SOCIETY FOR MUCOSAL IMMUNOLOGY

REVIEW ARTICLE

The role of retinoic acid in the production of immunoglobulin A

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Vitamin A and its derivative retinoic acid (RA) play important roles in the regulation of mucosal immunity. The effect of vitamin A metabolism on T lymphocyte immunity has been well documented, but its role in mucosal B lymphocyte regulation is less well described. Intestinal immunoglobulin A (IgA) is key in orchestrating a balanced gut microbiota composition. Here, we describe the contribution of RA to IgA class switching in tissues including the lamina propria, mesenteric lymph nodes, Peyer's patches and isolated lymphoid follicles. RA can either indirectly skew T cells or directly affect B cell differentiation. IgA levels in healthy individuals are under the control of the metabolism of vitamin A, providing a steady supply of RA. However, IgA levels are altered in inflammatory bowel disease patients, making control of the metabolism of vitamin A a potential therapeutic target. Thus, dietary vitamin A is a key player in regulating IgA production within the intestine, acting via multiple immunological pathways.

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INTRODUCTION

The beneficial effect of vitamin A on the immune system was first described in 1928, when it was demonstrated that rats on a vitamin A-deficient (VAD) diet developed infections in multiple organs, ultimately leading to death[1]. Over time, additional animal studies have demonstrated that vitamin A derivatives are important in providing protection against many pathogens, including respiratory syncytial virus[2], *Salmonella*[3], *Mycobacterium tuberculosis*[4], *Listeria monocytogenes*[5] and influenza virus[6]. In addition, the anti-infectious properties of dietary vitamin A have been observed in humans. Individuals with insufficient vitamin A intake have higher chances of developing tuberculosis when exposed to *Mycobacterium tuberculosis*[7] and enhanced risks for measles- and diarrhoeal disease-related mortality[8]. Taken together, these studies were the first to indicate the importance of dietary vitamin A in immunity and the protective functions of dietary vitamin A against a broad range of mucosal infections. Later studies recognized vitamin A as a key regulator in the production of protective immunoglobulin A (IgA) antibodies in mucosal tissues. In this review, we describe how the immune system uses vitamin A to promote the antibody response and preserve mucosal homeostasis. We further focus on the molecular mechanism by which vitamin A promotes IgA class switching in B lymphocytes, thereby preventing pathology.

Metabolism and transport of vitamin A and its derivatives

Vitamin A is involved in the regulation of multiple physiological processes, such as spermatogenesis, fertilization, pregnancy maintenance, morphogenesis, organogenesis, growth and cellular differentiation[9,10]. It is an essential fat-soluble molecule that needs to be obtained from the diet in the form of retinol, retinyl esters or carotenoids. The gastric and intestinal epithelial barriers, which are the first to come in contact with dietary vitamin A, absorb free retinol by passive transport, whereas the uptake of plant-derived β-carotene has been proposed to be facilitated by class B scavenger receptors (SRB1)[11,12]. The detailed mechanism of vitamin A transport and metabolism has been reviewed elsewhere[13]. Absorbed dietary retinoids are transported in chylomicrons in the form of retinyl esters via the lymphatic system. The majority of chylomicron retinyl ester (66–75%) is stored within the liver, while some (25–33%) is delivered to peripheral tissues[14]. When dietary vitamin A is scarce, retinol is released from the liver and transported through the periphery while bound to the retinol binding protein (RBP) complex. Within the periphery, retinol is taken up by target cells via the high-affinity retinoic acid 6 (STRA6) receptor, allowing cells to receive retinol for further metabolic processing[15].

Within the intestine, vitamin A is converted into its metabolically active form, retinoid acid (RA), within intestinal epithelial cells (IECs). First, retinol is converted into retinal under normal physiological conditions by microsomal retinol dehydrogenases (RDHs) in a reversible manner[16]. Then, the retinal is irreversibly converted into RA by aldehyde dehydrogenases (ALDHs)[17] (Fig. 1). Studies using rats have demonstrated that intestinal crypt epithelial stem cells, which express high levels of ALDH in humans[18,19], have the lowest concentration of retinol and the highest concentration of RA[20]. This suggests that crypt stem cells may be important in intestinal RA production. Multiple human ALDH isoforms exist, among which ALDH1a1, ALDH1a2, and ALDH1a3 are the most extensively studied in association with the immune system[17]. Within mice, these enzymes are referred to as retinaldehyde dehydrogenases (RALDH1, RALDH2, and RALDH3). In addition, multiple isomers of RA exist, but all-trans retinoic acid (ATRA) is considered to be the most biologically prevalent form, and ATRA, therefore, is the focus of this review[21,22].

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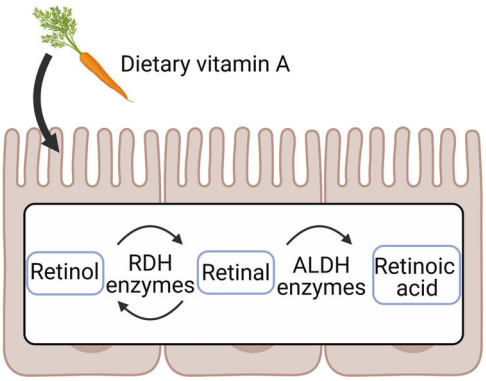

Fig. 1 Intestinal epithelial cells metabolize dietary vitamin A into retinoic acid. Dietary vitamin A is absorbed in human intestinal epithelial cells and processed via two enzymatic digestion steps. First, under normal physiological conditions, retinol is converted into retinal by microsomal retinol dehydrogenases (RDHs) in a reversible manner. Second, retinal is irreversibly converted by aldehyde dehydrogenases (ALDHs) into retinoic acid.

Production of retinoic acid in mucosal tissues

Since IECs are key in converting dietary vitamin A into RA, they are an important source of RA for the immune cells present within the lamina propria (LP). IECs were shown to be crucial in inducing the differentiation of tolerogenic dendritic cells (DCs), a process dependent on RA[23]. These tolerogenic DCs in turn are able to produce RA due to intracellular ALDH enzymatic activity and are therefore referred to as RA-producing DCs (RA-DCs). These RA-DCs are further characterized by the membrane expression of CD103 (Fig. 2). Under homeostatic conditions, mucosal DCs reside within the LP and interact with IECs when sampling luminal antigens[24–26] (Fig. 2). It has been demonstrated that the capacity of DCs to express ALDH enzymes relies on initial exposure to RA itself[27–29], for instance, provided by IECs (Fig. 2). Similarly, the number of $CD103^+$ ALDH-expressing DCs in the LP (LP-DCs) is decreased when RA signalling is disrupted within enterocytes[30]. IECs need to be in close proximity to DCs to provide them with RA[23]. In further support of this, CD103-expressing DCs have also been observed to interact with the epithelium in humans[31–33]. The ability of DCs to produce RA is characteristic of mucosal subsets, as DCs in the spleen or peripheral lymph nodes draining the skin do not exhibit ALDH expression[28,34,35]. It is thought that RA-DCs within the LP are not maintained by tissue-resident precursors but are derived from DC precursors that continuously seed the LP from the circulation, suggesting that the induction of RA-DCs is a continuous process[33]. These studies imply a key role for IECs in providing LP-DCs with a primary source of RA, allowing their local differentiation within the intestine after their arrival as precursors from the bloodstream. The presence of RA-DCs is not restricted to the LP, as RA-DCs have been observed in mucosal organized lymphoid tissues such as the gut-draining mesenteric lymph nodes (MLNs) and Peyer's patches (PPs). Similar to that of small intestinal DCs, the capacity of DCs within the MLNs to express ALDH enzymes is dependent on dietary vitamin A[28]. RA-DCs within the LP have migratory properties and have been described to migrate via the afferent lymphatics into the MLNs[33,36]. Moreover, there are reports of mucosal macrophages expressing ALDH enzymes as well, but to a lower extent than CD103 + RA-DCs[37]. Myeloid cells are well known for their role in facilitating adaptive immune responses. As a result, the observation that different myeloid cells, particularly DCs, possess the ability to produce RA has led to the question of whether mucosal DCs, by producing RA, influence adaptive immunity and contribute to gut homeostasis.

Retinoid acid and IgA differentiation in B cells

The link between RA-DCs and mucosal adaptive immunity was first studied in mice consuming VAD diets. In this context, it was observed that the absence of dietary vitamin A, which ultimately led to vitamin A-deficient mice, produced a dramatic decrease in the production of IgA antibodies in mucosal tissues[38]. IgA is predominantly produced within the mucosa[39] as a dimeric antibody by gut-resident plasma B cells. IgA binds to the polymeric immunoglobulin receptor (pIgR) expressed on IECs and is transported through the epithelial barrier into the gut lumen. During this process, IgA gains a secretory compound when it is released into the lumen. In this site, IgA plays an important role, as it interacts with the microbiota, promoting host–microbiota symbiosis and participating in homeostasis[40–43] as reviewed previously[44]. Additional studies have observed that RA is a key regulator in facilitating IgA production in mouse[45–48] and human[49,50] B lymphocytes. Despite the important role of RA in directing IgA production, B lymphocytes themselves are unable to produce RA, as they do not express the ALDH enzymatic machinery and are therefore dependent on external sources. It has been observed that mucosal RA-DCs are important in providing B cells with RA, leading to IgA production in vivo. Thus, when RA-DCs are unable to develop, for instance, when mice are deficient in vitamin A or RA production by IECs is hampered, there are reductions in the amounts of LP IgA^+ B lymphocytes and mucosal IgA secretion[23,30,35,38]. The direct role of RA-DCs in skewing B lymphocytes into IgA-secreting cells was observed in murine in vitro cultures, demonstrating that LP-DCs induce IgA class switch recombination (CSR) in naïve B lymphocytes in an RA-dependent mechanism. In addition to steering IgA isotype switching, RA has been proposed to stimulate the differentiation status of B cells by promoting plasmablast differentiation[50,51]. This is supported by a study that observed that RA enhances the expression of plasma cell-generating transcription factors in primary human B cells[51]. Furthermore, ageing is proposed to influence the immunological physiology in the gut and may therefore play a role in RA-mediated IgA biology (reviewed previously[52]). Taken together, these studies demonstrate a key role for RA-DCs in facilitating IgA production in the mucosa.

Molecular mechanism by which retinoic acid induces IgA production

Classic RA-dependent gene activation is mediated by the binding of RA to its nuclear retinoic acid receptor (RAR), which forms a dimer with retinoid X receptor (RXR) and functions as a transcription factor[53]. Three RAR (RARα, RARβ, and RARγ) and three RXR (RXRα, RXRβ, and RXRγ) isotypes, with different expression patterns, are involved in controlling the dynamics of RA signal transduction. In the absence of RA, the heterodimeric receptors are bound to retinoic acid response elements (RAREs) and provide a binding site for corepressors[54], which can indirectly make chromatin less accessible by allowing deacetylation[55] (Fig. 3). These RARE regions are found in gene promoters and characterized by two hexameric motifs, 5′-(A/G)G(G/T)TCA-3′, arranged as palindromes, direct repeats, or inverted repeats[56]. In the presence of RA, the RAR-RXR dimer undergoes a conformational change that eliminates the corepressor binding site, allowing chromatin acetylation[57]. As a result, binding can initiate RA-mediated gene activation (Fig. 3). The human genome contains almost 15,000 RARE sequences, of which 138 locations are highly conserved among vertebrates[58]. RA is a potent gene expression regulator that has been described to regulate the expression of up to 500 to 800 genes[59–61]. Primary CSR factors are essential for initiating somatic rearrangements in the immunoglobulin heavy chain gene in B cells, leading to isotype switching into, for instance, IgA. For class switching towards IgA, DNA breaks are specifically made in switch regions upstream of the α chain locus by the enzyme activation induced deaminase (AID), leading to switch region

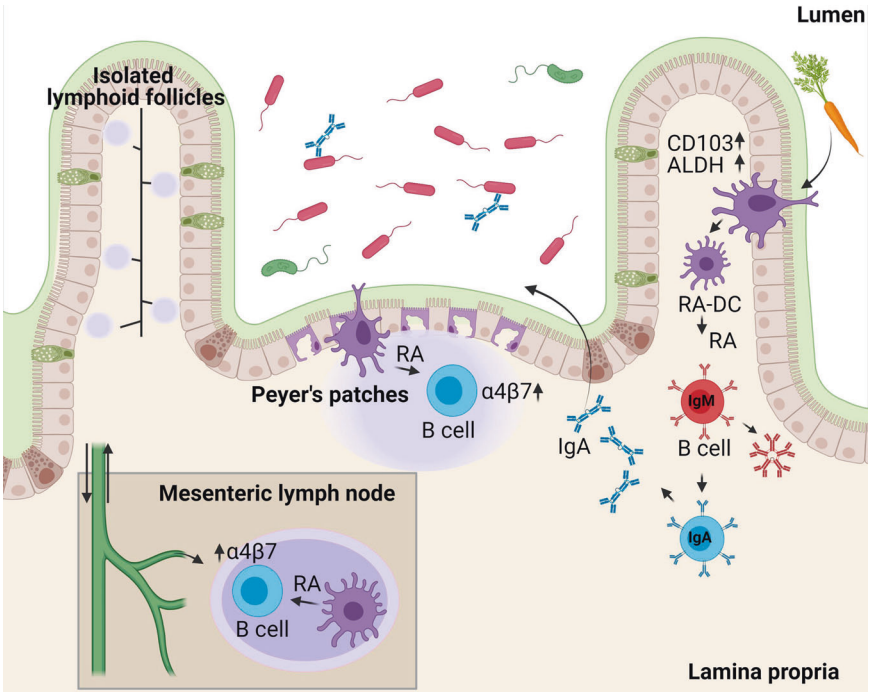

Fig. 2 Dietary vitamin A stimulates retinoic acid-producing dendritic cell differentiation in the gut. Dietary vitamin A is metabolized by intestinal epithelial cells (IECs) into retinoic acid (RA). Close interaction of intestinal dendritic cells (DCs) with the epithelium skews the DCs into an RA-DC phenotype characterized by the expression of CD103 and active ALDH enzymes. These local DCs provide RA to LP-residing IgM^+ B cells, allowing them to undergo class switching to IgA. Moreover, RA-DCs migrate from the LP into the mesenteric lymph nodes (MLNs) to provide RA to B cells. Dietary vitamin A is also important in the development of tolerogenic RA-DCs within Peyer's patches (PPs). Within organized lymphoid organs, the production of RA primes B lymphocytes to express the gut-homing receptor α4β7 and undergo IgA class switching. Activated B cells leave the PPs and MLNs, recirculate via the bloodstream and finally enter the gut using $\alpha_4\beta_7$, where they populate the LP and become resident IgA-secreting plasma cells. Locally, IgA is produced as a dimeric molecule and transported through the epithelial layer, where it binds to the microbiota, thereby regulating its composition.

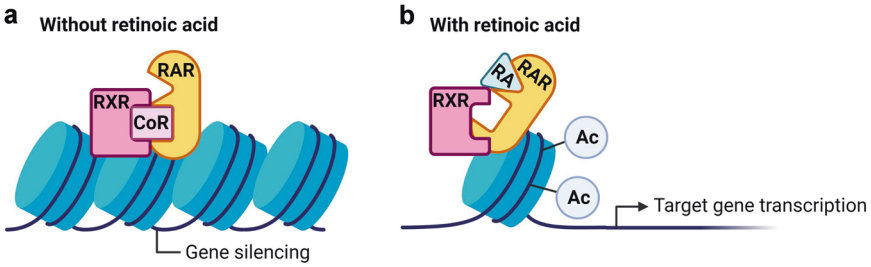

Fig. 3 Retinoic acid regulates the gene transcription of target genes. a In the absence of retinoic acid (RA), the nuclear factors retinoic acid receptor (RAR) and retinoid X receptor (RXR) form a dimer and bind to retinoic acid response element sequences within the DNA to provide a binding site for corepressors (CoRs). As a result, the deacetylated chromatin is not accessible for gene transcription. **b** After binding of RA to the RAR-RXR complex, the dimer changes its conformation to eliminate the corepressor binding site, allowing chromatin acetylation. As a result, the chromatin is acetylated, which allows access for transcription factors to regulate the gene expression of downstream target genes.

recombination and subsequent looping out of the constant regions of other isotypes (Cμ, Cδ, Cγ, and Cε). Primary CSR stimuli include CD40-ligand, TLR ligands and crosslinking of the BCR, which induce the expression of the enzyme AID to enable CSR[62,63]. However, secondary stimuli are required to direct CSR to predetermined immunoglobulin isotypes, but these stimuli cannot induce CSR on their own. As such, B cell differentiation towards the IgA isotype was shown to be dependent on RA when accompanied by primary CSR factors[45–49,64,65]. Thus, RA is considered a secondary CSR factor that stimulates IgA isotype B cell switching rather than initiating Cα gene transcription on its own[66]. Runt-related transcription factors (RUNX) are essential in Cα gene transcription, acting by binding to α Ig promoters. RA-induced mucosal and systemic IgA production is completely abrogated in RUNX2/3 double-knockout mice[67]. It is unclear how RA is involved in RUNX-induced IgA CSR, as there is no literature describing a direct physical link between RA and the RUNX family. Mechanistically, it has been suggested that RA receptors can directly bind the α switch region to promote the induction of DNA breaks by offering a binding site for AID[68]. Moreover, RARE sequences have been detected within the promoter region of immunoglobulin germline α itself[48], suggesting that RA may also influence the expression levels of the IgA heavy chain. Nevertheless, the induction of IgA CSR was observed to also occur independent of the RARE regions within the α chain promoter, as CSR was still efficient in the presence of mutations in these RARE sequences[69]. This suggests that RA enhances IgA CSR via epigenetic modulations, such as altering the chromatin density and thereby exposing AID binding sites, rather than acting as a transcriptional regulator.

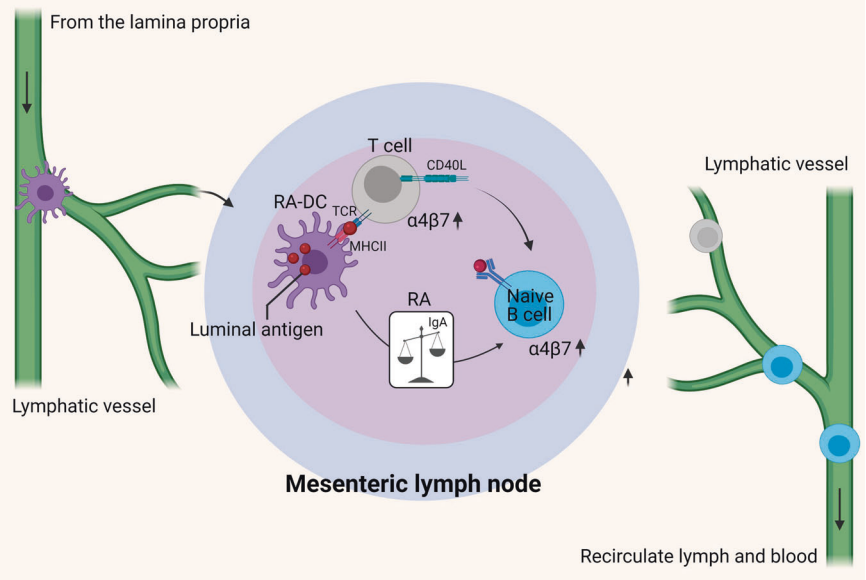

Fig. 4 Retinoic acid-producing dendritic cells facilitate the differentiation of IgA + B lymphocytes within the mesenteric lymph nodes in a T cell-dependent fashion. Antigen-loaded tolerogenic dendritic cells (DCs) migrate from the lamina propria (LP) into the mesenteric lymph nodes (MLNs) via lymphatic vessels. In the MLNs, luminal antigens are recognized by the B cell receptor on naïve B lymphocytes, which together with T cell help, initiates B cell activation. Moreover, RA initiates the expression of the gut-homing molecule $\alpha_4\beta_7$ on B and T lymphocytes. Subsequently, when activated B cells leave the MLNs, they move towards the circulation, after which they travel to the LP using α4β7 and populate this site as IgA-secreting plasma cells. T cell-independent B cell activation has also been reported but described to not be the predominant process in the MLNs.

Requirements for retinoic acid-mediated immunoglobulin A switching in mucosal organized tissues

RA-DCs are located in multiple mucosal organized tissues, including isolated lymphoid follicles (ILFs), the MLNs and PPs (Fig. 2). These organized tissues provide a structured network to allow interactions between immune cells to facilitate an adaptive immune response. In germinal centre (GC) structures, T cells play an important role in activating B cells, which is referred to as T cell-dependent (TD) B cell activation. Typically, TD antibody production starts when an antigen, presented by a mucosal antigen-presenting cell (APC), such as an RA-DC, is recognized by T-helper cells. At the same time, B lymphocytes recognize the antigen via their BCR, which initiates B cell activation. Subsequently, upon recognition of the antigenic peptide that is presented on B cell MHC-II, T-helper cells further activate B cells via CD40 ligation and interleukin-4 release[70]. TD B cell responses that develop in the follicles are characterized by somatic hypermutation and affinity maturation.

Isolated lymphoid follicles

ILFs are organized structures within the intestine that develop *de novo* in response to luminal stimuli[71]. Organized ILFs are dominated by the presence of B lymphocytes but also contain T cells, DCs[72] and stromal cells, allowing B cell differentiation[73]. ILFs harbour memory and naïve B lymphocytes and offer a suitable environment for GC-based B cell priming[74]. It has been suggested that ILFs contribute to the production of antigen-specific IgA[75,76]. Human ILFs contain immunoglobulin M (IgM)+ memory B cells that already express germline Cα transcripts, which is direct evidence of IgM-to-IgA CSR[77]. When entering GC pathways, these IgM+ memory B cells generate IgA+ memory B cells and IgA-secreting plasma cells, which is accompanied by hypermutation[77]. Additionally, it was demonstrated that B cells in an ILF express AID transcripts, supporting that IgA CSR takes place inside the ILF[74]. However, the extent to which RA orchestrates IgA induction in ILF-contained GCs must be addressed in future studies.

Mesenteric lymph nodes

The MLNs act as a firewall for nutrients and microbial substances entering the lymph in the intestinal LP. The MLNs contain B cell follicles and distinct T cell areas containing T cells and DCs. These DCs are important for tolerance induction to food proteins and prevent live commensal intestinal bacteria from spreading systemically[78]. It was demonstrated that antigen-loaded CD103$^+$ DCs from the LP migrate into the MLNs, where they drive T cell responses to soluble luminal antigens[79]. When mice are T cell deficient, they have a partial reduction in IgA+ B cells in the MLNs, suggesting that T cells contribute to the differentiation of IgA+ B cells within the MLNs[80]. Although the MLNs drain the LP and are important for orchestrating mucosal immunity, they are not the exclusive site of B cell IgA induction[81,82]. CD103 + DCs from the MLNs express ALDH enzymes, whereas their CD103- counterparts lack ALDH enzyme expression[37,38,83,84]. Although DC-mediated IgA production can occur the MLNs, mainly via TD mechanisms[82], the MLNs are still a minor site for IgA switching during homeostasis[81,85]. Mechanistically, CD103 + RA-DCs from the MLNs were shown to induce IgA CSR in naïve B lymphocytes, which was observed to be at least partly dependent on RA production[86] (Fig. 4). Similarly, the contribution of RA-mediated IgA production in the MLNs was supported by a reduction in antigen-specific IgA-secreting B lymphocyte numbers within the MLNs upon abrogation of RA signalling in the B cell lineage[47]. Although it is unclear to what extent the MLNs contribute to overall mucosal IgA levels, data point towards the involvement of RA-mediated IgA production in the MLNs.

Peyer's patches

PPs consist of multiple B cell follicles located underneath the surface of the epithelial cell barrier in the small intestine and have an important role in the production of antigen-specific IgA[87,88] (Fig. 5). Within PPs, B cells undergo somatic hypermutation and affinity maturation of IgA class-switched B cells, which is predominantly mediated by T cells[89]. In the subepithelial dome, luminal antigens are captured by microfold cells and

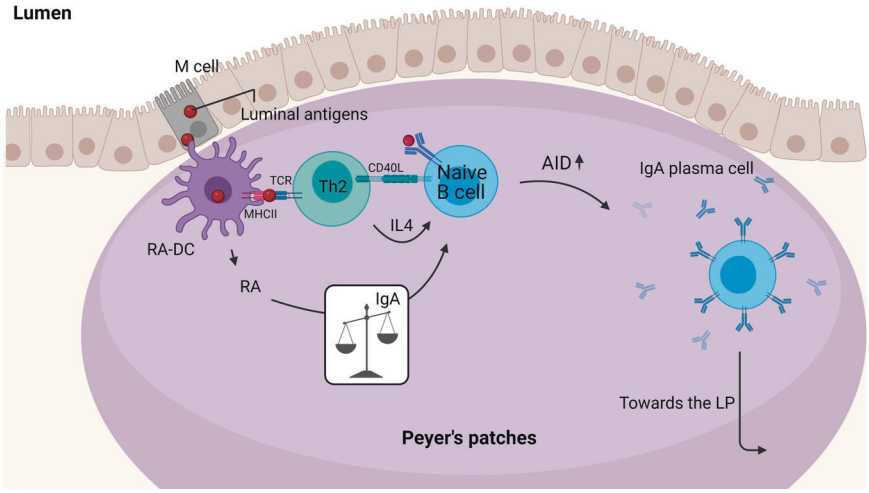

Fig. 5 Retinoic acid-producing dendritic cells facilitate the differentiation of IgA + B lymphocytes within Peyer's patches. Specialized microfold cells (M cells) transport microbes and luminal antigens to intestinal retinoic acid-producing dendritic cells (RA-DCs). These RA-DCs present antigenic peptides on major histocompatibility complex-2 (MHCII) to T lymphocytes, promoting their differentiation into T-helper 2 (Th2) cells. Th2 cells express CD40 ligand, which further activates B cells that have recognized their cognate antigen with the B cell receptor (BCR). Moreover, Th2 cells secrete multiple cytokines, including interleukin-4 (IL4), to facilitate B cell activation. Within PPs, the most predominant mechanism of IgA differentiation in B cells involves TD activation. It is suggested that local RA-DCs together with CD40 costimulation provided by T cells skew naïve B cells into an IgA isotype. Additionally, a fraction of TI IgA induction is also reported within PPs, but whether this requires the involvement of RA is still unclear.

transferred to DCs, which are required for intestinal IgA production in PPs[90] (Fig. 5). DCs present antigens to $CD4^+$ T cells in the perifollicular area, leading to the secretion of cytokines by effector T cells involved in IgA CSR[91,92]. The involvement of T lymphocytes in facilitating mucosal IgA CSR, induced by CD40L costimulation and cytokine production, is evident within PPs[93]. Recent work showed that T cell depletion in mice decreased the frequency of IgA+ B cells in PPs, supporting the involvement of T cells in IgA production[80]. With respect to the RA dependency of IgA production, PP-derived DCs have been observed to induce integrin α4β7 on lymphocytes in vitro, which reflects their capacity to produce RA[38,94]. Moreover, PP-derived DCs enhance the production of IgA in activated mouse B cells in vitro, which can be blocked by a RAR receptor antagonist[38,95], demonstrating that IgA differentiation in PPs is partly dependent on RA. Interestingly, PP-derived DCs were observed to express the mouse retinaldehyde dehydrogenase 3 (RALDH3) enzymes, in contrast to MLN-derived DCs, which express RALDH2[94]. Further support for the dependency for IgA production within PPs on RA was derived from studies in which RA signalling was silenced in mouse B lymphocytes. In these mice, the frequency of total B lymphocytes within PPs remained stable, whereas the numbers of IgA^+ and AID-expressing B cells were reduced[47]. The same study observed a reduction in antigen-specific IgA-secreting B lymphocyte numbers within PPs upon oral immunization[47]. Moreover, RA indirectly promotes IgA production by stimulating follicular DCs in the presence of bacterial products. As a result, follicular DCs produce multiple factors, including TGFβ, and thereby establish an IgA-promoting environment[96]. Together, these studies show the importance of RA in TD IgA production within PPs.

RETINOIC ACID-MEDIATED T CELL SKEWING INDIRECTLY REGULATES MUCOSAL IMMUNOGLOBULIN A PRODUCTION

While RA can have a direct effect on IgA CSR in B lymphocytes, it can also indirectly influence IgA production via its effect on T lymphocyte differentiation.

Retinoic acid regulates regulatory T cell and T-helper 17-cell differentiation

The production of RA by DCs in the steady state influences the differentiation of T cells. T cells exposed to RA become less sensitive to interleukin-6, interleukin-21 (IL21) and interleukin-23, which are required to develop a T-helper 17 (Th17) response[97–99]. As a result, naïve T cells are unable to differentiate into interleukin-17-producing cells when exposed to RA; rather, they differentiate into other subsets, such as regulatory T cells (Tregs) (Fig. 6)[98]. In addition, RA can suppress the T cell production of interleukin-4, IL21 and interferon-γ, cytokines that inhibit Treg formation (Fig. 6)[100]. As a result, RA-DCs allow the differentiation of Tregs and thereby modulate the balance between Treg and Th17 responses[99,101,102]. Although it is widely accepted that helper T cells facilitate B cell CSR, it was proposed that Tregs are important in mucosal IgA production as well[93]. Mice lacking Tregs displayed decreased numbers of IgA+ B cells, and these numbers were restored upon in vivo administration of Tregs[103]. There are multiple mechanisms by which CD4 + T cells contribute to the induction of mucosal IgA production. Tregs facilitate IgA production by secreting TGFβ within PPs, and this isotype switching is unrelated to antigens and independent of the microbiota[104]. Moreover, it was proposed that Tregs can transform into follicular T cells, which are responsible for GC formation[105] and IL21 secretion, thereby facilitating specific IgA production[104,106]. However, despite extensive studies showing a positive effect of RA on Treg differentiation, it remains unclear whether RA is involved in mucosal IgA production via the induction of Tregs or T follicular helper cells.

Retinoic acid regulates T-helper 1- and T-helper 2-cell differentiation

The contribution of RA to the regulation of T-helper 1 (Th1) and T-helper 2 (Th2) responses in the mucosa is less clear, as there are inconsistencies in the literature. It was observed that RA sustains stable expression of Th1 lineage-specific genes via RARα signalling. Furthermore, RA was found to be essential for limiting Th1-cell conversion into Th17 cells and preventing

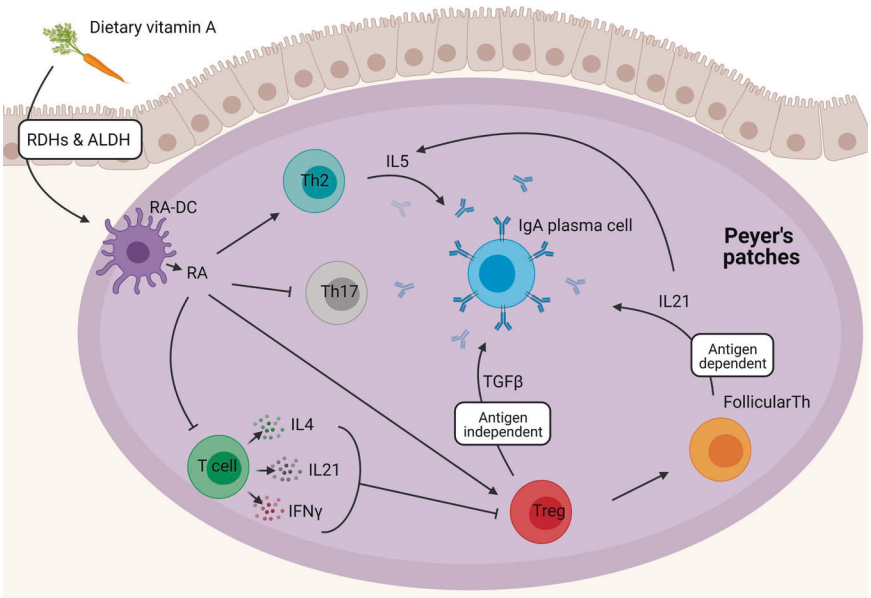

Fig. 6 Indirect effects of retinoic acid on T lymphocytes to stimulate IgA class switch recombination. Epithelial cells metabolize dietary vitamin A into retinoic acid (RA), which is required for dendritic cells (DCs) to differentiate into RA-producing DCs. Within Peyer's patches (PPs), RA secretion affects the differentiation of CD4 + T cells, thereby facilitating IgA class switching by B lymphocytes. In particular, RA regulates the balance of regulatory T cell (Treg) and T helper 17 (Th17)-cell differentiation. RA suppresses the production of interleukin-4 (IL4), interleukin-21 (IL21) and interferon-γ (IFNγ), which are cytokines that inhibit Treg formation, by T cells. As such, RA indirectly promotes the differentiation of Tregs. Tregs themselves are involved in the induction of antigen-independent IgA differentiation by secreting TGFβ. Moreover, Tregs can differentiate into T follicular helper cells (Tfhs), which are involved in antigen-dependent IgA production by secreting IL21 within PPs. RA also promotes the differentiation of T-helper 2 cells, which secrete interleukin-5 and, together with IL21, promote an IgA-inducing environment.

pathogenic responses in vivo[107]. In contrast, it was suggested that RA is important for Th2 responses, as RARα signalling within T cells leads to efficient activation, whereas RARα deficiency results in a cell-autonomous $CD4^+$ T cell activation defect[108]. Moreover, multiple studies have indicated that RA promotes Th2 rather than Th1 responses in mice[109,110]. The ability of RA to mediate mouse T cell differentiation in vitro towards Th2 cells was demonstrated to be dependent on the culture conditions[109], suggesting that the inconsistent data in the literature may be due to technical differences between experiments. Nevertheless, human T cells also differentiate towards a Th2 phenotype when cultured in the presence of RA, a process shown to be dependent on RARα signalling[111,112]. Overall, the data point towards a stimulatory effect of RA on Th2-cell differentiation. Th2 cells are well known for their roles in anti-parasitic immunity and allergy, functioning by producing a spectrum of effector cytokines, including interleukin-5 (IL5). Additionally, IL5 has been proposed to promote IgA production in multiple in vivo studies[113,114]. As an example, administration of recombinant murine IL5 promotes IgA synthesis in PP-derived cycling B cells[113]. IL5 itself cannot initiate IgA production[114] but rather cooperates with IL21 to promote the proliferation of B cells exposed to IgA-inducing factors, such as RA[115]. Accordingly, IgA induction by vitamin A is impaired in IL5 receptor-deficient mice[116], suggesting that RA and IL5 cooperate to induce IgA. However, despite extensive data supporting the enhancing effect of IL5 on mucosal IgA production in mice, a similar effect has not been demonstrated in humans. It is possible that RA may indirectly promote mucosal IgA production by facilitating a Th2 response, which, through the release of IL5 contributes, together with RA, to mucosal TD IgA production (Fig. 6). Nevertheless, future studies are required to demonstrate whether this also applies to humans.

RETINOIC ACID-MEDIATED IMMUNOGLOBIN A PRODUCTION OUTSIDE OF GERMINAL CENTRES

IgA differentiation of B cells is not only established with T cell help but can also occur in a T cell-independent (TI) manner. In contrast to TD B cell stimulation, TI stimulation induces a limited number of hypermutations in the Ig variable regions and has been proposed to occur outside GCs[117,118]. In addition to high-affinity IgA, low affinity IgA is produced in the LP[39]. Various studies have suggested that TI IgA responses produce natural polyreactive specificities with low affinity for commensal bacteria[119–121]. In vivo mouse experiments have frequently been used to study the contribution of TI stimulation to IgA+ B cell differentiation, but in humans, it was demonstrated that IgA+ memory B cells can differentiate independent of GCs[122]. Although there are reports describing that TI B cell activation can occur in the MLNs[86] and PP[39,86,117], a recent study proposed that TI IgA production in mucosal organized tissues is not the predominant mechanism[80]. We will therefore not further elaborate on the role of TI IgA production in mucosal lymphoid tissues but instead focus on RA-mediated IgA production outside of GCs. Typically, TI B cell activation requires DCs that have taken up luminal antigens. $CD103^+$ DCs can receive luminal antigens directly from goblet cells[25] or $CX3CR1^+$ macrophages[123] but can also take up these antigens via phagocytosis of luminal bacteria using their intraepithelial dendrites[124]. Once mucosal DCs are activated, they produce a range of cytokines, including BAFF and APRIL, to facilitate B cell activation independent of CD40 ligand[125,126]. Additionally, epithelial cells can secrete APRIL, depending on the composition of the microbiota, which further contributes to the creation of a suitable environment for B cells to undergo CSR[127]. Furthermore, B cells require TLR stimulation as well as BCR crosslinking with the corresponding antigen for TI antibody production. It is unclear whether IgA CSR occurs within the LP

itself, outside of organized lymphoid structures, as conflicting studies report on the absence/presence of markers for local CSR within LP B cells[118,128–130]. These inconsistencies in detecting markers for IgA CSR in the LP may be explained by the complexity of the techniques that have been used. Further support that IgA CSR occurs in the LP is derived from experiments using mouse models with dysfunctional T cell responses, which exhibited normal frequencies of IgA^+ B lymphocytes within the LP[117,118,131]. Similarly, mice that lack T cells have almost normal IgA coating of intestinal bacteria, except for a few atypical taxa[132], suggesting that a substantial part of the IgA repertoire produced against the microbiota is derived via TI activation[132]. Although these studies demonstrate that T cells are not essential for the development of IgA^+ B lymphocytes within the LP, they do not address whether IgA CSR actually takes place within the LP itself. In fact, B lymphocytes may become activated within gut lymphoid tissues, after which they migrate into the LP, where they complete CSR[117,133,134].

Indeed, IgM^+ B lymphocytes from the LP are pre-committed to class switching towards IgA, supporting the notion that the local RA-rich environment provided by DCs and stromal cells is important for inducing IgA+ B cell differentiation[129]. This is in line with the finding that IgM+ memory B cells express Iα-Cμ switch circle transcripts in the LP[77], suggesting that these B cells are primed to become IgA+ B cells. Naïve and IgM+ memory B cells may become activated in ILFs or PPs, where RA-DCs promote an IgA-inducing environment. As a result, B cells migrate into the LP, which also contains RA-DCs, to complete IgA CSR; this process has been shown in in vitro studies using LP-DCs and naïve B cells[135]. However, this does not represent what happens within the LP, as naïve B cells are not present in the LP. Moreover, low numbers of memory B cells can be found in the LP[74,77,136]. Mechanistically, stimulation of TLR5 on LP-DCs was shown to trigger the production of RA, together with IL5 and interleukin-6, which eventually skewed B lymphocytes towards IgA differentiation in vitro[38,135] (Fig. 7). IgA CSR may also occur in ILFs, which often do not have GC structures to provide TD B cell stimulation[77,137]. Similarly, AID expression and IgA CSR in B cells can still occur inside ILFs when T cells are lacking, showing that ILFs provide a TI stimulatory environment to promote IgA production[137]. Although unorganized ILFs retain AID expression, the extent to which they contribute to TI CSR is unclear, as IgM+ memory B cells from ILFs express markers associated with the post-GC response[77]. The contribution of ILFs to IgA production in the LP is relatively limited since mice without ILFs produce unaltered levels of faecal IgA[138]. Thus, it is likely that IgA CSR is predominantly initiated within the GC in an RA-dependent manner, with further completion within the LP, where RA-DCs facilitate B cell differentiation by providing an IgA-promoting environment.

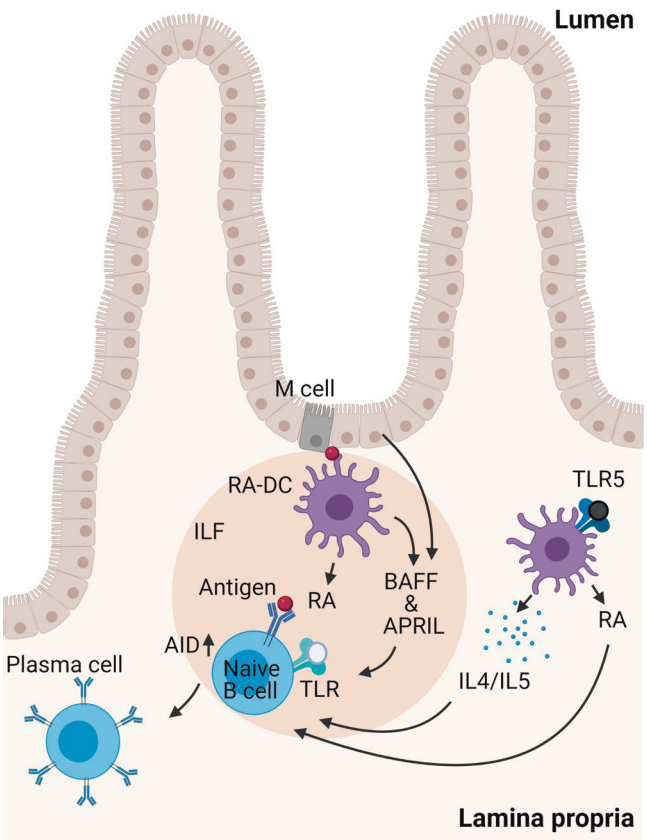

Fig. 7 Retinoic acid-producing dendritic cells facilitate the differentiation of IgA + B lymphocytes within isolated lymphoid follicles in a T cell-independent fashion. B cell activation within isolated lymphoid follicles (ILFs) is accomplished via multiple steps. Retinoic acid (RA)-producing dendritic cells (RA-DCs) retrieve antigens from microfold cells, which is necessary for B cell receptor (BCR) antigen recognition by B cells. Simultaneously, naïve B cells are stimulated via toll-like receptors (TLRs) and cofactors in the form of BAFF and APRIL produced by tolerogenic DCs and the epithelium. Secondary stimuli are produced after TLR5 activation by tolerogenic DCs. As a result, gut DCs secrete interleukin-4 (IL4), interleukin-5 (IL5) and RA to skew naïve B cells into an IgA isotype. Upon subsequent maturation, B lymphocytes leave the ILFs to become IgA-secreting plasma cells within the lamina propria (LP).

IMMUNOGLOBULIN A AND REGULATION OF MICROBIOTA HOMEOSTASIS

Vitamin A metabolism and the microbiota

Secreted mucosal IgA antibodies play an important role in mucosal tolerance. The tight interplay between the composition of the gut microbiota and production of mucosal IgA creates a homeostatic environment allowing commensal bacterial growth. Simultaneously, the mucosal immune system needs to balance its regulatory role with active readiness against pathogens[44,139]. Mucosal IgA recognizes and coats particular microbiota to prevent their translocation through the gut IEC layer. With the directed production of IgA, which recognizes the microbiota present within the gut lumen, host-microbe symbiosis is promoted, thereby safeguarding the composition and metabolic function of the gut microbiota[140]. The production of IgA against the gut microbiota is regulated by multiple processes, including direct sampling of luminal bacteria by DCs in the LP[141,142]. DCs within the MLNs and PPs harbour live commensals, which are required for B cells to undergo IgA differentiation in vitro[82]. Furthermore, commensal bacteria stimulate the enzymatic machinery in DCs to produce RA, which is important for DC-mediated skewing of B cells into an IgA isotype[38,46,47,143]. In line with this, multiple studies have demonstrated roles for RA in sustaining gut homeostasis and regulating the composition of the microbiota[144–147]. It was demonstrated that mice with low ALDH enzymatic activity secrete less IgA into the intestinal lumen. As a result, these mice show higher levels of bacterial translocation into the gut LP and MLNs than mice with high ALDH enzymatic activity[147]. Animals with insufficient intake of dietary vitamin A have an altered colonic microbiota diversity[144–146]. Similarly, loss of RA-DCs results in an altered microbiota composition, which makes mice more susceptible to intestinal *Citrobacter rodentium* infection[30]. Together, these data suggest that intestinal IgA antibody secretion, which is facilitated by RA-DCs, regulates mucosal tolerance under homeostatic conditions. As such, RA indirectly contributes to mucosal tolerance.

VITAMIN A METABOLISM AND INFLAMMATORY BOWEL DISEASE

Microbial homeostasis is lost in a variety of diseases, including inflammatory bowel disease (IBD). IBD is characterized by chronic inflammation of the gastrointestinal tract and disruption of the epithelial lining. IBD can be subdivided into two major forms, ulcerative colitis (UC) and Crohn's disease (CD)[148]. The production of mucosal IgA in these patients is altered compared to that in healthy donors. This was nicely illustrated in a study showing that the faecal bacteria of IBD patients were more abundantly opsonized with IgA compared to those of healthy individuals[149,150]; IBD patients also have increased levels of microbiota-specific IgA in the serum[151]. Transplantation of faecal IgA-coated bacterial strains from IBD patients into germ-free mice was shown to exacerbate DSS-induced colitis, suggesting that the IgA coating identifies colitogenic bacteria[150]. Accordingly, the percentage of IgA-opsonized bacteria in CD patients was found to strongly correlate with clinical indexes of disease activity[152]. Additionally, the composition of the microbiota of these patients was altered compared to that of healthy donors[153]. Together, these findings demonstrate a link among IBD pathogenesis, microbial composition and the ability of the immune system to produce specific IgA antibodies against the microbiota. It is, however, not clear which factors are causative for the alterations in the microbiota composition and which are consequential. Determining whether abnormalities in vitamin A metabolism can cause IBD development will require a closer look at possible associations of vitamin A pathway polymorphisms with IBD[154]. Such polymorphisms have been described; in particular, reduced ALDH1a1 and increased CYP26A1 levels have been linked to UC[155,156], whereas a CYP26B1 polymorphism resulting in higher levels of RA was associated with an increased risk of CD[155,157]. Moreover, ALDH activity is decreased in the intestinal DCs and macrophages of UC patients, both during active disease and in remission, compared to those of control individuals and CD patients[31]. It was suggested that the reduced serum vitamin A levels in UC patients correlate with a worse disease outcome[158]. However, additional studies have demonstrated altered vitamin A metabolism in CD patients as well, with decreased ALDH1A2 expression in IECs and an impaired ability to induce FoxP3-mediated differentiation in T lymphocytes[159]. Moreover, UC and CD onset is characterized by a damaged epithelial lining. As a result, it is possible that despite maintaining an intact ALDH enzymatic machinery, the epithelial cells of patients metabolize vitamin A inefficiently, as few viable epithelial cells are present. In contrast, the ALDH activity of gut DCs in CD patients was shown to be increased compared to that in healthy donors, which may reflect a compensatory mechanism[160].

Taken together, these data demonstrate that vitamin A metabolism can be altered during disease, with either reduced or enhanced RA production, making it difficult to therapeutically target this pathway. Nevertheless, a variety of studies have tested the efficacy of vitamin A (derivatives) in chronic intestinal inflammation, either in mouse models or in human clinical trials, and generally demonstrated a beneficial effect on disease pathology[161]. For instance, vitamin A-deficient mice developed more severe colitis and showed delayed recovery in different gut inflammation models, suggesting that RA has a protective effect in this context[147,162,163]. Moreover, the effect of vitamin A supplementation in UC patients was tested in a double-blinded randomized clinical trial, which demonstrated an RA-dependent increase in serum IgA levels and decreasing disease activity[164]. Nevertheless, due to the diverse processes by which RA affects the immune system, it is to be expected that therapeutic use of RA in IBD patients will result in a mixed treatment outcome, as IBD is a highly heterogenic disease.

CONCLUDING REMARKS

Vitamin A metabolism is a complex process that occurs in specific sites within the intestinal mucosa and plays a key role in sustaining gut homeostasis. Dietary vitamin A is processed by IECs, which allows the differentiation of RA-DCs. RA-DCs migrate to mucosal tissues, such as the MLNs and PPs, to provide B lymphocytes with exogenous RA to skew the B cells towards TD IgA differentiation. Moreover, RA-DCs regulate TI IgA production in non-organized tissues, such as the LP and ILFs. IgA is crucially involved in orchestrating the composition of the microbiota and sustaining healthy mucosal symbiosis. As a result, clinical trials have been initiated to study the effect of vitamin A on IBD pathogenesis, as IBD is characterized by altered IgA production and microbial dysbiosis. The mechanism by which RA-dependent IgA production can control microbial dysbiosis, which may underlie diseases such as IBD, is not yet understood.

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A.B. wrote the review. M.v.E. provided feedback on the content. R.M. provided feedback on the content and supervised the writing process.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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