

Vitamin effects on the immune system: vitamins A and D take centre stage

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Abstract | Vitamins are essential constituents of our diet that have long been known to influence the immune system. Vitamins A and D have received particular attention in recent years as these vitamins have been shown to have an unexpected and crucial effect on the immune response. We present and discuss our current understanding of the essential roles of vitamins in modulating a broad range of immune processes, such as lymphocyte activation and proliferation, T-helper-cell differentiation, tissue-specific lymphocyte homing, the production of specific antibody isotypes and regulation of the immune response. Finally, we discuss the clinical potential of vitamin A and D metabolites for modulating tissue-specific immune responses and for preventing and/or treating inflammation and autoimmunity.

“A vitamin is a substance that makes you ill if you don’t eat it.” (Albert Szent-Gyorgyi, Nobel Prize in Physiology or Medicine, 1937).

The statement by Albert Szent-Gyorgyi epitomizes the impact of vitamins on the body’s vital organs, including the immune system. Vitamins (*vital amines*) are organic compounds that are required in trace amounts in the diet because they cannot be synthesized in sufficient quantities by an organism¹. Vitamins and their metabolites are essential for a large number of physiological processes, fulfilling diverse functions as hormones and antioxidants, as regulators of tissue growth and differentiation, in embryonic development and in calcium metabolism, among others¹.

In addition, vitamins have a role in the immune system, which extends to both innate and adaptive immune responses. Although some vitamins, such as vitamins C and E and members of the B complex, can act in a relatively nonspecific manner in the immune system (for example, as antioxidants)^{2–4}, other vitamins, such as vitamins A and D, can influence the immune response in highly specific ways (see [Supplementary information S1](#) (table)). Here we review the most important effects of vitamins on the immune system, with special emphasis on vitamins A and D, which have received particular attention owing to recent discoveries of their multi-faceted interactions with the immune system. Vitamins A and D are notably distinct from other vitamins in that their respective bioactive metabolites, retinoic acid and 1,25-dihydroxyvitamin D₃

(1,25(OH)₂VD₃), have hormone-like properties. Both of these metabolites are synthesized from their vitamin precursors by different tissues and cells in the body and exert their effects on target cells remotely by binding to nuclear-hormone receptors.

Vitamin metabolism in the immune system

Vitamin D. Vitamin D₃ (VD₃), the most physiologically relevant form of vitamin D, is synthesized in the skin from 7-dehydrocholesterol⁵, a process which depends on sunlight, specifically ultraviolet B radiation (wavelengths of 270–300 nm). Alternatively, it can be acquired in the diet or in vitamin supplements⁵ (FIG. 1a). VD₃ is then converted in the liver to 25-dihydroxyvitamin D₃ (25(OH)VD₃), which is the main circulating form of VD₃. Finally, 25(OH)VD₃ is metabolized in the kidneys to 1,25(OH)₂VD₃, the most physiologically active VD₃ metabolite⁵. In addition to being processed in the liver and the kidneys, VD₃ can also be metabolized by cells of the immune system^{5,6} (FIG. 1a). In this way, 1,25(OH)₂VD₃ is concentrated locally in those lymphoid microenvironments that contain physiologically high concentrations of VD₃, thereby increasing its specific action and also limiting potentially undesirable systemic effects, such as hypercalcaemia and increased bone resorption⁷.

Activated T cells (and probably also B cells) can only perform the final step of converting 25(OH)VD₃ to 1,25(OH)₂VD₃ (REFS 6,8). However, macrophages and some dendritic cells (DCs), such as monocyte-derived DCs and dermal DCs, express the two sets of enzymes needed to convert VD₃ into 1,25(OH)₂VD₃ (REFS 6,7,9).

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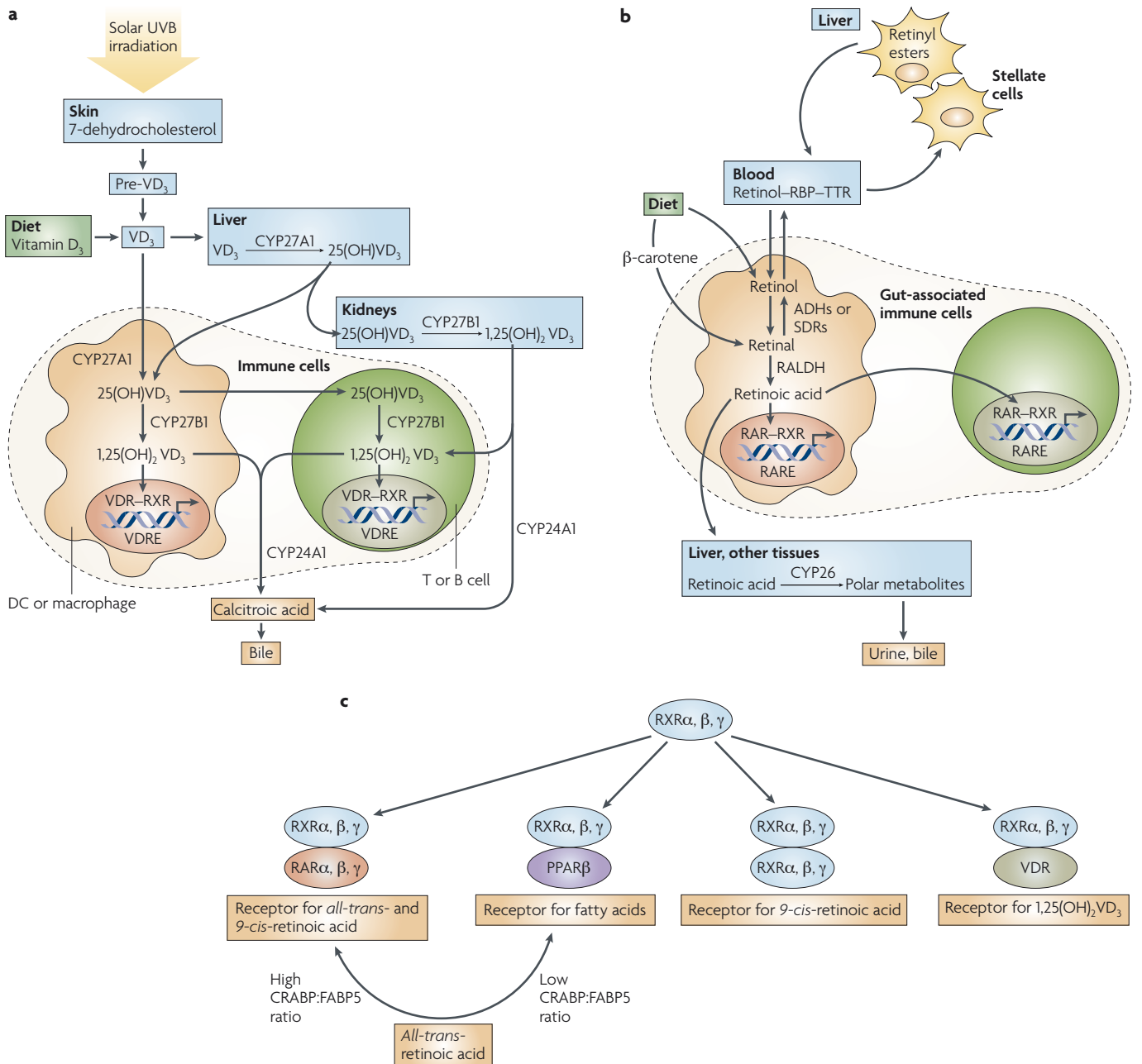


Figure 1 | Overview of vitamin A and D metabolism. **a** | Vitamin D₃ (VD₃) is acquired in the diet or synthesized in the skin and hydroxylated in the liver to 25(OH)VD₃, the main circulating form. 25(OH)VD₃ is then hydroxylated in the kidneys by the cytochrome P450 protein CYP27B1 to become 1,25(OH)₂VD₃, the physiologically most active metabolite, which then reaches the blood where it has multiple systemic effects. Cells of the immune system, including macrophages, dendritic cells (DCs), T and B cells express the enzymes CYP27A1 and/or CYP27B1, and therefore can also hydroxylate 25(OH)VD₃ to 1,25(OH)₂VD₃. 1,25(OH)₂VD₃ acts on immune cells in an autocrine or paracrine manner by binding to the vitamin D receptor (VDR). 24-hydroxylase (CYP24A1) catabolizes 1,25(OH)₂VD₃ to its inactive metabolite, calcitric acid, which is excreted in the bile. **b** | Vitamin A (also known as retinol) is obtained from the diet and transported in the blood as a complex with retinol-binding protein (RBP) and transthyretin (TTR). In the liver, retinol is esterified to retinyl esters and stored in stellate cells. In other tissues, including gut-associated immune cells, retinol is oxidized to retinal by alcohol dehydrogenases (ADHs) or short chain dehydrogenase/reductases (SDRs). Retinal is then oxidized to all-trans-retinoic acid in an irreversible reaction that is catalysed by retinal dehydrogenases (RALDHs). Retinoic acid acts on immune cells by binding to the retinoic acid receptor (RAR). Retinoic acid is catabolized in the liver and in other tissues by the enzyme CYP26 and its metabolites are eliminated in the bile and urine. **c** | Retinoid X receptors (RXRs) can form RXR–RAR (receptor for all-trans- and 9-cis-retinoic acid), RXR–PPARβ (peroxisome-proliferator-activated receptor β) (receptor for fatty acids), RXR–RXR (receptor for 9-cis-retinoic acid), or RXR–VDR (receptor for 1,25(OH)₂VD₃) complexes. The ratio between cellular retinoic acid-binding proteins (CRABPs) and fatty acid-binding protein 5 (FABP5) might determine whether retinoic acid signals through RAR–RXR or PPARβ–RXR, leading to different functional outcomes. RARE, retinoic acid response element; VDRE, VD response element.

Finally, the enzyme 24-hydroxylase, which is most abundant in the kidney and intestine¹⁰, catabolizes 1,25(OH)₂VD₃ to its inactive metabolite, calcitric acid, which is then excreted in the bile.

Vitamin A. Vitamin A is obtained from the diet either as *all-trans*-retinol, retinyl esters or β-carotene^{11,12} (FIG. 1b). *All-trans*-retinol is esterified to retinyl esters and stored in the liver, mostly in the stellate cells^{11,12}. In the tissues, *all-trans*-retinol and β-carotene are oxidized to *all-trans*-retinal by alcohol dehydrogenases or short chain dehydrogenase reductases, which are ubiquitously expressed enzymes^{11,12}. *All-trans*-retinal is then oxidized to *all-trans*-retinoic acid through an irreversible reaction catalysed by retinal dehydrogenases (RALDHs), the expression of which is tightly controlled. A related metabolite of *all-trans*-retinal, 9-*cis*-retinoic acid, can be formed either by spontaneous isomerization of *all-trans*-retinoic acid or through oxidation of 9-*cis*-retinal by RALDH¹³. However, 9-*cis*-retinoic acid has not been shown to be synthesized *in vivo*¹³.

In adult mammals, RALDH can be found in some gut-associated cells, including intestinal epithelial cells (IECs)^{14,15} and gut-associated DCs, such as DCs from Peyer's patches and mesenteric lymph nodes^{15,16}. Interestingly, DCs from Peyer's patches express RALDH-1 mRNA and protein, whereas DCs from mesenteric lymph nodes express mRNA encoding RALDH-2. Although the functional relevance of this differential RALDH isoform expression is currently unclear, it indicates that there might be more than one pathway or environmental stimulus that renders DCs capable of synthesizing retinoic acid from vitamin A. In addition, IECs also express RALDH-1 and can metabolize vitamin A to retinoic acid *in vitro*¹⁴, which indicates that they are another potential source of retinoic acid in the gut mucosa. The relative contributions and *in vivo* relevance of these different sources of retinoic acid in the gut are yet to be determined.

Nuclear receptors for vitamin metabolites

Locally produced 1,25(OH)₂VD₃ can act on immune cells in an autocrine or paracrine manner. On complexing with 1,25(OH)₂VD₃, the nuclear vitamin D receptor (VDR) heterodimerizes with nuclear receptors of the retinoic X receptor (RXR) family — which has three main isoforms: α, β and γ — and binds to VD₃ response elements (VDREs) in the promoters of VD₃-responsive genes (FIG. 1c).

Similarly, retinoic acid exerts its multiple effects by binding to nuclear receptors of the retinoic acid receptor (RAR) family, which also has three main isoforms: α, β and γ. These form RAR–RXR heterodimers, which interact with retinoic acid response elements (RAREs) within the promoters of retinoic acid-responsive genes^{11,12}. RAR proteins are ubiquitously expressed and are also upregulated by retinoic acid^{11,12}. As mentioned above, RXR proteins can also pair with VDR proteins or form RXR–RXR homodimers, which are specific receptors for 9-*cis*-retinoic acid but not for *all-trans* retinoic acid (hereafter referred to as retinoic

acid) (although 9-*cis*-retinoic acid can also signal through RAR–RXR heterodimers). In addition, RXR proteins are partners for other nuclear receptors, such as thyroid hormone receptor, peroxisome proliferator-activated receptor (PPAR) and liver X receptor, among others¹⁷. Therefore, it is possible that, given their common RXR nuclear binding partners, some ligands, such as 1,25(OH)₂VD₃ and retinoic acid, might antagonize each other's effects¹⁸.

Notably, retinoic acid can also bind and signal through the PPARβ (also known as PPARδ) nuclear receptor¹⁹. Whether signalling occurs through RAR or PPARβ depends on the ratio of cellular retinoic acid-binding proteins (CRABPs) to fatty acid-binding protein 5 (FABP5), which ultimately determines the partitioning of retinoic acid between the two types of receptors¹⁹. In this model, a high CRABP:FABP5 ratio promotes RAR signalling by CRABP-mediated 'channelling' of retinoic acid to RAR, which results in growth inhibition and apoptosis in some cell lines, whereas a low ratio favours FABP5-mediated delivery of retinoic acid to PPARβ and survival in the same cells¹⁹. However, although this is a potentially attractive mechanism for regulating retinoic acid responses, its significance in the immune system is yet to be determined.

Immunomodulatory role of vitamin D

The influence of VD₃ metabolites in the immune system, particularly of 1,25(OH)₂VD₃, has been known for more than 20 years^{20,21}. *In vitro*, 1,25(OH)₂VD₃ exerts a marked inhibitory effect on adaptive immune cells (FIG. 2). It inhibits T-cell proliferation^{20,21}, the expression of interleukin-2 (IL-2)^{21–23} and interferon-γ (IFNγ) mRNA and protein in T cells^{24,25}, and CD8 T-cell-mediated cytotoxicity²⁶. The decrease in the production of IL-2 and IFNγ by 1,25(OH)₂VD₃ is partially mediated by binding of the VDR–RXR complex to the VDRE in the promoters of genes encoding IL-2 (REF. 27) and IFNγ (REF. 28). The anti-proliferative effect could be explained, at least in part, by the decrease in IL-2 production, as proliferation is partially rescued by adding exogenous IL-2 (REFS 21, 22, 29). These inhibitory effects of 1,25(OH)₂VD₃ are most pronounced in the memory T-cell compartment³⁰, which is concomitant with the higher expression of VDR in effector and memory T cells compared with naive T cells³¹. Moreover, 1,25(OH)₂VD₃ enhances nonspecific T-cell suppressor activity, as measured by the ability of 1,25(OH)₂VD₃-treated T cells to suppress primary mixed-lymphocyte reactions and cytotoxic T-cell responses²⁶.

Overall, the net result of 1,25(OH)₂VD₃ action on T cells is to block the induction of T-helper-1 (T_H1)-cell cytokines, particularly IFNγ, while promoting T_H2-cell responses, an effect mediated both indirectly by decreasing IFNγ production and directly by enhancing IL-4 production⁷. The activity of 1,25(OH)₂VD₃ on effector T-cell differentiation is further enhanced by its effect on antigen-presenting DCs, in which it suppresses the synthesis of IL-12, a cytokine that promotes T_H1-cell responses^{32,33}. Furthermore, 1,25(OH)₂VD₃ also inhibits T_H17-cell responses, probably owing in part to its capacity

Stellate cells

(Also known as Ito cells). Types of pericytes found in the hepatic perisinusoidal space that are the main reservoirs of retinol in the liver.

Intestinal epithelial cells

(IECs). A tight monolayer of cells covering the luminal surface of the intestine. They are specialized in the absorption of nutrients and also serve as a mechanical and immunological barrier with the external environment (the intestinal lumen).

Peyer's patches

Groups of lymphoid nodules present in the small intestine (usually the ileum). They are found massed together on the intestinal wall, opposite the line of attachment of the mesentery. Peyer's patches consist of a dome area, B-cell follicles and interfollicular T-cell areas.

Mesenteric lymph nodes

(MLNs). Lymph nodes located at the base of the mesentery. They collect lymph (including cells and antigens) draining from the intestinal mucosa.

T_H17 cells

(T helper 17 cells). A subset of CD4⁺ T helper cells that produce interleukin-17 (IL-17) and are thought to be important in inflammatory and autoimmune diseases. Their generation involves TGFβ, IL-6, IL-23 or IL-21, IL-1β and the transcription factor RORγt.

to inhibit IL-6 and IL-23 production^{34,35}, and induces the reciprocal differentiation and/or expansion of forkhead box protein 3 (FOXP3)⁺ regulatory T (T_{Reg}) cells^{35–37}.

In addition to its inhibitory effects on T cells, 1,25(OH)₂VD₃ decreases B-cell proliferation, plasma-cell differentiation and IgG secretion^{8,20} (FIG. 2). It has been suggested that the effect of 1,25(OH)₂VD₃ on B cells might be indirectly mediated through the effect it has on antigen-presenting-cell (APC) function and/or T-cell help³⁸. Indeed, there are conflicting reports concerning the expression of VDR by B cells^{8,31,39}, leaving it unclear whether 1,25(OH)₂VD₃ can act directly on B cells.

Interestingly, 1,25(OH)₂VD₃ inhibits mitogen-stimulated IgG production by B cells from patients with inactive systemic lupus erythematosus (SLE), but not the spontaneous IgG production by cells from patients with active SLE⁴⁰. Thus, it is possible that fully differentiated memory B cells and/or antibody-secreting cells (ASCs) are refractory to 1,25(OH)₂VD₃-mediated inhibition. In addition, serum levels of 1,25(OH)₂VD₃ are significantly decreased in patients with SLE, especially during active disease⁸. So, it is tempting to speculate that a decreased level of 1,25(OH)₂VD₃ could have an exacerbating role in SLE pathogenesis by releasing a basal physiologic or 'tonic' brake in humoral immunity.

Cells of the innate immune system can also be inhibited by 1,25(OH)₂VD₃ (FIG. 2), which is known to inhibit the differentiation, maturation and immunostimulatory

capacity of DCs by decreasing the expression of MHC class II molecules and of CD40, CD80 and CD86 (REFS 7,9,33,41). Furthermore, VDR-deficient mice have increased numbers of mature DCs in skin-draining lymph nodes⁴¹. In addition, 1,25(OH)₂VD₃ decreases the synthesis of IL-12 (REFS 32,33) and simultaneously increases the production of IL-10 by DCs³³. The net result is a decrease in T_H1-cell responses and probably an induction of IL-10-producing T regulatory type 1 (T_R1) cells⁷.

Although 1,25(OH)₂VD₃ primarily has inhibitory effects on the adaptive immune response, some of its effects on innate immune cells are stimulatory. For example, 1,25(OH)₂VD₃ can stimulate human monocyte proliferation *in vitro*⁴² and has been shown to increase the production of both IL-1 and the bactericidal peptide cathelicidin by monocytes and macrophages^{5,23}.

Unexpectedly, in spite of the potentially important role of 1,25(OH)₂VD₃ in maintaining immune homeostasis, VDR-deficient mice have a normal composition of immune-cell populations and reject allogeneic and xenogeneic transplants at the same rate as wild-type mice⁴³. However, to carefully dissect the *in vivo* effects of VD₃ metabolites on the immune response, it will be necessary to study the effect of VD₃ in animal models in which VDR deficiency is restricted to T cells, B cells and myeloid cells.

T_{Reg} cells
(Regulatory T cells). Specialized types of CD4⁺ T cells that can suppress the effector responses of other immune cells. These cells provide a crucial mechanism for the maintenance of peripheral self-tolerance and are characterized by the expression of the transcription factor forkhead box P3.

Systemic lupus erythematosus (SLE). An autoimmune disease in which autoantibodies specific for DNA, RNA or proteins associated with nucleic acids form immune complexes. These complexes damage small blood vessels, especially in the kidneys. Patients with SLE generally have abnormal B- and T-cell function as well as rashes, arthritis, kidney disease and central-nervous-system involvement.

Antibody-secreting cells (ASCs). Cells specialized in secreting immunoglobulins. Although they originate from activated B cells, ASCs lose the expression of surface immunoglobulins and other B-cell markers and upregulate plasma cell markers, such as CD138 in mice or CD27 in humans.

T_R1 cells
(T regulatory type 1 cells)
A population of regulatory T cells that arises in the periphery after an encounter with antigen in the presence of interleukin-10 (IL-10) and that regulates immune responses through the secretion of IL-10 and transforming growth factor-β. They suppress T-cell responses, downregulate the expression of co-stimulatory molecules and pro-inflammatory cytokines by antigen-presenting cells and favour the production of IgD, IgA and IgG by B cells.

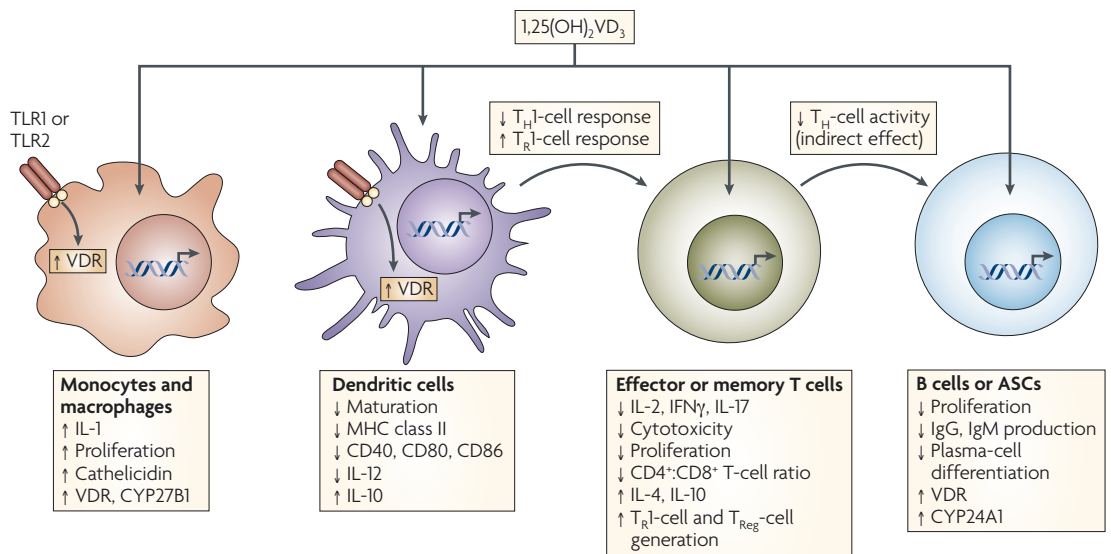


Figure 2 | Mechanisms of vitamin D immunomodulation. Systemic or locally produced 1,25(OH)₂VD₃ exerts its effects on several immune-cell types, including macrophages, dendritic cells (DCs), T and B cells. Macrophages and DCs constitutively express vitamin D receptor (VDR), whereas VDR expression in T cells is only upregulated following activation. In macrophages and monocytes, 1,25(OH)₂VD₃ positively influences its own effects by increasing the expression of VDR and the cytochrome P450 protein CYP27B1. Certain Toll-like-receptor (TLR)-mediated signals can also increase the expression of VDR. 1,25(OH)₂VD₃ also induces monocyte proliferation and the expression of interleukin-1 (IL-1) and cathelicidin (an antimicrobial peptide) by macrophages, thereby contributing to innate immune responses to some bacteria. 1,25(OH)₂VD₃ decreases DC maturation, inhibiting upregulation of the expression of MHC class II, CD40, CD80 and CD86. In addition, it decreases IL-12 production by DCs while inducing the production of IL-10. In T cells, 1,25(OH)₂VD₃ decreases the production of IL-2, IL-17 and interferon-γ (IFNγ) and attenuates the cytotoxic activity and proliferation of CD4⁺ and CD8⁺ T cells. 1,25(OH)₂VD₃ might also promote the development of forkhead box protein 3 (FOXP3)⁺ regulatory T (T_{Reg}) cells and IL-10-producing T regulatory type 1 (T_R1) cells. Finally, 1,25(OH)₂VD₃ blocks B-cell proliferation, plasma-cell differentiation and immunoglobulin production. ASCs, antibody-secreting cells.

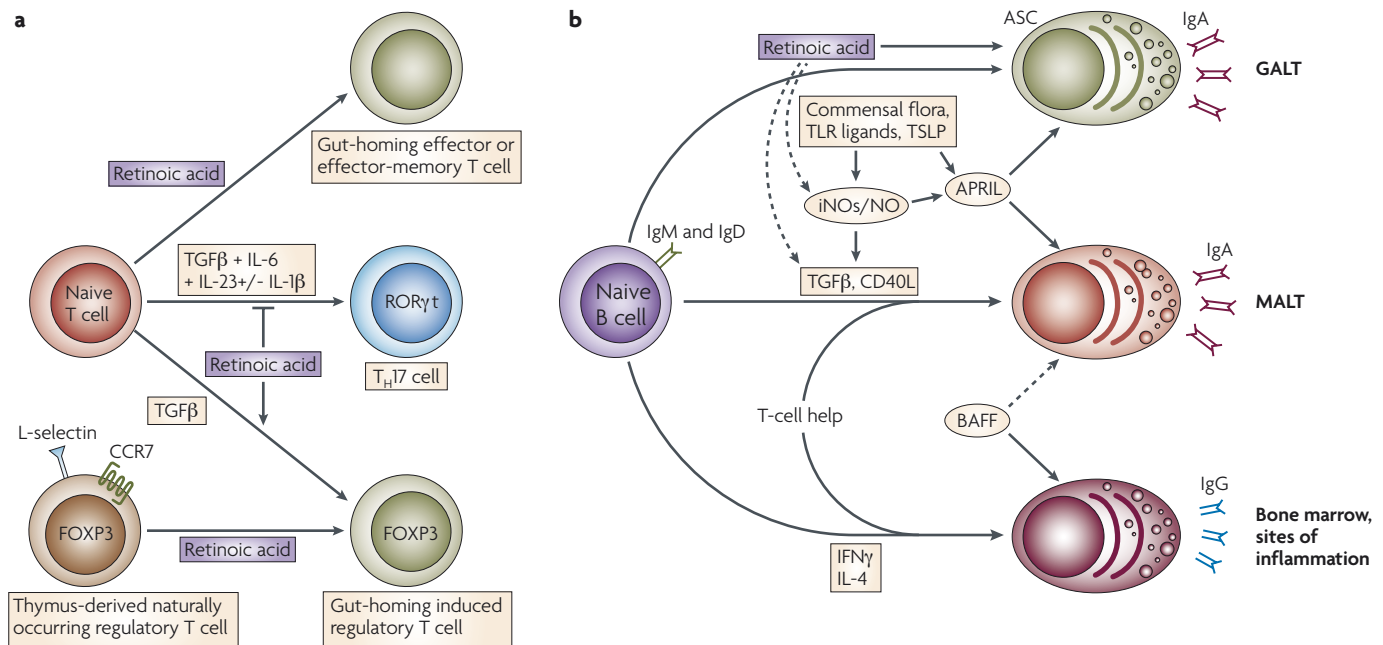


Figure 3 | Effects of vitamin A metabolites on gut mucosal immunity. a | In addition to upregulating the expression of gut-homing receptors, retinoic acid has also been reported to promote T-helper-2 (T_H2)-cell differentiation. Moreover, retinoic acid blocks the differentiation of T helper 17 (T_H17) cells and induces forkhead box protein 3 (FOXP3)⁺ regulatory T (T_{Reg}) cells in the presence of transforming growth factor- β (TGF β) by reciprocally downregulating receptor-related orphan receptor- γ t (ROR γ t) and inducing FOXP3 expression in T cells, respectively. Retinoic acid also enhances the TGF β -driven induction of T_{Reg} cells and induces gut-homing receptor expression in both naturally occurring and induced T_{Reg} cells. T_H17 -cell differentiation requires TGF β , interleukin-6 (IL-6), IL-23 and, in humans, IL-1 β . **b** | B cells activated in non-mucosal lymphoid tissues, such as peripheral lymph nodes and spleen, mostly become IgG⁺ antibody-secreting cells (ASCs) and home to the bone marrow and sites of inflammation. By contrast, B cells activated in mucosal-associated lymphoid tissues (MALT) give rise to IgA⁺ ASCs. In MALT (including the gut-associated lymphoid tissue; GALT), TGF β and CD40 ligand (CD40L) are essential for the generation of T-cell-dependent IgA responses, whereas BAFF (B-cell-activating factor) and APRIL (a proliferation-inducing ligand) are important for T-cell-independent IgA responses. APRIL is induced by Toll-like receptor (TLR) signals, commensal flora and thymic stromal lymphopoietin (TSLP). Inducible nitric oxide synthase (iNOS), which is also upregulated by TLR signals and commensal flora, produces nitric oxide (NO), allows proper TGF β signalling and induces the production of APRIL and BAFF by dendritic cells. Thus, iNOS and NO are essential for both T-cell-dependent and -independent IgA responses. In the GALT, retinoic acid might contribute directly to the differentiation of T-cell-independent (and probably also T-cell-dependent) IgA⁺ ASCs. In addition, retinoic acid might contribute indirectly to T-cell-dependent and -independent IgA responses by inducing iNOS expression.

Immunomodulatory role of vitamin A

Effects on adaptive immune-cell subsets. Vitamin A metabolites can also affect some aspects of the adaptive immune response (FIG. 3). Retinoic acid enhances cytotoxicity⁴⁴ and T-cell proliferation⁴⁵, the latter probably mediated, at least in part, by enhancing IL-2 secretion and signalling in T cells⁴⁵. Consistent with an *in vivo* role for vitamin A in T-cell function, vitamin A-deficient mice have defects in T_H -cell activity⁴⁶. A possible mechanism for this observation is that in the setting of vitamin A deficiency, retinoic acid does not compete with 1,25(OH)₂VD₃ for their common nuclear binding partner RXR and, therefore, the inhibitory effects of 1,25(OH)₂VD₃ on T-cell function (including T_H -cell activity) are not offset by retinoic acid.

Retinoic acid can inhibit B-cell proliferation^{47,48}, although it has also been found to enhance B-cell activation under some conditions^{49,50}. In addition, retinoic acid inhibits B-cell apoptosis. These effects are mediated through binding of vitamin A metabolites to RAR receptors⁵¹.

Notably, it has been reported that a distinct set of vitamin A metabolites classified as retro-retinoids can also affect general lymphocyte functions such as B-cell proliferation⁵² and T-cell activation and proliferation⁵². 14-hydroxy-retroretinol (14HRR) has a positive effect on proliferation, whereas anhydroretinol blocks B-cell proliferation and induces apoptosis in T cells⁵³. Retro-retinoids do not signal through RAR or RXR and, as 14HRR and anhydroretinol can antagonize each other's effects, it has been suggested that they might compete for a common, as-yet unknown receptor⁵³.

Retinoic acid can also modulate antigen presentation by exerting direct effects on DC function. For example, retinoic acid increases the expression of matrix metalloproteinases, thereby increasing the migration of tumour-infiltrating DCs to the draining lymph nodes, which have the potential to boost tumour-specific T-cell responses⁵⁴. In addition, in the presence of inflammatory stimuli, such as tumour-necrosis factor (TNF), retinoic acid enhances DC maturation and antigen-presenting capacity, both of which are effects mediated

by RXR receptors⁵⁵. However, it should be noted that DCs pre-treated with retinoic acid can apparently store this metabolite⁵⁰, which when released could ultimately act directly on T cells and/or other cells and contribute to the final outcome of an immune response.

Vitamin A metabolites also modulate more specific functional aspects of the immune response, such as the T_H1 – T_H2 -cell balance and the differentiation of T_{Reg} cells and T_H17 cells (FIG. 3a). Vitamin A deficiency correlates with decreased T_H2 -cell responses⁵⁶ and, conversely, vitamin A supplementation blocks the production of T_H1 -cell cytokines *in vitro* and *in vivo*^{57,58}. These effects of vitamin A on T_H1 – T_H2 -cell differentiation are mediated by retinoic acid. In fact, retinoic acid promotes T_H2 -cell differentiation by inducing *IL4* gene expression⁵⁹. Moreover, retinoic acid blocks the expression of the T_H1 -cell master regulator T-bet and induces T_H2 -cell-promoting transcription factors, such as GATA3 (GATA-binding protein 3), macrophage-activating factor (MAF) and signal transducer and activator of transcription 6 (STAT6)^{57,60}. Interestingly, vitamin A supplementation was correlated with an increase in disease severity in a mouse model of asthma, whereas vitamin A deficiency had the opposite effect, which was associated with a decrease in T_H2 -cell cytokines⁶¹. It has been proposed that retinoic acid exerts its T_H2 -cell-promoting effect indirectly through the modulation of APCs⁶². However, retinoic acid can also act directly on T cells to induce T_H2 -cell differentiation^{57,60} through RAR proteins⁵⁷.

Several types of T cells that have dominant immunomodulatory effects have been described. The best characterized are T_{Reg} cells that express the transcription factor FOXP3. Although transforming growth factor- β (TGF β) drives the generation of induced T_{Reg} cells in peripheral tissues⁶³, it was recently demonstrated by several groups that this process can be significantly enhanced by retinoic acid^{64,65} (FIG. 3a). In addition, DCs from the gut-associated lymphoid tissue (GALT) or small intestinal lamina propria also enhance T_{Reg} -cell differentiation in a retinoic acid-dependent manner^{16,66}. Notably, one recent study indicated that macrophages and not DCs are responsible for inducing T_{Reg} cells in the intestinal lamina propria⁶⁷, and that DCs are mainly involved in the induction of T_H17 cells at this site^{67,68}. The reasons for these seemingly discrepant results are unclear. In addition to inducing FOXP3, retinoic acid also upregulates gut-homing receptors on T_{Reg} cells, targeting these cells to the gut mucosa^{65,66}. The relative contribution of *in situ*-generated gut T_{Reg} cells to both oral and peripheral tolerance is yet to be determined.

The differentiation of T_{Reg} cells and T_H17 cells is reciprocally regulated by cytokine signals⁶⁹. Exposure of activated CD4⁺ T cells to TGF β alone induces T_{Reg} cells, whereas the combination of TGF β with IL-6, IL-1 β and IL-23 or IL-21 blocks FOXP3 induction and induces T_H17 -cell differentiation^{69,70}. However, exposure of CD4⁺ T cells to retinoic acid together with TGF β and IL-6 negates the T_H17 -cell-promoting effect of IL-6 by enhancing the induction of T_{Reg} cells and blocking the induction of retinoic-acid-receptor-related orphan receptor- γ t (ROR γ t), a key transcription factor for T_H17 -cell differentiation^{64,71}.

It should be noted that this effect is only observed when retinoic acid is used over a certain threshold concentration⁶⁸. In fact, low concentrations of retinoic acid seem to be necessary for T_H17 -cell differentiation⁶⁸. So, retinoic acid has a dual role in maintaining immunological tolerance: it favours the induction of T_{Reg} cells and can simultaneously either block or enhance T_H17 -cell differentiation, depending on its concentration.

Effects on immunoglobulin isotypes. An important feature of activated B cells is their capacity to undergo immunoglobulin class-switching and to give rise to different antibody isotypes. T_H1 - and T_H2 -cell cytokines differentially influence antibody class-switching: the T_H1 -cell cytokine IFN γ promotes switching to IgG2a and IgG3, whereas the T_H2 -cell cytokine IL-4 induces the production of IgG1 and IgE and suppresses the generation of IgG2b and IgG3 (REF. 72). DCs can also modulate B-cell activation and antibody class-switching⁷³, which could occur indirectly by influencing T_H -cell differentiation⁷⁴. However, DCs can also directly promote the induction of specific immunoglobulin isotypes. GALT-resident DCs efficiently induce the generation of IgA⁺ ASCs when cultured with activated B cells *in vitro*^{75,76}. Several cytokines and other bioactive factors are involved in the capacity of GALT-resident DCs to induce IgA⁺ ASCs, including TGF β 1 (REFS 16,67,74,77), IL-6 (REFS 75,76), APRIL (a proliferation-inducing ligand)⁷⁸ and nitric oxide⁷⁹.

GALT- and lamina propria-resident DCs also contribute to the generation of IgA⁺ ASCs by a mechanism depending, at least in part, on retinoic acid^{68,76} (FIG. 3b). The presence of retinoic acid efficiently induces IgA secretion by lipopolysaccharide (LPS)-activated splenocytes⁸⁰ or by LPS-stimulated B cells cultured with spleen DCs⁶⁸. However, the presence of retinoic acid during the activation of purified B cells is not sufficient to impart these effects^{68,76}. Retinoic acid-induced IgA secretion requires either IL-5 (REFS 76,80) or IL-6 (REFS 75,76), which are known to possess a general adjuvant role in IgA production^{73,81,82}. In fact, both retinoic acid and IL-6 are required for GALT-resident DCs to induce optimal IgA production by mouse and human B cells *in vitro*^{75,76}. Vitamin A-depleted mice have decreased numbers of IgA⁺ ASCs in the small bowel lamina propria^{76,83}, consistent with an *in vivo* role for retinoic acid in gut mucosal IgA responses. In addition, oral administration of a RAR agonist significantly increases serum IgA levels in rats⁸⁴. Interestingly, exposure to retinoic acid together with IL-6 or IL-5 is not sufficient to induce IgA⁺ ASCs from *in vitro*-activated naive B cells in the absence of DCs⁷⁶. Therefore, it is likely that DCs provide some necessary B-cell survival and differentiation signals that are not tissue-specific.

It was recently shown that inducible nitric oxide synthase (iNOS; also known as NOS2A) and nitric oxide are crucial for the generation of IgA⁺ ASCs⁷⁹. Interestingly, the promoter of the *iNOS* gene contains a RARE that is directly activated by retinoic acid bound to its nuclear RAR α –RXR heterodimeric receptor⁸⁵. Moreover, intra-peritoneal administration of either retinoic acid or a

Gut-associated lymphoid tissue

(GALT). Lymphoid structure associated with the intestinal mucosa, including cryptopatches, isolated lymphoid follicles, Peyer's patches and caecal and colonic patches.

Small intestinal lamina propria

Connective tissue between the intestinal epithelium and the intestinal muscularis mucosae layer, which contains various myeloid and lymphoid cells, including macrophages, dendritic cells, T cells and B cells.

RAR agonist enhances LPS-induced iNOS expression in several organs and increases plasma levels of nitrate and nitrite in rats⁸⁶. Therefore, retinoic acid might also indirectly contribute to IgA secretion by inducing iNOS and nitric oxide expression.

Precursors of retinoids in the diet (which include β -carotene and retinyl esters) are absorbed from the gut lumen and can be metabolized to produce retinoic acid in IECs¹⁴ and in DCs that reside near the germinal centres in Peyer's patches¹⁵. The proximity of Peyer's patches to the gut bacterial flora and to other potential sources of retinoic acid and cytokines, such as IECs, could help to explain the predominance of IgA class-switching that occurs in Peyer's patches compared with mesenteric lymph nodes⁸⁷. Consistent with a role for vitamin A in gut IgA production, rats or mice depleted of vitamin A have decreased levels of total IgA in intestinal lavages and decreased mucosal antigen-specific IgA responses, which correlates with decreased protection against infections and oral bacterial toxins^{56,88,89}. Furthermore, vitamin A supplementation prevents the decline in IgA levels observed in malnourished mice⁵⁸. In addition to a direct effect on ASCs, it should be considered that vitamin A deficiency could also decrease IgA secretion in the gut by reducing the expression of the polymeric immunoglobulin receptor, leading to a decrease in the secretion of dimeric IgA to the gut lumen^{88,89}. Moreover, although vitamin A-deficient mice have decreased numbers of IgA⁺ ASCs in the small bowel^{76,83}, their serum IgA levels are normal⁷⁶, which indicates that retinoids are not absolutely required for generating IgA⁺ ASCs in other mucosal compartments.

Vitamin metabolites and lymphocyte homing

Although naive lymphocytes migrate mainly through secondary lymphoid organs, effector and memory lymphocytes acquire 'traffic' molecules that endow them with the capacity to migrate to select extralymphoid tissues and to sites of inflammation. Of the extralymphoid compartments, the gastrointestinal mucosa and the skin are the two main body surfaces exposed to environmental antigens and are also the two paradigmatic tissues for which tissue-specific adhesion and chemoattractant receptors (also known as homing receptors) have been characterized in detail. For example, effector and memory lymphocytes migrating to the small bowel require expression of the $\alpha_4\beta_7$ -integrin and CC-chemokine receptor 9 (CCR9), whereas those migrating to the skin rely on the expression of ligands for E- and P-selectin and CCR4 or CCR10 (REF. 73) (FIG. 4a). It has been demonstrated that the lymphoid microenvironment in which lymphocytes are activated determines the set of homing receptors that they acquire — for example, T cells activated in skin-draining lymph nodes acquire skin-homing receptors whereas those activated in the GALT acquire gut-homing receptors⁹⁰. In the lymphoid microenvironment, DCs are essential for efficient T-cell activation⁹¹. Several groups have shown that DCs from Peyer's patches and mesenteric lymph nodes are sufficient to induce the expression of $\alpha_4\beta_7$ -integrin and CCR9 and therefore imprint gut-homing

capacity on activated mouse T cells^{92–96} and mouse and human B cells^{68,76}, whereas lymphocytes activated by mouse DCs from peripheral lymph nodes preferentially acquire skin-homing receptors^{95,96}.

How do GALT-resident DCs imprint lymphocytes with a gut-homing phenotype? More than 25 years ago it was shown that rats suffering from both protein-caloric and vitamin A deficiencies exhibited impaired migration of recently activated mesenteric lymphocytes to the small intestinal mucosa⁹⁷. Protein-caloric malnutrition without vitamin A deficiency did not affect lymphocyte migration⁹⁷. Adoptive transfer experiments showed that impaired lymphocyte migration was observed only when donor lymphocytes were from protein-caloric-deficient and vitamin A-deficient rats and not when wild-type cells were transferred to protein-caloric-deficient and vitamin A-deficient recipients⁹⁷. This suggests that vitamin A deficiency mainly affected lymphocyte migratory capacity, but not the target tissues⁹⁷. More recently, it was described that vitamin A-depleted rats had a marked decrease in the number of IgA⁺ ASCs and CD4⁺ T cells in the ileum⁸³. The molecular basis for these observations was recently determined in a study that showed that mice depleted of vitamin A had decreased numbers of effector and memory T cells in the gut mucosa, but not elsewhere¹⁵. The vitamin A metabolite retinoic acid was sufficient to induce the expression of $\alpha_4\beta_7$ -integrin and CCR9 by activated T cells, even in the absence of DCs¹⁵. Blocking retinoic acid receptors of the RAR family significantly decreased the induction of $\alpha_4\beta_7$ -integrin expression by T cells by GALT-resident DCs, which shows that retinoic acid is essential for the gut-imprinting capacity of the DCs¹⁵. Consistently, GALT-resident DCs, unlike DCs from other tissues, express RALDH enzymes, which are essential for retinoic acid biosynthesis¹⁵. Together, these results indicate that retinoic acid is pivotal for the imprinting of gut-homing T cells.

Although vitamin A deficiency decreases the number of T and B cells in the small bowel lamina propria^{15,76,83,97}, it does not affect lymphocyte migration to the colon⁹⁷. Analogously, GALT-resident DCs imprint T and B cells with homing capacity for the small bowel, but they do not induce colon-homing T cells⁹³. Therefore, retinoic acid is neither necessary nor sufficient to imprint colon-homing lymphocytes. The molecular signals that are responsible for lymphocyte homing to the colon and the reasons why T-cell migration to this compartment is controlled differently from homing to the small bowel are still to be determined.

Regarding the migration of ASCs, it has been proposed that CCR10 might have a role in the homing of IgA⁺ ASCs to the colon, mammary glands and probably to other mucosal compartments⁷³ (FIG. 4b). However, it is currently unclear how CCR10 expression is induced by ASCs. Recent reports indicate that IgA⁺ ASCs might acquire CCR10 expression in colonic patches or in iliac lymph nodes following rectal immunization⁹⁸ and that the expression of this receptor can also be induced by 1,25(OH)₂VD₃ in human ASCs³⁹. However, 1,25(OH)₂VD₃ does not induce CCR10 expression in

Colonic patches

Structures resembling Peyer's patches that are scattered throughout the colon. They have been implicated in the generation of colonic immune responses.

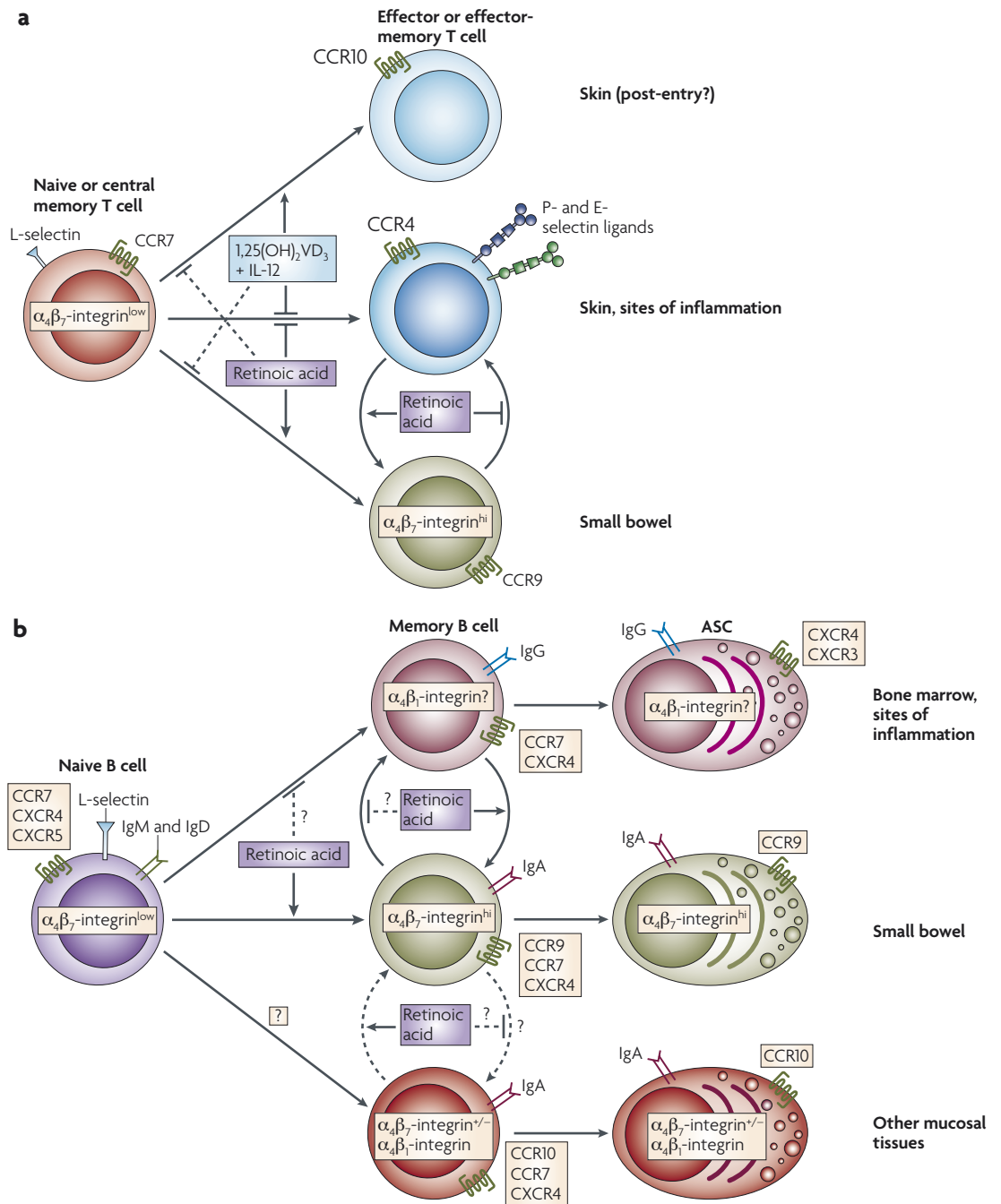


Figure 4 | Roles of retinoic acid and 1,25(OH)₂VD₃ in tissue-specific lymphocyte homing. **a** | Retinoic acid produced by gut-associated lymphoid tissue (GALT)-resident dendritic cells and probably by other cells, such as intestinal epithelial cells, potentially induces the expression of the gut-homing receptors $\alpha_4\beta_7$ -integrin and CC-chemokine receptor 9 (CCR9) by activated CD4⁺ and CD8⁺ T cells. Retinoic acid also blocks the induction of skin-homing receptors by T cells, including CCR4 and the ligands for E- and P-selectin. Effector and memory T cells exhibit plasticity in their homing commitment: skin-homing T cells can become gut-homing T cells and vice versa if they are restimulated either with or without retinoic acid, respectively. In the presence of interleukin-12 (IL-12), 1,25(OH)₂VD₃ (the main circulating vitamin D₃ (VD₃) metabolite) induces the expression of skin-associated CCR10 by human (but not mouse) T cells. However, 1,25(OH)₂VD₃ blocks the induction of E-selectin ligands and therefore inhibits skin-homing. So, it is possible that 1,25(OH)₂VD₃ induces CCR10 expression after T cells have homed to the skin to retain them in the epidermis (in which the CCR10 ligand CCL27 is expressed). 1,25(OH)₂VD₃ also antagonizes the upregulation of gut-homing receptors, whereas retinoic acid reciprocally blocks the induction of CCR10 expression by 1,25(OH)₂VD₃. **b** | Like T cells, B cells also exhibit plasticity in their homing commitment and can either acquire or lose gut-homing potential when reactivated with or without retinoic acid, respectively. Retinoic acid induces the expression of $\alpha_4\beta_7$ -integrin and CCR9 on activated B cells and antibody-secreting cells (ASCs). It is unknown whether retinoic acid alters the homing of B cells and ASCs to other tissues, such as the bone marrow or sites of inflammation. ASCs in mucosal tissues (mostly IgA⁺ ASCs) also express CCR10, although it is unclear where and how this receptor is upregulated by these cells.

murine ASCs *in vitro* and VDR-deficient mice have normal numbers of CCR10⁺ IgA⁺ ASCs³⁹, which indicates that 1,25(OH)₂VD₃ might not be necessary for the induction of CCR10 expression by B cells *in vivo*, at least in mice.

In vitro-generated tissue-tropic lymphocytes retain marked plasticity; skin-homing T cells can be converted to gut-homing T cells and vice versa if they are re-stimulated with or without GALT-resident DCs, respectively^{95,96}. Similarly, previously activated B cells can be re-educated and acquire or lose gut-homing potential when they are restimulated with or without retinoic acid, respectively⁷⁶. The decrease in $\alpha_4\beta_7$ -integrin and CCR9 expression observed in T and B cells that are activated in the absence of retinoic acid might be a default differentiation mechanism. However, it is also possible that other factors can actively contribute to the downregulation of gut-homing-receptor expression by lymphocytes. As both RAR and VDR must form heterodimers with RXR to signal, it is possible that 1,25(OH)₂VD₃ could actively antagonize the effects of retinoic acid by competing for the same nuclear partner¹⁸. Consistent with this possibility, 1,25(OH)₂VD₃ blocks the retinoic acid-induced upregulation of gut-homing receptors on human T cells^{6,99}. However, whether this retinoic acid antagonism by 1,25(OH)₂VD₃ has a regulatory role in the imprinting of gut-homing lymphocytes *in vivo* remains unknown.

In addition to imprinting a gut-homing phenotype on lymphocytes, retinoic acid and GALT-resident DCs also block the upregulation of the skin-homing receptors CCR4 and ligands for P- and E-selectin by T cells^{15,95}. Therefore, acquisition of a skin-homing phenotype might be the default pathway for T-cell activation in the absence of retinoic acid or when RAR signalling is blocked¹⁰⁰. Nonetheless, as VD₃ as well as 1,25(OH)₂VD₃ can be synthesized in the skin¹⁰¹, it is conceivable that, like retinoic acid in the gut, 1,25(OH)₂VD₃ might have a reciprocal role in imprinting lymphocyte homing to the skin. In agreement with this possibility, it was recently shown that 1,25(OH)₂VD₃ synergizes with IL-12 to induce the expression of skin-associated CCR10 by human T cells⁶. However, it was also shown that 1,25(OH)₂VD₃ actually blocks the upregulation of ligands for E-selectin^{6,99} and the expression of fucosyltransferase-VII (REF. 99), an enzyme essential for the synthesis of selectin ligands¹⁰². This was correlated with decreased homing of T cells to the inflamed skin in a model of contact hypersensitivity induced by oxazolone⁹⁹. Although these data indicate that 1,25(OH)₂VD₃ might block skin-homing, it should be noted that these experiments were carried out without IL-12 supplementation, which could potentially counteract the negative effect of 1,25(OH)₂VD₃ on the expression of E-selectin ligands and skin-homing. It is also possible that 1,25(OH)₂VD₃ induces CCR10 expression by T cells after they have homed to the skin to increase their retention in this tissue. In fact, because keratinocytes express the CCR10 ligand CC-chemokine ligand 27 (CCL27), it has been proposed that CCR10 upregulation might promote T-cell trafficking to and/or retention in the epidermis⁶.

Finally, 1,25(OH)₂VD₃ might also affect leukocyte migration by blocking chemokine synthesis at effector sites. For instance, 1,25(OH)₂VD₃ decreased the expression of CCL2, CCL3, CXCL10 and subsequent monocyte infiltration in experimental autoimmune encephalomyelitis (EAE)¹⁰³. Similarly, a 1,25(OH)₂VD₃ analogue decreased the production of the chemokines CCL2, CCL5, CXCL10 and consequent T_H1-cell infiltration in non-obese diabetic (NOD) mice, a model of type 1 diabetes¹⁰⁴.

Effects of antioxidant vitamins on immunity

It has been known for more than 30 years that some vitamins with antioxidant properties, including vitamin A, vitamin B6 (pyridoxine), vitamin C (ascorbic acid) and particularly vitamin E, have protective effects on animal models of atherosclerosis and ischaemia-reperfusion injury (IRI)²⁻⁴. Vitamin E collectively refers to eight related compounds (tocopherols and tocotrienols), of which α -tocopherol has the greatest bioavailability and is the best characterized¹⁰⁵. Vitamin E decreases the release of reactive oxygen species by monocytes¹⁰⁶ and the expression of CD11b and very late antigen 4 (VLA4), thereby decreasing monocyte adhesion to the endothelium¹⁰⁶. Vitamin E also blocks the release of pro-inflammatory cytokines, including IL-1, IL-6, TNF and the chemokine IL-8, by monocytes and macrophages^{107,108}. Moreover, vitamin E prevents the upregulation of the adhesion molecules vascular cell-adhesion molecule 1 (VCAM1) and intercellular adhesion molecule 1 (ICAM1) on the endothelium induced by oxidized low-density lipoprotein (LDL)¹⁰⁹ and IL-1 β ¹¹⁰, as well as the upregulation of E-selectin and some chemokines¹⁰⁸. Reactive oxygen species activate the nuclear factor- κ B (NF- κ B) pathway¹⁰⁶, which initiates many pro-inflammatory events. Therefore, the therapeutic antioxidant effect of these vitamins could be explained, at least in part, by their capacity to decrease NF- κ B activation.

Vitamin E can also act directly on T cells by decreasing IFN γ production¹¹¹ and CD95L (also known as FASL)¹¹² expression, thereby helping to decrease inflammation and immune-mediated tissue damage. These effects on macrophages and T cells are believed to be important for the protective effect for vitamin E in animal models of atherosclerosis^{108,113} and IRI^{114,115}. Consistent with a potential physiological role for vitamin E in preventing atherosclerosis, hyperlipidemic mice that are deficient in α -tocopherol transfer protein, which is important for transporting α -tocopherol and for preventing its degradation, have more severe atherosclerosis¹¹⁶.

It was recently shown that vitamin C prevents oxidative damage during ischaemia reperfusion in rats¹¹⁷ and humans⁴. Notably, it was shown that vitamin C but not vitamin E prevented leukocyte adhesion to the microvascular endothelium in hamster models of oxidative endothelial stress induced by cigarette smoke or oxidized LDL^{118,119}. Differences in lipophilicity might potentially have an impact in the distribution and/or location of vitamins C and E and partially account for the differential effect of these vitamins. Therefore, it is possible that combined supplementation of vitamins C and E could

Experimental allergic encephalomyelitis

(EAE). An experimental model of the human disease multiple sclerosis. Autoimmune disease is induced in experimental animals by immunization with myelin or peptides derived from myelin. The animals develop a paralytic disease with inflammation and demyelination in the brain and spinal cord.

Type 1 diabetes

A chronic autoimmune disease that is characterized by the T-cell-mediated destruction of β cells (which secrete insulin) in the pancreas. Patients with type 1 diabetes develop hyperglycaemia and can develop diabetes-associated complications in multiple organ systems, owing to a lack of insulin. Diabetes in non-obese diabetic mice is a model of type 1 diabetes.

Atherosclerosis

A chronic disorder of the arterial wall characterized by endothelial damage that gradually induces deposits of cholesterol, cellular debris, calcium and other substances. These deposits eventually lead to plaque formation and arterial stiffness.

Ischaemia-reperfusion injury

(IRI). Cellular damage caused by the return of blood supply to a tissue after a period of inadequate blood supply. The absence of oxygen and nutrients causes cellular damage, such that restoration of the blood flow results in inflammation.

offer synergistic benefits. The antioxidant and/or anti-atherogenic role for other vitamins, such as vitamin B6 and vitamin K, is less well documented and somewhat controversial, with evidence both in favour^{120,121} and against¹²² a protective role for these vitamins in atherosclerosis.

Finally, it should be noted that many of the encouraging results in animal models have not been consistently translated into a significant therapeutic benefit in controlled clinical trials of vitamin supplementation for the prevention of cardiovascular diseases and IRI¹²¹. Therefore, it remains to be determined whether antioxidant vitamins will prove to be useful for the treatment and/or prevention of these ailments in humans.

Vitamin metabolites in immunotherapy

Vitamin D. Given that 1,25(OH)₂VD₃ has a physiological protective role in dampening or limiting potentially pathogenic immune responses at the cellular level, one might predict that interfering with its effects could predispose to hypersensitivity or autoimmunity⁷. Consistent with this idea, levels of serum 1,25(OH)₂VD₃ are often decreased in patients with type I diabetes¹²³ and SLE^{8,124}, and 1,25(OH)₂VD₃ levels are inversely correlated with disease activity in patients with rheumatoid arthritis¹²⁵. Moreover, VD₃ deficiency accelerates intestinal inflammation in IL-10-deficient mice, which develop inflammatory bowel disease¹²⁶. VD₃ deficiency might also predispose to type I diabetes^{7,127}. However, although children with rickets (typically caused by insufficient dietary supply of vitamin D) have a higher incidence of diabetes than VD₃-sufficient children⁷, they are also more susceptible to infection^{7,128}, which suggests that VD₃ might also be important for protective immune responses. This effect could be partially mediated by the capacity of 1,25(OH)₂VD₃ to increase the bactericidal capacity of macrophages^{5,23}.

Given its immunomodulatory properties, 1,25(OH)₂VD₃ or its analogues might be clinically useful for the treatment of inflammatory and autoimmune diseases. Administration of 1,25(OH)₂VD₃ or an analogue prevented proteinuria and prolonged life span in a mouse model of experimental SLE^{129,130}. In addition, 1,25(OH)₂VD₃ prevented EAE in mice^{103,131,132}, an effect that was dependent on IL-10 and IL-10 receptor signalling¹³². As 1,25(OH)₂VD₃ in combination with glucocorticoids induces IL-10-producing T_R1 cells¹³³, it is possible that 1,25(OH)₂VD₃ might exert its therapeutic effect, at least in part, through the generation of T_R1 cells. However, induction of FOXP3⁺ T_{Reg} cells might also have an important role^{35–37}. Other models of experimental autoimmunity in which 1,25(OH)₂VD₃ has shown a therapeutic benefit include insulinitis in NOD mice¹²⁷, prostatitis³⁴ and rheumatoid arthritis^{7,134}. 1,25(OH)₂VD₃ can also block cutaneous contact hypersensitivity⁹⁹, an effect that could be mediated in part by blocking the induction of skin-homing-receptor expression by lymphocytes⁹⁹. Consistent with its anti-inflammatory role, a 1,25(OH)₂VD₃ analogue has been successfully used as a therapy for psoriasis^{135,136}. However, it should be pointed out that topical skin application of 1,25(OH)₂VD₃ also has the potential to trigger allergic dermatitis by increasing T_H2 cell-mediated responses¹³⁷.

It has been proposed that 1,25(OH)₂VD₃ could also be used as an adjuvant in immunomodulatory therapy in transplantation. 1,25(OH)₂VD₃ and a 1,25(OH)₂VD₃ analogue prolonged the survival of mouse cardiac allografts^{138,139}, and decreased the rates of allograft rejection and increased survival in a rat model of liver transplantation¹⁴⁰ and in fully mismatched mouse pancreatic islet transplants¹⁴¹. In addition, a 1,25(OH)₂VD₃ analogue provided significant protection from graft-versus-host disease (GVHD) in rats¹⁴². Moreover, 1,25(OH)₂VD₃ and a 1,25(OH)₂VD₃ analogue significantly prevented chronic allograft rejection in a rat model of renal transplantation¹⁴³ and delayed chronic allograft rejection in a mouse model of aortic transplantation¹³⁹.

Interestingly, polymorphisms in VDR are associated with a higher incidence of GVHD in patients who undergo bone-marrow transplantation¹⁴⁴, which indicates that 1,25(OH)₂VD₃ might also have a role in suppressing alloreactive immune responses in humans. In agreement with this possibility, 1,25(OH)₂VD₃ supplementation has been shown to have a beneficial effect by improving allograft function of human renal transplants¹⁴⁵, which is especially relevant considering that renal insufficiency is associated with decreased 1,25(OH)₂VD₃ synthesis^{146,147}. Importantly, although 1,25(OH)₂VD₃ helps to prevent transplant rejection, it does not seem to interfere significantly with protective immune responses against pathogens¹⁴⁸. Therefore, it is possible that once 1,25(OH)₂VD₃ induces a 'homeostatic' immunomodulatory threshold, it does not exert further immunosuppression.

Although 1,25(OH)₂VD₃ could be a potentially useful immunomodulatory agent for clinical use, it can cause some serious adverse effects, in particular the induction of hypercalcaemia and bone resorption⁷. Therefore, multiple drug development efforts are aimed at finding 1,25(OH)₂VD₃ analogues that exert immunomodulation without causing significant hypercalcaemia⁷. Indeed, long-term administration of a 1,25(OH)₂VD₃ analogue efficiently decreased serum levels of IL-2 and IgG in mice without significant adverse side effects¹⁴⁹, and some 1,25(OH)₂VD₃ analogues have been successfully used in autoimmune disease models, such as EAE, to decrease the doses of conventional immunosuppressive drugs⁷. These results indicate that 1,25(OH)₂VD₃ analogues may be an effective and safer alternative to 1,25(OH)₂VD₃ for immune modulation.

Vitamin A. Given the crucial role of retinoic acid in imprinting a gut-homing capacity on T and B cells^{15,76}, as well as its potential to promote the differentiation of IgA⁺ ASCs^{76,80}, it is not surprising that vitamin A deficiency is associated with impaired intestinal immune responses^{56,88,89} and increased mortality associated with gastrointestinal and respiratory infections¹⁵⁰. Conversely, vitamin A supplementation correlates with a significant decrease in diarrhoea and mortality in HIV-infected or malnourished children^{76,151,152}. Therefore, retinoic acid or RAR agonists could be used for targeting T- and B-cell responses to the gut mucosa for vaccination purposes.

Rheumatoid arthritis

An immunological disorder that is characterized by symmetrical polyarthritis, often progressing to crippling deformation after years of synovitis. It is associated with systemic immune activation, with the presence of acute-phase reactants in the peripheral blood and with rheumatoid factor (immunoglobulins specific for IgG), which form immune complexes that are deposited in many tissues.

Inflammatory bowel disease

(IBD). A chronic condition of the intestine that is characterized by severe inflammation and mucosal destruction. The most common forms in humans are ulcerative colitis and Crohn's disease, which are believed to be T helper 2 (T_H2)- and T_H1-type diseases, respectively. However, interleukin-23 and T_H17 cells have also recently been shown to be involved in the pathology of IBD.

Graft-versus-host disease

(GVHD). An immune response mounted against the recipient of an allograft by immunocompetent donor T cells that are derived from the graft. Typically, it is seen in the context of allogeneic bone-marrow transplantation.

In addition, given that retinoic acid can potentiate the TGF β -mediated induction of T_{Reg} cells while antagonizing the differentiation of pro-inflammatory T_H17 cells⁶⁴, treatment with retinoic acid together with TGF β could be a useful strategy to generate T_{Reg} cells for treating inflammatory pathologies affecting not only the intestine, but also peripheral tissues. In fact, retinoic acid and RAR-agonists have been successfully used in some models of autoimmune inflammation, such as EAE^{153,154}, adjuvant arthritis^{155,156} and experimental nephritis¹⁵⁷. Retinoids have also been successfully used to treat psoriasis¹⁵⁸ and they are effective in treating and/or preventing contact dermatitis in mice and humans^{159,160}. In these models, therapeutic effects partially correlated with the induction of T_H2-cell responses¹⁵³ and decreased expression of $\alpha_4\beta_1$ -integrin on effector T cells¹⁵⁷, but the role of retinoic acid in the induction of T_{Reg} cells and the inhibition of T_H17 cells in these settings has yet to be assessed.

Concluding remarks

Although 1,25(OH)₂VD₃ clearly exerts immunomodulatory activity *in vitro* and *in vivo*, its relative physiological role in maintaining immune tolerance and in shaping immune responses is still unclear. Moreover, as retinoic acid and 1,25(OH)₂VD₃ can potentially antagonize each other's effects^{6,18,99}, it will be important to dissect the interplay between 1,25(OH)₂VD₃, retinoic acid and other mechanisms of immunomodulation *in vivo*.

1,25(OH)₂VD₃ can upregulate CCR10 on human T cells and ASCs^{6,39} while blocking the expression of skin- and gut-homing receptors^{6,99}. However, the *in vivo* relevance of the effects of 1,25(OH)₂VD₃ on CCR10 expression by T cells that are infiltrating the skin and by IgA⁺ ASCs that are migrating to the gut lamina propria remains to be determined. In addition, although GALT-resident DCs enhance IgA secretion and induce gut-homing effector lymphocytes *ex vivo*, it will be

important to determine the relative *in vivo* contribution of DCs versus other potential sources of retinoic acid in the gut (such as IECs), and to determine whether there are retinoic acid-independent mechanisms of imprinting a gut-homing phenotype. Moreover, as GALT-resident DCs can also enhance the differentiation of TGF β -induced T_{Reg} cells, it will be necessary to determine the *in vivo* scenarios in which GALT-resident DCs and retinoic acid promote either effector or suppressive T-cell responses. Along these same lines, it will be important to study the contribution of these *in situ*-generated gut T_{Reg} cells compared with their systemic counterparts in maintaining immune tolerance at intestinal and extra-intestinal sites.

Aside from the antioxidant effects of vitamins C and E that have been demonstrated in animal models of cardiovascular disease and IRI, there is a lack of published information on the impact of these and other vitamins, such as vitamin B6 and K^{120–122}, on the adaptive immune system and in other inflammatory settings, such as autoimmune diseases. Whether these vitamins will offer a therapeutic benefit in the settings of human cardiovascular diseases, IRI and other pathologies remains unclear.

Although many open questions remain, there is promise that vitamin A and D metabolites or their analogues have the potential to be used in clinical settings for therapeutic benefit. In particular, it will be important to assess the impact of using 1,25(OH)₂VD₃ analogues as an adjuvant immunomodulatory therapy in the setting of autoimmune diseases and in transplant recipients. It will also be important to determine the net effects of retinoic acid or synthetic RAR-agonists, especially in the intestine, where these agents appear to have a role in enhancing immune responses. The capacity of vitamin A metabolites to foster gut-homing T cells might improve strategies of mucosal vaccination or aid in decreasing pathogenic immunity by potentiating the induction of T_{Reg} cells.

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