cell-independent (TI) antigens, either type 1 (polyclonal activators, such as lipopolysaccharide (LPS), CpG, poli-IC) or type 2 (polysaccharides, such as capsular bacterial polysaccharides), and become short-lived IgM-ASCs, except in the peritoneal cavity and the gut mucosa, tissues that can also support TI IgA responses.[34]

Some ASCs producing IgM or IgG (and, to a lesser extent, IgE or IgA) stay in the lymph node medulla or spleen red pulp as short-lived ASCs.[35] However, most ASCs leave lymphoid tissues and travel to the bone marrow, where a small fraction becomes long-lived plasma cells.[35,36] CXCR4 and probably also $\alpha 4\beta 1$, LFA-1, P-selectin ligands, and CD22 are important for ASC lodging retention and/or survival in the bone marrow.[37–39]

Some ASCs also home to sites of inflammation; this migration event is probably mediated by CXCR3.[35]

Peyer's patches are the main secondary lymphoid organs where B cells differentiate into IgA-ASCs,[40] although IgA-ASCs can also differentiate in MLNs and the peritoneal cavity[2] (**Figure 2**). That IgA-ASCs migrate preferentially to the gut was originally described more than 30 years ago.[41–43] Although the presence of the specific antigen in the target tissue may play a role in the accumulation of IgA-ASCs in the gut (especially in late stages), their initial accumulation in the intestinal lamina propria is clearly antigen-independent, suggesting that it is regulated at the level of cell entry.[44,45] Indeed, intestinal ASCs express $\alpha4\beta7$[46] and this integrin (as well as its ligand MAdCAM-1) is critical for the homing of IgA-ASCs to the gut.[32] In addition, the chemokine receptor CCR10 is expressed on all ASCs located in mucosal compartments,[36,47–49] and the CCR10 ligand CCL28/MEC is expressed by most mucosal epithelial cells[48,50] and selectively attracts IgA-ASCs.[49,51] In fact, IgA-ASCs require CCL28 to home efficiently to the colon lamina propria.[52] To add even more complexity, a subset of IgA-ASCs also responds to the CCR9 ligand CCL25/TECK,[53] and ASCs require CCR9 to home to the small intestine, at least in some experimental settings.[52,54] It is noteworthy that although one report suggested that IgA-ASCs need both CCR9 and CCR10 for optimal migration to the small bowel,[52] another study showed that CCR9 and CCR10 play redundant roles in IgA-ASC homing to the small intestine during rotavirus infection.[55] The reasons for these discrepancies remain to be determined, although it is possible that inflammation upregulates CCL28 in the small bowel, thus making the homing of ASCs less dependent on CCR9.

Homing of IgA-ASCs to the intestinal lamina propria offers an efficient mechanism for locally delivering dimeric IgA that binds to the pIgR expressed on intestinal epithelial cells (IECs) to be finally secreted into the gut lumen. However, it should be considered that serum dimeric IgA can also bind directly to pIgR expressed on hepatocytes, transported to the bile ducts, and secreted via the bile into the gut lumen.[56] Such a mechanism may, in principle, decrease the relative importance of gut homing on intestinal IgA secretion. However, although hepatobiliary IgA transport is very efficient in mice and rats, it does not play a significant role in humans, which is probably due to species differences in the cellular localization and availability of pIgR, as well as the relative paucity of dimeric IgA in human serum, as compared to mice.[56,57] Nevertheless, in spite of this potentially compensatory mechanism, memory B cells from $\beta7$ integrin-deficient mice exhibit a decreased capacity to confer protection against rotavirus infections,[7] implying that gut homing plays an important role in protective gut IgA responses.

T cell-dependent B-cell responses also generate long-lived B_{Mem}.[34,58] It is thought that B_{Mem} recirculate through both lymphoid and peripheral tissues, but their migratory behavior has been difficult to study owing to their physiologically low abundance and lack of specific markers in mice. However, B_{Mem} conferring protection against intestinal pathogens express $\alpha4\beta7$, indicating that these cells, like their T-cell counterparts, respond to tissue-specific imprinting signals.[15,16] It is noteworthy that IgA-ASCs have a short half-life in the intestinal mucosa, at least in the steady state,[59] and whether there are long-lived mucosal plasma cells is still an open question.[60] Thus, it is likely that IgA-ASCs need to be constantly replenished in the gut by recently activated naive B cells or B_{Mem}.

MECHANISMS OF IMPRINTING OF GUT-HOMING B CELLS AND ASC

A great deal of evidence indicates that the tissue where the antigen is encountered influences the traffic pattern that lymphocytes acquire. For example, pathogens entering through the skin preferentially generate B and T lymphocytes with skin-homing receptors.[61–65] Analogously, oral vaccination induces higher levels of the gut-homing integrin $\alpha4\beta7$ on B and T cells than parenteral administration of the same antigen[13,14,66–73] (**Figure 2**). The functional relevance of this observation is suggested by the fact that among B_{Mem}, only $\alpha4\beta7^{+}$ (but not $\alpha4\beta7^{Neg}$) cells confer protection against intestinal rotavirus,[7,15] indicating that gut tropism is required for immune defense against at least some tissue-restricted pathogens.

In the lymphoid microenvironment, dentritic cells (DCs) are essential for efficient T-cell activation.[74,75] However, DCs can also influence B-cell responses by enhancing their differentiation to ASCs and their survival.[76,77] It has also been shown that DCs can present unprocessed antigens to B cells *in vivo*[78–80] and affect B-cell function in a tissue-specific fashion. For instance, PP-DCs promote class switching to IgA by activated B cells.[81–83] Several reports have shown that DCs from PPs and MLNs (gut-associated lymphoid tissue (GALT)-DCs) are sufficient to induce $\alpha4\beta7$ and CCR9 and gut-homing capacity on activated T cells.[84–89] We have recently shown that, analogously, GALT-DCs but not DCs from other lymphoid organs imprint gut homing on activated B cells[90] (**Figure 2**). Moreover, work from several groups has recently shown that subcapsular macrophages can also present lymph-borne antigens and activate naive B cells in PLNs.[91–93] It will be interesting to determine whether a similar mechanism operates in MLNs and whether subcapsular macrophages can also imprint tissue-specific homing and/or promote specific IgA class switching in that tissue. In support of this possibility, macrophages can secrete B-cell activating factor (BAFF),[76] which can induce TI IgA class switching. Furthermore, intestinal lamina propria macrophages were reported to secrete retinoic acid (RA),[94] which is sufficient to imprint gut homing on activated B cells[90] (see below).

What is the molecular mechanism controlling gut homing? Twenty-five years ago, it was shown that malnourished vitamin A-deficient rats exhibit impaired migration of recently activated mesenteric lymphocytes to the intestinal mucosa.[95] More recently, it was described that vitamin A-deficient rats show a marked decrease in the number of IgA-ASCs and CD4 T cells in the ileum.[96] However, the molecular basis for these observations was determined only recently in a seminal study by Iwata *et al.* that showed that mice depleted of vitamin A have a dramatic reduction in the number of effector/memory T cells in the gut mucosa but not elsewhere.[97] Concomitant *in vitro* experiments

showed that the vitamin A metabolite retinoic acid (RA) is sufficient to induce $\alpha4\beta7$ and CCR9 on activated T cells, even in the absence of DCs.[97] In agreement with a major role for RA in gut-homing imprinting, blocking RA receptors of the RAR family significantly decreased the induction of $\alpha4\beta7$ by GALT-DCs.[97] Consistently, GALT-DCs, unlike DCs from other tissues, express retinal dehydrogenases (RALDH), which are essential enzymes for RA biosynthesis.[97]

Although GALT-DCs are sufficient to induce gut-homing molecules on lymphocytes *in vitro*, it is formally possible that other sources of RA may contribute *in vivo*. For example, IECs express RALDH[97] and can synthesize RA *in vitro*.[98] Also, IECs, and perhaps other cells in the gut, may act as "RA donors" for DCs. In fact, it has been described recently that RA can be passively stored by DCs and used to imprint gut-homing lymphocytes.[99] Moreover, IP immunization efficiently generates gut-homing T cells,[86,100,101] and recently activated B cells (plasmablasts) may also be imprinted with gut tropism in the peritoneal cavity.[102] Thus, it is possible that gut-homing lymphocytes can be imprinted in regions other than GALT. Indeed, some nonintestinal viral infections also induce $\alpha4\beta7^+$ T cells[103] and there may be constitutive sources of RA in the lung mucosa[104] and liver.[105] Also, it remains to be determined whether RA synthesis can be induced in nonintestinal tissues under inflammatory or infectious conditions and whether there are additional RA-independent mechanisms of gut-homing imprinting.[106]

It is also unknown how IgA-ASCs are imprinted to home to mucosal compartments other than the small bowel. CCR10 plays a role in IgA-ASC homing to colon,[52] mammary glands,[47] and probably other mucosal compartments,[49] but this chemokine receptor has not been reported to be upregulated in murine B cells *in vivo* or *in vitro*, so it is currently unknown where and how CCR10 is induced.

Peritoneal B1 B cells can also give rise to intestinal IgA-ASCs, although the extent of their contribution remains controversial, ranging from 1 to 50% of all intestinal lamina propria IgA-ASCs, depending on the experimental system and readout. Earlier studies showed that almost half of the IgA-ASCs arise from peritoneal B1 B cells.[107,108] Consistently, reconstitution experiments with bone marrow from T cell-deficient mice and peritoneal cavity B1 B cells, as well as mice deficient in B1 B cells, showed that virtually all intestinal lamina propria IgA-ASCs and secretory IgA against commensal flora were of B1 origin, suggesting that these cells account for most of the TI IgA responses, at least in the absence of T cells.[109] However, another study showed that, in gnotobiotic mice, conventional B2 B cells contribute most of the bacteria-specific intestinal IgA.[110] Moreover, in humans, peritoneal B1 B cells do not seem to be a significant source of lamina propria (LP) IgA-ASCs.[111] Thus, the relative contribution of B1 B cells to the pool of intestinal IgA-ASCs and their relevance to gut immunity remain to be determined.

It is also unknown which traffic molecules B1 cells need to home to the gut mucosa.[112] It is likely that B1-derived ASCs rely, at least in part, on the same homing imprinting mechanisms as B2-derived IgA-ASCs. A recent study showed that the peritoneal cavity environment can "instruct" B1 and B2 B cells to home back to the peritoneum via induction of CXCR5 expression,[102] which is critical for lodging of B1 B cells in body cavities.[113] Interestingly, the peritoneal environment also imprinted gut-homing capacity and IgA class switching/secretion on plasmablasts.[102] It remains to be determined whether this "peritoneal imprinting" relies on similar mechanisms such as GALT imprinting. In addition, TLR ligands also play an important role during peritoneal B1 B-cell mobilization by transiently downregulating integrin expression on B1 cells.[114]

Can B cells change or modulate their homing potential? We and others have shown that gut- or skin-homing T cells can be "reprogrammed" in their migratory potential. Skin-homing T cells can be converted to gut-homing T cells and vice versa if they are stimulated with or without GALT-DCs or RA, respectively.[88,89] Similarly, human B cells may also exhibit homing plasticity,[73] and we have shown that they can be re-educated and acquire or lose gut-homing potential when they are restimulated with or without RA, respectively[90] (**Figure 3**). Given that terminally differentiated plasma cells do not divide, it is likely that homing plasticity occurs at the level of B_{Mem}. Also, B_{Mem} expressing either IgM or another immunoglobulin isotype different from IgA may switch to IgA when reactivated in mucosal-associated lymphoid tissues (MALTs). In fact, sequential immunoglobulin switching from IgG2b to IgA or, in humans, from IgA1 to IgA2, has been described.[115] On the other hand, IgA^+ B_{Mem} remain IgA^+ regardless of where they are reactivated, although, given their homing plasticity,[90] they probably lose their gut-tropic capacity when reactivated in a nonintestinal context outside GALT.

DIFFERENTIATION OF IgA-ASC IN MUCOSAL TISSUES: MALTS VS. GALT

Peyer's patches were the first secondary lymphoid organs proposed as an inductive site for IgA-ASCs.[40] Hallmark molecular markers of immunoglobulin class switching, such as activation-induced cytidine deaminase (AID, essential for immunoglobulin class switching[116]), and specific markers for IgA class switching, such as germline alpha transcripts and postswitch circular DNA transcripts,[117] are found at much higher levels in PPs as compared to MLNs and the peritoneal cavity.[118] In addition, IgA class switching is also higher in PPs as compared to the nasal-associated lymphoid tissues or bronchus-associated lymphoid tissues.[119] It is noteworthy that IgA class switching has also been reported in human and murine colon lamina propria[115,120] as well as in murine small intestinal lamina propria.[121] However, other studies have failed to find molecular markers of IgA class switching in the small intestinal lamina propria of mice or humans,[118,122,123] arguing against a significant role of *in situ* IgA class switching in this compartment. In this regard, it should be considered that IgA class switching also takes place in the isolated lymphoid follicles (ILF) of the small intestine, which could contaminate lamina propria preparations.[123] However, ILF do not seem to efficiently generate IgA responses against orally administered antigens.[124,125]

Several different and sometimes seemingly contradictory pathways have been implicated for inducing IgA-ASCs

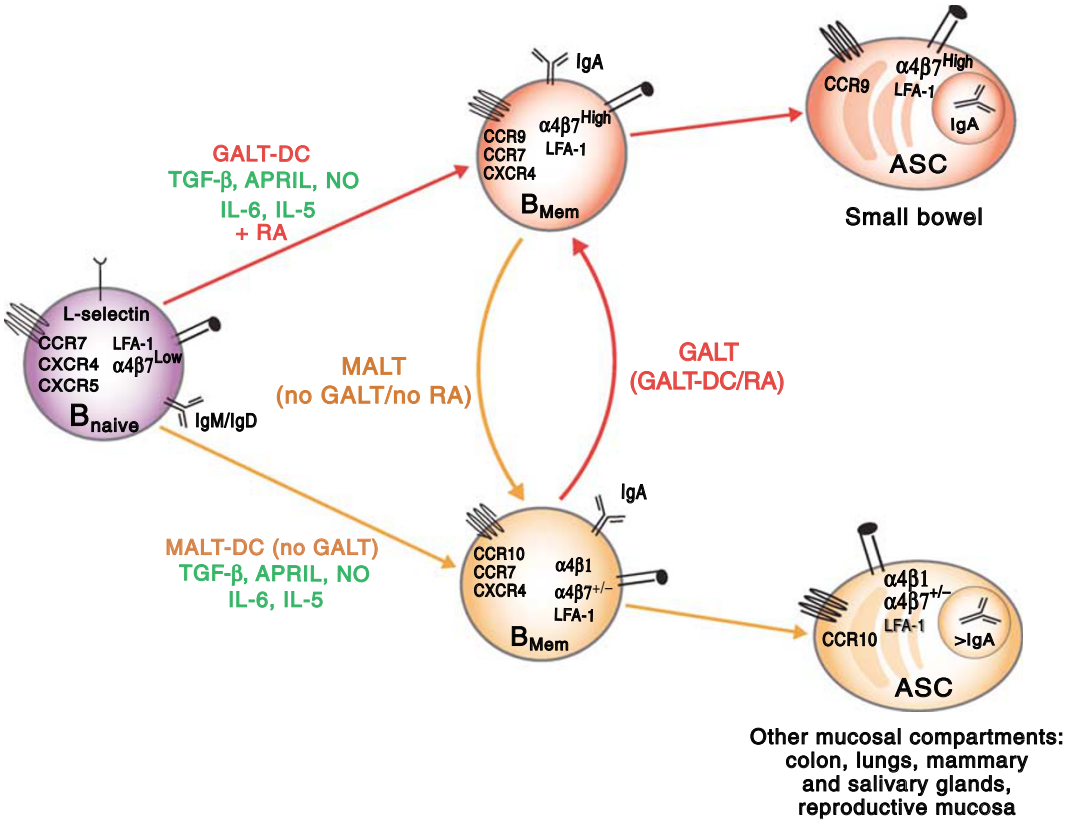

Figure 3 Plasticity in lymphocyte homing. B cells show plasticity regarding their homing commitment. As ASCs are terminally differentiated and do not divide, it is likely that the capacity to be reprogrammed in their homing potential resides at the level of B_{Mem}. If activated B cells that do not express gut-homing receptors are restimulated in the presence of RA, they readily upregulate $\alpha 4\beta 7$ and CCR9. On the other hand, B cells with gut-homing capacity lose $\alpha 4\beta 7$ and CCR9 if they are reactivated without RA. It is possible that IgA^{Neg} B_{Mem} give rise to IgA-ASCs and IgA^+ B_{Mem} upon restimulation in the MALTs. However, gut-homing IgA^+ $B_{Mem,}$ restimulated outside the MALTs, remain IgA^+, although they lose their gut-homing capacity. The latter may be a potential mechanism to help targeting IgA^+ B_{Mem} and IgA-ASCs to mucosal tissues outside the gut. ASC, antibody-secreting cells; $B_{Mem,}$ memory B cells; MALT, mucosal-associated lymphoid tissue.

at mucosal tissues (**Table 1**). The *in vivo* interplay and the relative roles of these mechanisms are probably determined by the context in which B cells are activated, e.g., the nature of the antigen, T cell help, site of activation, type of responder B cells (B1 or B2), and the contribution of Toll-like signals. In addition, as discussed below, the experimental conditions used may also have a significant impact in the final readout.

Role of TGF-β1

IgA responses to TD antigens require transforming growth factor (TGF)-β1, as well as CD4 T-cell "help," the latter provided in the form of CD40L as well as cytokines such as IL-5 (**Figure 4A**). TGF-β is critical for IgA responses *in vivo*, as TGF-β1 knockout mice have impaired systemic and mucosal IgA responses.[126] Moreover, mice whose B cells are deficient in TGF-βRII lack serum IgA[127] and do not mount systemic[127] or mucosal[128] antigen-specific IgA responses. Consistently, mice with B cells deficient in Smad2, which is critically involved in TGF-β signaling, show decreased levels of serum IgA and have impaired antigen-specific IgA responses.[129] In addition, the cytokines IL-5[130–133] and IL-6[134–138] fulfill a complementary role in IgA production by promoting differentiation of ASCs and IgA secretion. Indeed, IgA levels are increased in mice overexpressing IL-6,[139] and IL-5 plays also an additional indirect role in IgA production by promoting B1 B-cell differentiation.[140–143]

Although TGF-β plays a central role in the differentiation of IgA-ASCs, the relevant sources of TGF-β in the gut are less clear. TGF-β1 mRNA and/or protein have been detected in rodent jejunal enterocytes,[144] small and large bowel intestinal epithelium,[145] and intestinal lamina propria cells[145,146] (**Figure 4A**). However, TGF-β1 mRNA and protein are expressed at very low quantities in the steady-state noninflamed human intestine and become upregulated only during inflammation, e.g., in active inflammatory bowel disease.[146,147] It is noteworthy that TGF-β mRNA transcripts have been detected in PP-DCs and $CD103^+$ MLN-DCs.[148,149] Moreover, $CD103^+$ MLN-DCs and LP-DCs have been described to secrete active TGF-β, as evidenced by their capacity to induce $foxp3^+$ T_{REG}, which is abrogated by blocking TGF-β.[94,149] However, parallel studies by others showed that TGF-β1 needed to be added exogenously to obtain a significant induction of T_{REG} in this system,[150,151] suggesting that LP-DCs and MLN-DCs are not a significant source of active TGF-β1 in the gut. Thus, the actual contribution of gut-associated DCs to the pool of active TGF-β1 needs further clarification. An additional possibility is that gut-associated DCs are in charge of activating latent TGF-β1. In this regard, it has been shown that the

Table 1 Mechanisms involved in IgA-ASC differentiation in GALTs

	TD	TI	Effect *in vitro*	Effect *in vivo*	IgA class switching[a]	ASC differentiation IgA secretion	Role in other MALTs[b]	References
TGF-β1	↑	—?	↑or↓[c]	↑	↑	— or↓	↑	126–128, 153–160
iNOS/NO	↑	↑	↑	↑	↑[d]	—	↑	161
APRIL	—	↑	↑	↑	↑	—	↑?	115,164,165, 167–169
BAFF	—	↑	↑	↑?	↑	—	↑?	164–169
RA	↑?	↑	↑	↑[e]	↑?	?	—	90,176–178, 180–182
IL-5, IL-6, IL-10	↑	↑[f]	↑	↑	—	↑	↑	90,130–139, 153
VIP	↑?	↑?	↑	↑?	↑	?	—	171–173

APRIL, a proliferation-inducing ligand; ASC, antibody-secreting cell; BAFF, B-cell activating factor; GALT, gut-associated lymphoid tissue; iNOS, inducible nitric oxide synthase; NO, nitric oxide; RA, retinoic acid; TD, thymus-dependent; TI, thymus-independent; VIP, vasoactive intestinal peptide.
[a]The effect can be direct or indirect in IgA class switching.
[b]Includes lymphoid tissues associated with bronchus (BALT), mammary and salivary glands, and reproductive mucosa.
[c]Whether TGF-β1 increases or decreases the generation of IgA-ASCs *in vitro* depends on the culture conditions (discussed in the text).
[d]iNOS/NO indirectly promotes TD and TI IgA class-switching by increasing B-cell responsiveness to TGF-β1 and by inducing APRIL and BAFF secretion by DCs, respectively.
[e]RA increases IgA-ASCs in the gut by imprinting gut homing and by promoting IgA-ASC differentiation.
[f]Since IL-5 is produced by T cells, this cytokine is probably more relevant for TD IgA responses.
↑Promotes IgA-ASC differentiation; ↓decreases IgA-ASC differentiation; — not demonstrated effect on IgA-ASC differentiation; ? effect unclear; TD/TI thymus-dependent-independent B-cell responses.

integrin αvβ8 is expressed on DCs and that it is critical to activate TGF-β *in vivo*,[152] suggesting yet another potential mechanism by which gut-associated DCs (as well as DCs from other mucosal tissues) may promote IgA class switching.

While the essential *in vivo* role of TGF-β in IgA class switching is well demonstrated, its *in vitro* effects on IgA class switching/secretion seem to vary significantly depending on the experimental system. For instance, although TGF-β1 consistently induces IgA production in LPS-activated splenocytes,[153] it suppresses the spontaneous IgA secretion by nonstimulated mononuclear cells from MLNs and PPs and almost completely blocks IL-5-induced IgA secretion in LPS-activated bulk splenocytes.[90,153] Paradoxically, TGF-β1 potentiates IL-5-induced IgA secretion in T cell-depleted MLN cells activated by LPS.[154,155] Interestingly, the timing of IL-5 addition seems to be important in whether or not IL-5 synergizes with TGF-β1 for IgA secretion.[156] Moreover, TGF-β1 can only be present during the initial phase of *in vitro* human B-cell cultures, because it inhibits IgA secretion at later stages.[157] Nonetheless, it has been demonstrated that TGF-β1 can truly promote IgA class switching at the clonal level,[158,159] whereas IL-5 is mostly involved in post-switch differentiation/secretion.[155] Finally, while TGF-β1 promotes molecular IgA class switching regardless of the activation stimuli, IgA secretion is induced only when B cells are activated with LPS but not by B-cell antigen receptor (BCR) crosslinking.[160] Thus, TGF-β1 may have very different and even opposite effects on IgA production/secretion depending on seemingly minor differences in culture conditions, which may account, at least in part, for some discrepant reports on the role of TGF-β1 and IL-5 in IgA class-switching/secretion.[90,153]

In summary, TGF-β1 promotes germ-line IgA class switching and is essential for TD IgA responses *in vivo*. However, it may block ASC differentiation and IgA secretion in some *in vitro* settings. On the other hand, IL-5 and IL-6 are probably not directly involved in IgA class switching, but they contribute to ASC differentiation and immunoglobulin secretion (including IgA), thus complementing the role of TGF-β1.

Role of nitric oxide

It was recently described that nitric oxide (NO) plays a critical role in IgA class switching and in the generation of IgA-ASCs[161] (**Figure 4**). Mice deficient in the inducible isoform of nitric oxide synthase (iNOS) had a marked reduction in serum and intestinal IgA, but not in other immunoglobulin isotypes. A similar effect on IgA levels was observed when mice were treated with NO scavengers. It is noteworthy that iNOS was critical for both TD and TI IgA responses. TD IgA responses were affected because iNOS-deficient mice expressed decreased levels of TGF-βRII and Smad proteins on B cells.[161] In addition, NO probably also affects DC function, as iNOS-deficient GALT-DCs had a significantly reduced capacity to produce APRIL (a proliferation-inducing ligand) and BAFF (B-cell activating factor/Blys), which may explain the lack of TI IgA responses (discussed below). Interestingly, iNOS expression is dependent on commensal flora and TLR signals, because DC from germ-free or MyD88$^{-/-}$ mice express very low levels of this enzyme.[161] In addition, given that serum IgA levels in iNOS knockout mice can be rescued by adoptively transferring wild-type LP-DCs,[161] iNOS expression by mucosal DCs[161] seems to be sufficient to allow IgA class switching/secretion. However, these results do

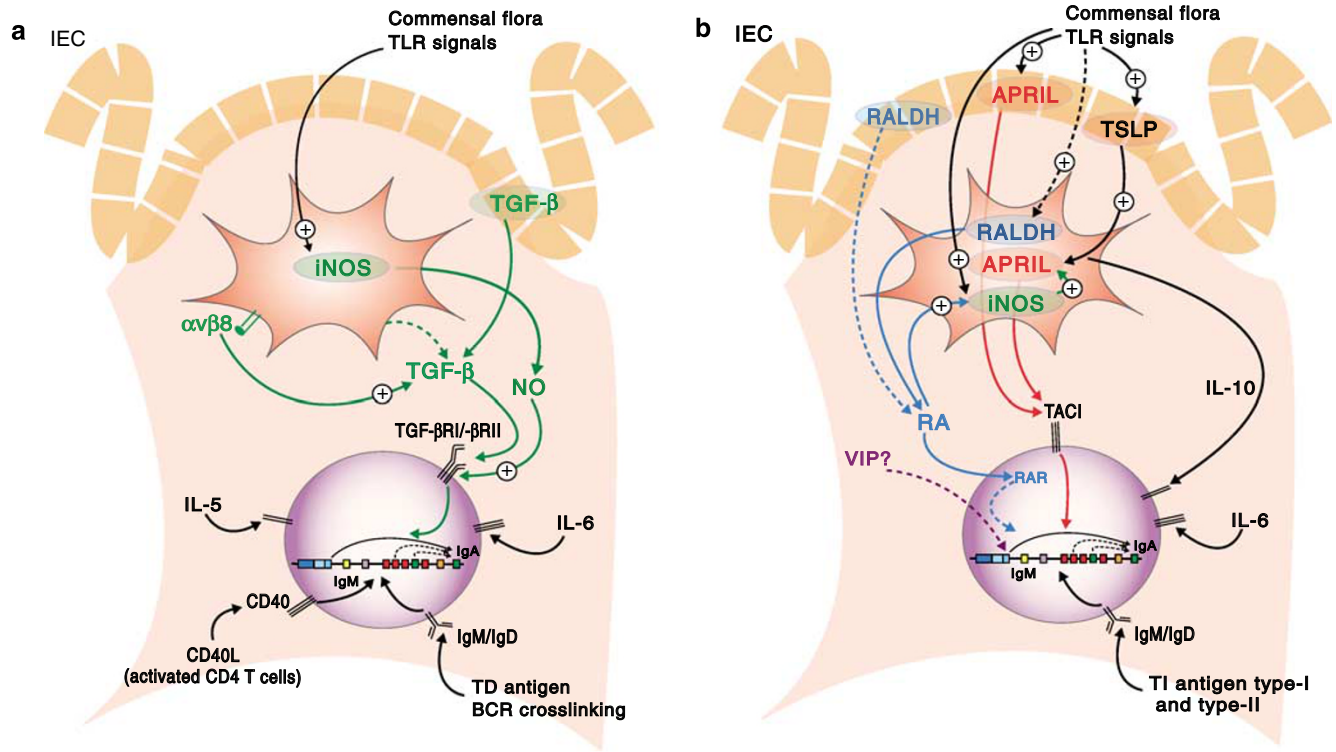

Figure 4 Mechanisms inducing IgA class switching in GALT. IgA class switching is a complex process. (**a**) IgA responses to TD antigens require interaction between CD40-CD40L (the latter expressed by activated CD4 T cells) and also TGF-β1. IECs and GALT-DCs are among the potential sources of TGF-β1 in the gut mucosa. DCs also express the integrin $\alpha v\beta 8$, which is essential for TGF-β1 activation *in vivo*. (**b**) Gut IgA responses to TI antigens require APRIL binding to TACI on B cells. APRIL is produced by IEL upon stimulation by commensal flora or TLR signals. These stimuli also induce TSLP secretion by IEL, which in turn induces more APRIL expression by GALT-DCs. IECs and GALT-DCs can also metabolize vitamin A into RA. The latter imprints gut-homing lymphocytes, but in concert with IL-5 or IL-6, can also induce differentiation of IgA-ASCs. RA may induce IgA class switching directly or by upregulating iNOS and TGF-β secretion. VIP can also induce TGF-β-independent IgA class switching *in vitro*, although its significance *in vivo* remains to be determined. Mucosal DCs (including GALT-DCs) express the inducible form of iNOS, which is induced by commensal flora and TLR signals. iNOS generates NO, which is critical for both TD and TI IgA responses. Indeed, NO synthesis is essential for proper TGF-β1 signaling on B cells and also for APRIL synthesis by GALT-DCs. Interestingly, IgA class switching can start from naive IgM-expressing B cells or sequentially after IgG class switching or, in humans, IgA1 class switching (the latter generates protease-resistant IgA2). Dotted lines: not clearly demonstrated. (+): synthesis induction or activation. APRIL, a proliferation-inducing ligand; ASC, antibody-secreting cells; DC, dentritic cell; GALT, gut-associated lymphoid tissue; IEC, intestinal epithelial cell; IEL, intraepithelial lymphocytes; iNOS, inducible nitric oxide synthase; NO, nitric oxide; TACI, transmembrane activator and CAML interactor; TD, thymus-dependent; TI, thymus-independent; TSLP, thymus stromal lymphopoietin; VIP, vasoactive intestinal peptide.

not exclude the possibility that NO secretion by other cells in the gut may also contribute to or be sufficient for sustaining IgA production. Also, although iNOS is necessary for IgA induction by GALT-DCs,[161] it has not been shown whether its expression is sufficient to confer IgA-inducing capacity to non-mucosal DCs.

APRIL and BAFF

As discussed above, in addition to TD IgA responses to proteins, B cells can respond to TI antigens. A fraction of TI IgA-ASCs derives from B1 B cells. In fact, in contrast to conventional B2 B cells, B1 B cells do not seem to require BCR stimulation to become IgA-ASCs.[162] However, the production of "natural" TI intestinal IgA against commensal flora requires the presence of PPs or MLNs, as shown in mice deficient in LN and GALT (alymphoplasia *aly/aly* mice) reconstituted with wild-type bone marrow, which lack intestinal IgA.[163] It is noteworthy that serum IgA is present in *aly/aly* mice, which lack intestinal IgA indicating that extraintestinal IgA switching can occur in the absence of GALT and PLN.[163]

It has been determined that the cytokine APRIL is important for the induction of TI IgA-ASCs (**Figure 4B**). APRIL knockout mice show decreased mucosal TI IgA responses, and both APRIL and BAFF are sufficient to induce CD40-independent IgA class switching on murine naive B2 B cells, even without BCR stimulation, [164,165] although in this case, either TGF-β1, IL-4, or IL-10 need to be supplemented to the cultures.[167] APRIL and BAFF bind to the receptor TACI (transmembrane activator and CAML interactor), and TACI (but not BAFF-R) is essential for induction of IgA class switching by APRIL and BAFF in murine and human B cells.[164,165,169] However, although both APRIL and BAFF can induce IgA class switching *in vitro*, BAFF-deficient mice show only a mild decrease in IgA (despite a significant decrease in most other immunoglobulin isotypes),[166] which is consistent with a major role of APRIL–TACI but not BAFF–TACI interactions in IgA class-switching *in vivo*. It is noteworthy that IgA induction by TGF-β1 plus LPS is largely TACI- and BAFF-R-independent.[165] In fact TACI-deficient mice have impaired TI but normal TD IgA immune responses[168] (**Table 1**).

BAFF and APRIL are expressed by cytokine- or TLR-activated DCs and monocytes, endowing them with the capacity to induce TI IgA class switching.[167] APRIL is also induced in IECs by commensal flora and TLR signals, especially in the distal part of the intestine[115] (**Figure 4B**). TLR signals also stimulate the production of the cytokine thymus stromal lymphopoietin (TSLP) by IECs, which in turn induces APRIL and IL-10 secretion by LP-DCs, further contributing to their IgA-inducing capacity.[115]

In human B cells, BAFF preferentially induces IgA1, while APRIL induces IgA2 (but not IgA1) class switching.[115,167] In addition, APRIL induces IgA2 class switching starting from either IgM or sequentially from IgG1 or IgA1 class-switched cells.[115] The latter may represent a mechanism by which IgA1 induced in PP germinal centers becomes more resistant to intestinal proteases by switching locally to IgA2 in the colon lamina propria.[115] It is noteworthy that TACI is mutated in some common variable immunodeficiencies as well as in some isolated IgA immunodeficiencies, underscoring the relevance of IgA-inducing mechanisms in human pathologies.[170]

Vasoactive intestinal peptide

Vasoactive intestinal peptide (VIP), which is highly expressed in postsynaptic parasympathetic nerve fibers and intrinsic neurons in the intestinal lamina propria, induces IgA1 and IgA2 secretion in anti-CD40-stimulated human B cells in a TGF-β-independent fashion[171–173] (**Figure 4B**). In fact, TGF-β1, as well as several other cytokines, did not induce IgA in this system,[173] illustrating yet another example of the variable effect of TGF-β1 on IgA secretion *in vitro*. Interestingly, VIP may also indirectly contribute to IgA secretion by inducing nitric oxide synthase activity and NO production.[174,175] It remains to be determined whether VIP plays a physiological role in IgA secretion and how it might influence other pathways that induce IgA secretion in the gut.

Role of GALT-DCs and RA

In addition to their imprinting effect on gut-homing T and B cells,[85,86,90] GALT-DCs can efficiently induce IgA-ASCs *in vitro*.[81–83,90] Moreover, GALT-DCs can induce IgA secretion by BCR-stimulated B cells independent of T cells,[90] as has been shown in other settings.[109] This effect may be mediated, at least in part, by their capacity to produce active TGF-β1[94,148,149,152] and secrete APRIL.[115] However, GALT-DCs (and LP-DCs) can also induce and/or contribute to IgA class switching/secretion by secreting RA (**Figure 4B**). RA induces IgA secretion in LPS-activated splenocytes.[176] Interestingly, although RA also induces TGF-β activity in LPS-activated splenocytes,[176–178] the IgA-inducing effect of RA is only partially dependent on this cytokine.[176] Instead, RA-induced IgA secretion requires either T cells or exogenous IL-5,[180] and IL-5 is also essential for RA-induced IgA switching in LPS-activated splenocytes.[181] Moreover, GALT-DC require RA and IL-6 for optimal IgA induction *in vitro*.[83,90] Interestingly, RA and IL-6 (and/or IL-5) can also synergize to induce IgA secretion in human B cells.[90] Thus, by inducing gut-homing potential and IgA secretion, RA links lymphocyte migration and function in the gut.[90,112]

Consistent with the effect of RA on IgA secretion *in vitro*, oral administration of an RA-receptor agonist significantly increases serum IgA levels in rats.[182] Moreover, treatment with either RA or an RA receptor agonist potentiates LPS-induced iNOS expression in several organs and also increases plasma levels of nitrate/nitrite in rats.[183,184] Of note, the iNOS gene promoter has an RA response element that is directly activated by RA bound to its nuclear RARα/RXR heterodimeric receptor.[184,185] As discussed above, iNOS expression is critical for the *in vivo* production of IgA. Thus, in addition to increasing IgA class switching, RA may indirectly contribute to IgA secretion by inducing iNOS expression.

Precursors of retinoids (β-carotene and retinyl esters) in the diet are absorbed from the gut lumen and can be converted to RA in IECs[98] and DCs near the germinal centers in PPs.[97] This proximity could explain the predominant IgA class switching observed in PPs as compared to MLNs.[118] Consistent with a role of vitamin A in gut IgA production, rats depleted of vitamin A have decreased levels of total IgA in intestinal lavages and decreased mucosal antigen-specific IgA responses.[186–190] Also, vitamin A-depleted mice show impaired IgA secretion and protection at mucosal sites,[191,192] as well as impaired IgA responses to oral toxins.[193] However, it should be kept in mind that vitamin A deficiency may have other effects on the immune system. In fact, the greater susceptibility to intestinal infections and toxins observed in these animals may also be explained, at least in part, by decreased pIgR expression and therefore decreased IgA secretion to the gut and other mucosal compartments.[186,190,192] Moreover, although vitamin A-deficient mice have a greatly reduced number of IgA-ASCs in the small bowel,[90,96] they have normal serum IgA levels,[90] indicating that retinoids are not absolutely required for IgA production in other tissues.

In summary, given the critical role of RA in T- and B-cell gut-homing imprinting,[90,97] as well as its IgA-ASC-promoting potential in the gut,[90,176] it is not surprising that vitamin A deficiency is associated with impaired intestinal immune responses[186–194] and increased mortality.[195] Moreover, vitamin A supplementation correlates with a significant decrease in diarrhea and mortality in HIV-infected or malnourished children.[196–199]

CONCLUSION

Although our understanding of the mechanisms inducing IgA-ASC homing to the small bowel has increased significantly, it is still unknown how IgA-ASCs are imprinted to migrate to other mucosal compartments, such as colon, lungs, and salivary and mammary glands. In this regard, the chemokine receptor CCR10 has been proposed as a "common" mucosal homing receptor for IgA-ASCs. However, where and how CCR10 is induced is still a mystery. It is also unclear to what extent B1 B cells contribute to the generation of gut IgA-ASCs and whether they obey the same gut-homing imprinting signals as conventional B2 B cells.

As we have discussed, IgA class switching and differentiation of IgA-ASCs is a complex process. TGF-β1 and iNOS/NO play a critical role in the generation of IgA-ASCs *in vivo*. However, in the gut mucosa, other mechanisms may work independently of or in complement to TGF-β1 to generate IgA-ASCs.

APRIL–TACI interactions can induce TGF-β1-independent and TI IgA class switching in the intestine. In addition, both VIP and RA also induce TGF-β1-independent IgA class switching. Therefore, it will be important to determine the relative *in vivo* contributions and interrelationships among all these IgA-inducing mechanisms. A systematic dissection of each pathway in different settings of B-cell activation (e.g., TD, TI, mucosal, systemic, with and without TLR signals) will be required to sort out these questions.

ACKNOWLEDGMENTS

We are grateful to Susan Davis for valuable editorial assistance and to Drs Scott Snapper and Andrea Cerutti for critical reading of this manuscript. JRM is indebted to Ingrid Ramos for constant support. JRM is supported by grants from Crohn's & Colitis Foundation of America (CCFA), Cancer Research Institute (CRI) and Center for the Study of Inflammatory Bowel Disease (CSIBD, DK43351). UHvA is supported by grants from the NIH.

DISCLOSURE

The authors declared no conflict of interest.

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