Published in final edited form as:
*Nat Rev Immunol*. 2008 September ; 8(9): 685–698. doi:10.1038/nri2378.

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

**J. Rodrigo Mora**[*], **Makoto Iwata**[‡], and **Ulrich H. von Andrian**[§]

[*]Gastrointestinal Unit, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114, USA.

[‡]Laboratory of Biodefense Research, Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University, Kagawa, 769-2193, Japan.

[§]Immune Disease Institute & Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115, USA.

## 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 organism[1]. 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 others[1].

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



Correspondence to J.R.M. or U.H.v.A., j_rodrigo_mora@harvard.hms.edu; uva@harvard.hms.edu.

**DATABASES**

**Entrez Gene:** http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
α4β7| CCR4 | CCR9 | CCR10 | FOXP3| IFNγ | IL-2| iNOS | PPARβ | RAR | RXR | TNF | VDR

**FURTHER INFORMATION**

**Ulrich H. von Adrian's homepage:** http://www.cbr.med.harvard.edu/labs/vonandrian
**Rodrigo Mora's homepage:** http://www.massgeneral.org/gastroenterology/research/research_mora.html

**SUPPLEMENTARY INFORMATION**

**See online article:** S1 (table)

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 ($1,25(OH)_2VD_3$), 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 $D_3$ ($VD_3$), the most physiologically relevant form of vitamin D, is synthesized in the skin from 7-dehydrocholesterol[5], 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 supplements[5] (FIG. 1a). $VD_3$ is then converted in the liver to 25-dihydroxyvitamin $D_3$ ($25(OH)VD_3$), which is the main circulating form of $VD_3$. Finally, $25(OH)VD_3$ is metabolized in the kidneys to $1,25(OH)_2VD_3$, the most physiologically active $VD_3$ metabolite[5]. In addition to being processed in the liver and the kidneys, $VD_3$ can also be metabolized by cells of the immune system[5,6] (FIG. 1a). In this way, $1,25(OH)_2VD_3$ is concentrated locally in those lymphoid microenvironments that contain physiologically high concentrations of $VD_3$, thereby increasing its specific action and also limiting potentially undesirable systemic effects, such as hypercalcaemia and increased bone resorption[7].

Activated T cells (and probably also B cells) can only perform the final step of converting $25(OH)VD_3$ to $1,25(OH)_2VD_3$ (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 $VD_3$ into $1,25(OH)_2VD_3$ (REFS 6,7,9).

Finally, the enzyme 24-hydroxylase, which is most abundant in the kidney and intestine[10], catabolizes $1,25(OH)_2VD_3$ to its inactive metabolite, calcitroic 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 RALDH[13]. 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 $1,25(OH)_2VD_3$ can act on immune cells in an autocrine or paracrine manner. On complexing with $1,25(OH)_2VD_3$, 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 $VD_3$ response elements (VDREs) in the promoters of $VD_3$-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 acid11,12. 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 others[17]. Therefore, it is possible that, given their common RXR nuclear binding partners, some ligands, such as $1,25(OH)_2VD_3$ and retinoic acid, might antagonize each other's effects[18].

Notably, retinoic acid can also bind and signal through the PPARβ (also known as PPARδ) nuclear receptor[19]. 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 $VD_3$ metabolites in the immune system, particularly of $1,25(OH)_2$ $VD_3$, has been known for more than 20 years[20,21]. *In vitro*, $1,25(OH)_2VD_3$ 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 cells24,25, and CD8 T-cell-mediated cytotoxicity[26]. The decrease in the production of IL-2 and IFNγ by $1,25(OH)_2VD_3$ 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 $1,25(OH)_2VD_3$ 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 cells[31]. Moreover, $1,25(OH)_2VD_3$ enhances nonspecific T-cell suppressor activity, as measured by the ability of $1,25(OH)_2VD_3$-treated T cells to suppress primary mixed-lymphocyte reactions and cytotoxic T-cell responses[26].

Overall, the net result of $1,25(OH)_2VD_3$ 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 production[7]. The activity of $1,25(OH)_2$ $VD_3$ 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 responses32,[33]. Furthermore, $1,25(OH)_2VD_3$ also inhibits $T_H17$-cell responses, probably owing in part to its capacity 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, $1,25(OH)_2VD_3$ decreases B-cell proliferation, plasma-cell differentiation and IgG secretion[8,20] (FIG. 2). It has been suggested that the effect of $1,25(OH)_2VD_3$ 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 cells8,31,39, leaving it unclear whether $1,25(OH)_2VD_3$ can act directly on B cells.

Interestingly, $1,25(OH)_2VD_3$ 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 SLE[40]. Thus, it is possible that fully differentiated memory B cells and/or antibody-secreting cells (ASCs) are refractory to $1,25(OH)_2VD_3$-mediated inhibition. In addition, serum levels of $1,25(OH)_2VD_3$ are significantly decreased in patients with SLE, especially during active disease[8]. So, it is tempting to speculate that a decreased level of $1,25(OH)_2VD_3$ 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 $1,25(OH)_2VD_3$ (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 nodes[41]. In addition, $1,25(OH)_2VD_3$ 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 ($T_R1$) cells[7].

Although $1,25(OH)_2VD_3$ primarily has inhibitory effects on the adaptive immune response, some of its effects on innate immune cells are stimulatory. For example, $1,25(OH)_2VD_3$ 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 $1,25(OH)_2VD_3$ 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 mice[43]. However, to carefully dissect the *in vivo* effects of $VD_3$ metabolites on the immune response, it will be necessary to study the effect of $VD_3$ in animal models in which VDR deficiency is restricted to T cells, B cells and myeloid cells.

## 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 cytotoxicity[44] and T-cell proliferation[45], the latter probably

mediated, at least in part, by enhancing IL-2 secretion and signalling in T cells[45]. Consistent with an *in vivo* role for vitamin A in T-cell function, vitamin A-deficient mice have defects in $T_H$-cell activity[46]. A possible mechanism for this observation is that in the setting of vitamin A deficiency, retinoic acid does not compete with $1,25(OH)_2VD_3$ for their common nuclear binding partner RXR and, therefore, the inhibitory effects of $1,25(OH)_2VD_3$ 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 receptors[51].

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 proliferation[52]. 14-hydroxy-retroretinol (14HRR) has a positive effect on proliferation, whereas anhydroretinol blocks B-cell proliferation and induces apoptosis in T cells[53]. Retroretinoids 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 responses[54]. 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 receptors[55]. However, it should be noted that DCs pre-treated with retinoic acid can apparently store this metabolite[50], 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 responses[56] 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 expression[59]. 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 cytokines[61]. It has been proposed that retinoic acid exerts its $T_H2$-cell-promoting effect indirectly through the modulation of APCs[62]. However, retinoic acid can also act directly on T cells to induce $T_H2$-cell differentiation[57,60] through RAR proteins[57].

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 (TGFβ) drives the generation of induced $T_{Reg}$ cells in peripheral tissues[63], 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 propria[67], 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 signals[69]. Exposure of activated $CD4^+$ T cells to TGFβ alone induces $T_{Reg}$ cells, whereas the combination of TGFβ with IL-6, IL-1β 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 TGFβ 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-γt (RORγt), 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 concentration[68]. In fact, low concentrations of retinoic acid seem to be necessary for $T_H17$-cell differentiation[68]. 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 IFNγ 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 differentiation[74]. 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 TGFβ1 (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 splenocytes[80] 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 effects68،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 production73،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 rats[84]. 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 DCs[76]. 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 $IgA^+$ ASCs[79]. Interestingly, the promoter of the *iNOS* gene contains a RARE that is directly activated by retinoic acid bound to its nuclear

RARα–RXR heterodimeric receptor[85]. Moreover, intraperitoneal administration of either retinoic acid or a RAR agonist enhances LPS-induced iNOS expression in several organs and increases plasma levels of nitrate and nitrite in rats[86]. 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 IECs[14] 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 toxins56,[88,89]. Furthermore, vitamin A supplementation prevents the decline in IgA levels observed in malnourished mice[58]. 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 normal[76], 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 α4β7-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 receptors[90]. In the lymphoid microenvironment, DCs are essential for efficient T-cell activation[91]. 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 mucosa[97]. Protein-caloric malnutrition without vitamin A deficiency did not affect lymphocyte migration[97]. 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 recipients[97]. This suggests that vitamin A deficiency mainly affected lymphocyte migratory capacity, but not the target tissues[97]. 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 ileum[83]. 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 elsewhere[15]. 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 DCs[15]. 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 DCs[15]. Consistently, GALT-resident DCs, unlike DCs from other tissues, express RALDH enzymes, which are essential for retinoic acid biosynthesis[15]. 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 colon[97]. Analogously, GALT-resident DCs imprint T and B cells with homing capacity for the small bowel, but they do not induce colon-homing T cells[93]. 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 compartments[73] (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 immunization[98] and that the expression of this receptor can also be induced by $1,25(OH)_2VD_3$ in human ASCs[39]. However, $1,25(OH)_2VD_3$ does not induce CCR10 expression in murine ASCs *in vitro* and VDR-deficient mice have normal numbers of $CCR10^+$ $IgA^+$ ASCs[39], which indicates that $1,25(OH)_2VD_3$ 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, respectively[76]. 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 $1,25(OH)_2VD_3$ could actively antagonize the effects of retinoic acid by competing for the same nuclear partner[18]. Consistent with this possibility, $1,25(OH)_2VD_3$ blocks the retinoic acid-induced upregulation of gut-homing receptors on human T cells6,[99]. However, whether this retinoic acid antagonism by $1,25(OH)_2VD_3$ 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 blocked[100]. Nonetheless, as $VD_3$ as well as $1,25(OH)_2VD_3$ can be synthesized in the skin[101], it is conceivable that, like retinoic acid in the gut, $1,25(OH)_2VD_3$ might have a reciprocal role in imprinting lymphocyte homing to the skin. In agreement with this possibility, it was recently shown that $1,25(OH)_2VD_3$ synergizes with IL-12 to induce the expression of skin-associated CCR10 by human T cells[6]. However, it was also shown that $1,25(OH)_2VD_3$

actually blocks the upregulation of ligands for E-selectin6,99 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 $1,25(OH)_2VD_3$ 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 $1,25(OH)_2VD_3$ on the expression of E-selectin ligands and skin-homing. It is also possible that $1,25(OH)_2VD_3$ 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 epidermis[6].

Finally, $1,25(OH)_2VD_3$ might also affect leukocyte migration by blocking chemokine synthesis at effector sites. For instance, $1,25(OH)_2VD_3$ decreased the expression of CCL2, CCL3, CXCl10 and subsequent monocyte infiltration in experimental autoimmune encephalomyelitis (EAE)[103]. Similarly, a $1,25(OH)_2VD_3$ 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 diabetes[104].

## 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 macrophages107,108. 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 lipo protein (LDL)109 and IL-1β110, as well as the upregulation of E-selectin and some chemokines108. Reactive oxygen species activate the nuclear factor-κB (NF-κB) 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-κB activation.

Vitamin E can also act directly on T cells by decreasing IFNγ production[111] 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 atherosclerosis108,113 and IRI114,115. 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 atherosclerosis[116].

It was recently shown that vitamin C prevents oxidative damage during ischaemia reperfusion in rats[117] and humans[4]. 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 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 against[122] 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 IRI[121]. 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 $1,25(OH)_2VD_3$ 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 autoimmunity[7]. Consistent with this idea, levels of serum $1,25(OH)_2VD_3$ are often decreased in patients with type I diabetes123 and SLE8,[124], and $1,25(OH)_2VD_3$ levels are inversely correlated with disease activity in patients with rheumatoid arthritis[125]. Moreover, $VD_3$ deficiency accelerates intestinal inflammation in IL-10-deficient mice, which develop inflammatory bowel disease[126]. $VD_3$ deficiency might also predispose to type I diabetes7,[127]. However, although children with rickets (typically caused by insufficient dietary supply of vitamin D) have a higher incidence of diabetes than $VD_3$-sufficient children[7], they are also more susceptible to infection[7,128], which suggests that $VD_3$ might also be important for protective immune responses. This effect could be partially mediated by the capacity of $1,25(OH)_2VD_3$ to increase the bactericidal capacity of macrophages[5,23].

Given its immunomodulatory properties, $1,25(OH)_2VD_3$ or its analogues might be clinically useful for the treatment of inflammatory and auto immune diseases. Administration of $1,25(OH)_2VD_3$ or an analogue prevented proteinuria and prolonged life span in a mouse model of experimental SLE129,[130]. In addition, $1,25(OH)_2VD_3$ prevented EAE in mice103,131,132, an effect that was dependent on IL-10 and IL-10 receptor signalling[132]. As $1,25(OH)_2VD_3$ in combination with glucocorticoids induces IL-10-producing $T_R1$ cells[133], it is possible that $1,25(OH)_2VD_3$ 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 role35–37. Other models of experimental autoimmunity in which $1,25(OH)_2VD_3$ has shown a therapeutic benefit include insulitis in NOD mice127, prostatitis34 and rheumatoid arthritis7,[134]. $1,25(OH)_2VD_3$ can also block cutaneous contact hypersensitivity99, an effect that could be mediated in part by blocking the induction of skin-homing-receptor expression by lymphocytes[99]. Consistent with its anti-inflammatory role, a $1,25(OH)_2VD_3$ analogue has been successfully used as a therapy for psoriasis135,[136]. However, it should be pointed out that topical skin application of $1,25(OH)_2VD_3$ also has the potential to trigger allergic dermatitis by increasing $T_H2$ cell-mediated responses[137].

It has been proposed that $1,25(OH)_2VD_3$ could also be used as an adjuvant in immunomodulatory therapy in transplantation. $1,25(OH)_2VD_3$ and a $1,25(OH)_2VD_3$ analogue prolonged the survival of mouse cardiac allografts138,139, and decreased the rates of allograft rejection and increased survival in a rat model of liver transplantation140 and in fully mismatched mouse pancreatic islet transplants[141]. In addition, a $1,25(OH)_2VD_3$ analogue provided significant protection from graft-versus-host disease (GVHD) in rats[142]. Moreover, $1,25(OH)_2VD_3$ and a $1,25(OH)_2VD_3$ analogue significantly prevented chronic allograft

rejection in a rat model of renal transplantation[143] and delayed chronic allograft rejection in a mouse model of aortic transplantation[139].

Interestingly, polymorphisms in VDR are associated with a higher incidence of GVHD in patients who undergo bone-marrow transplantation[144], which indicates that $1,25(OH)_2VD_3$ might also have a role in suppressing alloreactive immune responses in humans. In agreement with this possibility, $1,25(OH)_2VD_3$ supplementation has been shown to have a beneficial effect by improving allograft function of human renal transplants[145], which is especially relevant considering that renal insufficiency is associated with decreased $1,25(OH)_2VD_3$ synthesis146,[147]. Importantly, although $1,25(OH)_2VD_3$ helps to prevent transplant rejection, it does not seem to interfere significantly with protective immune responses against pathogens[148]. Therefore, it is possible that once $1,25(OH)_2VD_3$ induces a 'homeostatic' immunomodulatory threshold, it does not exert further immunosuppression.

Although $1,25(OH)_2VD_3$ 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 resorption[7]. Therefore, multiple drug development efforts are aimed at finding $1,25(OH)_2VD_3$ analogues that exert immunomodulation without causing significant hypercalcaemia[7]. Indeed, long-term administration of a $1,25(OH)_2VD_3$ analogue efficiently decreased serum levels of IL-2 and IgG in mice without significant adverse side effects[149], and some $1,25(OH)_2VD_3$ analogues have been successfully used in autoimmune disease models, such as EAE, to decrease the doses of conventional immunosuppressive drugs[7]. These results indicate that $1,25(OH)_2VD_3$ analogues may be an effective and safer alternative to $1,25(OH)_2VD_3$ 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 infections[150]. 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.

In addition, given that retinoic acid can potentiate the TGFβ-mediated induction of $T_{Reg}$ cells while antagonizing the differentiation of pro-inflammatory $T_H17$ cells[64], treatment with retinoic acid together with TGFβ 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 nephritis[157]. Retinoids have also been successfully used to treat psoriasis[158] 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 responses[153] and decreased expression of $\alpha_4\beta_7$-integrin on effector T cells[157], 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 $1,25(OH)_2VD_3$ 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 $1,25(OH)_2VD_3$ can potentially antagonize each

other's effects[6,18,99], it will be important to dissect the interplay between $1,25(OH)_2VD_3$, retinoic acid and other mechanisms of immunomodulation *in vivo*.

$1,25(OH)_2VD_3$ 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 $1,25(OH)_2VD_3$ 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 TGFβ-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 $1,25(OH)_2VD_3$ 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.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We thank S. Davis for editorial assistance and E. Villablanca for critical reading of this manuscript. J.R.M. is grateful to I. Ramos for constant support. J.R.M. is supported by grants from the Crohn's & Colitis Foundation of America, the Cancer Research Institute, the Howard M. Goodman Fellowship and the Center for the Study of IBD (DK 43351). M.I. is supported by the Grants-in-Aid from Japan Society for the Promotion of Science and the Ministry of Education, Culture, Sports, Science and Technology, the Naito Foundation and the Uehara Memorial Foundation. U.H.v.A. is supported by National Institutes of Health grants AI061663, AI069259, AI072252, HL56949 and AR42689.

## Glossary

| | |
|---|---|
| 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. |
| $T_{Reg}$ 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. |
| 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. |

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

## References


1. Rosenberg IH. Challenges and opportunities in the translation of the science of vitamins. Am. J. Clin. Nutr 2007;85:325S–327S. [PubMed: 17209220]
2. Beetens JR, Coene MC, Verheyen A, Zonnekeyn L, Herman AG. Influence of vitamin C on the metabolism of arachidonic acid and the development of aortic lesions during experimental atherosclerosis in rabbits. Biomed. Biochim. Acta 1984;43:S273–S276. [PubMed: 6440540]
3. Llesuy S, et al. Effect of vitamins A and E on ischemia-reperfusion damage in rabbit heart. Mol. Cell. Biochem 1995;145:45–51. [PubMed: 7659077]

4. Pleiner J, et al. Intra-arterial vitamin C prevents endothelial dysfunction caused by ischemia-reperfusion. Atherosclerosis 2008;197:383–391. [PubMed: 17645881]
5. Holick MF. Vitamin D deficiency. N. Engl. J. Med 2007;357:266–281. [PubMed: 17634462]
6. Sigmundsdottir H, et al. DCs metabolize sunlight-induced vitamin $D_3$ to 'program' T cell attraction to the epidermal chemokine CCL27. Nature Immunol 2007;8:285–293. [PubMed: 17259988] This paper reports that $1,25(OH)_2VD_3$ is synthesized by dermal DCs and induces CCR10 expression by human T cells, presumably increasing their epidermotropism.
7. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin $D_3$: basic concepts. J. Steroid. Biochem. Mol. Biol 2005;97:93–101. [PubMed: 16046118]
8. Chen S, Sims GP, Chen XX, Gu YY, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin $D_3$ on human B cell differentiation. J. Immunol 2007;179:1634–1647. [PubMed: 17641030]
9. Fritsche J, Mondal K, Ehrnsperger A, Andreesen R, Kreutz M. Regulation of 25-hydroxyvitamin $D_3$–1α-hydroxylase and production of 1α, 25-dihydroxyvitamin $D_3$ by human dendritic cells. Blood 2003;102:3314–3316. [PubMed: 12855575]
10. Akeno N, Saikatsu S, Kawane T, Horiuchi N. Mouse vitamin D-24-hydroxylase: molecular cloning, tissue distribution, and transcriptional regulation by 1α, 25-dihydroxyvitamin $D_3$. Endocrinology 1997;138:2233–2240. [PubMed: 9165006]
11. Blomhoff R, Blomhoff HK. Overview of retinoid metabolism and function. J. Neurobiol 2006;66:606–630. [PubMed: 16688755]
12. Moise AR, Noy N, Palczewski K, Blaner WS. Delivery of retinoid-based therapies to target tissues. Biochemistry 2007;46:4449–4458. [PubMed: 17378589]
13. de Lera AR, Bourguet W, Altucci L, Gronemeyer H. Design of selective nuclear receptor modulators: RAR and RXR as a case study. Nature Rev. Drug Discov 2007;6:811–820. [PubMed: 17906643]
14. Lampen A, Meyer S, Arnhold T, Nau H. Metabolism of vitamin A and its active metabolite *all-trans*-retinoic acid in small intestinal enterocytes. J. Pharmacol. Exp. Ther 2000;295:979–985. [PubMed: 11082432]
15. Iwata M, et al. Retinoic acid imprints gut-homing specificity on T cells. Immunity 2004;21:527–538. [PubMed: 15485630] This paper reports for the first time that retinoic acid is necessary and sufficient to induce gut-homing receptors on T cells. It is also shown that gut-associated DCs can metabolize dietary vitamin A into retinoic acid, which explains their capacity to imprint gut-homing lymphocytes.
16. Coombes JL, et al. A functionally specialized population of mucosal $CD103^+$ DCs induces $Foxp3^+$ regulatory T cells via a TGF-β- and retinoic acid-dependent mechanism. J. Exp. Med 2007;204:1757–1764. [PubMed: 17620361] This paper shows that $CD103^+$ DCs from mesenteric lymph nodes induce $T_{Reg}$ cells by a mechanism involving TGFβ and retinoic acid.
17. Germain P, et al. International union of pharmacology. LXIII. Retinoid X receptors. Pharmacol. Rev 2006;58:760–772. [PubMed: 17132853]
18. Bastie JN, et al. 1α 25-dihydroxyvitamin $D_3$ transrepresses retinoic acid transcriptional activity via vitamin D receptor in myeloid cells. Mol. Endocrinol 2004;18:2685–2699. [PubMed: 15284334]
19. Schug TT, Berry DC, Shaw NS, Travis SN, Noy N. Opposing effects of retinoic acid on cell growth result from alternate activation of two different nuclear receptors. Cell 2007;129:723–733. [PubMed: 17512406] This paper demonstrates that the intracellular ratio between cellular retinoic acid binding protein (CRABP) and fatty acid binding protein (FABP) determines whether retinoic acid acts through RAR or PPARβ nuclear receptors, which translates as distinct functional outcomes.
20. Lemire JM, Adams JS, Sakai R, Jordan SC. 1α, 25-dihydroxyvitamin $D_3$ suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells. J. Clin. Invest 1984;74:657–661. [PubMed: 6611355]
21. Rigby WF, Stacy T, Fanger MW. Inhibition of T lymphocyte mitogenesis by 1,25-dihydroxyvitamin $D_3$ (calcitriol). J. Clin. Invest 1984;74:1451–1455. [PubMed: 6332829] References 20 and 21 are among the first reports to clearly demonstrate that $1,25(OH)_2VD_3$ can exert a powerful immunomodulatory effect on T and B cells *ex vivo*.
22. Lemire JM, et al. 1,25-dihydroxyvitamin $D_3$ suppresses human T helper/inducer lymphocyte activity *in vitro*. J. Immunol 1985;134:3032–3035. [PubMed: 3156926]

23. Bhalla AK, Amento EP, Krane SM. Differential effects of 1,25-dihydroxyvitamin $D_3$ on human lymphocytes and monocyte/macrophages: inhibition of interleukin-2 and augmentation of interleukin-1 production. Cell. Immunol 1986;98:311–322. [PubMed: 3489547]
24. Reichel H, Koeffler HP, Tobler A, Norman AW. 1α,25-dihydroxyvitamin $D_3$ inhibits γ-interferon synthesis by normal human peripheral blood lymphocytes. Proc. Natl Acad. Sci. USA 1987;84:3385–3389. [PubMed: 3033646]
25. Rigby WF, Yirinec B, Oldershaw RL, Fanger MW. Comparison of the effects of 1,25-dihydroxyvitamin $D_3$ on T lymphocyte subpopulations. Eur. J. Immunol 1987;17:563–566. [PubMed: 3106071]
26. Meehan MA, Kerman RH, Lemire JM. 1,25-dihydroxyvitamin $D_3$ enhances the generation of nonspecific suppressor cells while inhibiting the induction of cytotoxic cells in a human MLR. Cell. Immunol 1992;140:400–409. [PubMed: 1531943]
27. Alroy I, Towers TL, Freedman LP. Transcriptional repression of the interleukin-2 gene by vitamin $D_3$: direct inhibition of NFATp/AP-1 complex formation by a nuclear hormone receptor. Mol. Cell. Biol 1995;15:5789–5799. [PubMed: 7565732]
28. Cippitelli M, Santoni A. Vitamin $D_3$: a transcriptional modulator of the interferon-γ gene. Eur. J. Immunol 1998;28:3017–3030. [PubMed: 9808170]
29. Matsui T, Nakao Y, Koizumi T, Nakagawa T. & Fujita, T 1,25-dihydroxyvitamin $D_3$ regulates proliferation of activated T-lymphocyte subsets. Life Sci 1985;37:95–101. [PubMed: 2989641]
30. Muller K, Bendtzen K. Inhibition of human T lymphocyte proliferation and cytokine production by 1,25-dihydroxyvitamin $D_3$. Differential effects on $CD45RA^+$ and $CD45R0^+$ cells. Autoimmunity 1992;14:37–43. [PubMed: 1299346]
31. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin $D_3$ receptor in the immune system. Arch. Biochem. Biophys 2000;374:334–338. [PubMed: 10666315]
32. D'Ambrosio D, et al. Inhibition of IL-12 production by 1,25-dihydroxyvitamin $D_3$. Involvement of NF-κB downregulation in transcriptional repression of the p40 gene. J. Clin. Invest 1998;101:252–262. [PubMed: 9421488]
33. Penna G, Adorini L. 1α, 25-dihydroxyvitamin $D_3$ inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J. Immunol 2000;164:2405–2411. [PubMed: 10679076]
34. Penna G, et al. Treatment of experimental autoimmune prostatitis in nonobese diabetic mice by the vitamin D receptor agonist elocalcitol. J. Immunol 2006;177:8504–8511. [PubMed: 17142748]
35. Daniel C, Sartory NA, Zahn N, Radeke HH, Stein JM. Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1 / Th 17 to a Th2 and regulatory T cell profile. J. Pharmacol. Exp. Ther 2008;324:23–33. [PubMed: 17911375]
36. Gorman S, et al. Topically applied 1,25-dihydroxyvitamin $D_3$ enhances the suppressive activity of $CD4^+$ $CD25^+$ cells in the draining lymph nodes. J. Immunol 2007;179:6273–6283. [PubMed: 17947703]
37. Penna G, et al. Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for induction of $CD4^+$ $Foxp3^+$ regulatory T cells by 1,25-dihydroxyvitamin $D_3$. Blood 2005;106:3490–3497. [PubMed: 16030186]
38. Muller K, Heilmann C, Poulsen LK, Barington T, Bendtzen K. The role of monocytes and T cells in 1,25-dihydroxyvitamin $D_3$ mediated inhibition of B cell function *in vitro*. Immunopharmacology 1991;21:121–128. [PubMed: 1880018]
39. Shirakawa AK, et al. 1,25-dihydroxyvitamin $D_3$ induces CCR10 expression in terminally differentiating human B cells. J. Immunol 2008;180:2786–2795. [PubMed: 18292499]
40. Chong PJ, et al. 1,25 dihydroxyvitamin-$D_3$ regulation of immunoglobulin production in peripheral blood mononuclear cells of patients with systemic lupus erythematosus. J. Autoimmun 1989;2:861–867. [PubMed: 2695100]
41. Griffin MD, et al. Dendritic cell modulation by 1α, 25 dihydroxyvitamin $D_3$ and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity *in vitro* and *in vivo*. Proc. Natl Acad. Sci. USA 2001;98:6800–6805. [PubMed: 11371626]

42. Ohta M, Okabe T, Ozawa K, Urabe A, Takaku F. 1α, 25-dihydroxyvitamin $D_3$ (calcitriol) stimulates proliferation of human circulating monocytes *in vitro*. FEBS Lett 1985;185:9–13. [PubMed: 3838944]
43. Mathieu C, et al. *In vitro* and *in vivo* analysis of the immune system of vitamin D receptor knockout mice. J. Bone Miner. Res 2001;16:2057–2065. [PubMed: 11697802]
44. Dennert G, Lotan R. Effects of retinoic acid on the immune system: stimulation of T killer cell induction. Eur. J. Immunol 1978;8:23–29. [PubMed: 346357]
45. Ertesvag A, Engedal N, Naderi S, Blomhoff HK. Retinoic acid stimulates the cell cycle machinery in normal T cells: involvement of retinoic acid receptor-mediated IL-2 secretion. J. Immunol 2002;169:5555–5563. [PubMed: 12421932]
46. Carman JA, Smith SM, Hayes CE. Characterization of a helper T lymphocyte defect in vitamin A-deficient mice. J. Immunol 1989;142:388–393. [PubMed: 2463304]
47. Blomhoff HK, et al. Vitamin A is a key regulator for cell growth, cytokine production, and differentiation in normal B cells. J. Biol. Chem 1992;267:23988–23992. [PubMed: 1429735]
48. Ballow M, Xiang S, Wang W, Brodsky L. The effects of retinoic acid on immunoglobulin synthesis: role of interleukin 6. J. Clin. Immunol 1996;16:171–179. [PubMed: 8734361]
49. Ertesvag A, Aasheim HC, Naderi S, Blomhoff HK. Vitamin A potentiates CpG-mediated memory B-cell proliferation and differentiation: involvement of early activation of p38MAPK. Blood 2007;109:3865–3872. [PubMed: 17209053]
50. Saurer L, McCullough KC, Summerfield A. *In vitro* induction of mucosa-type dendritic cells by all-trans retinoic acid. J. Immunol 2007;179:3504–3514. [PubMed: 17785784] This paper shows that DCs can efficiently store retinoic acid, which can be ‘released’ during lymphocyte activation.
51. Lomo J, et al. RAR-, not RXR, ligands inhibit cell activation and prevent apoptosis in B-lymphocytes. J. Cell. Physiol 1998;175:68–77. [PubMed: 9491782]
52. Buck J, Derguini F, Levi E, Nakanishi K, Hammerling U. Intracellular signaling by 14-hydroxy-4, 14-retro-retinol. Science 1991;254:1654–1656. [PubMed: 1749937]
53. O’Connell MJ, et al. Retro-retinoids in regulated cell growth and death. J. Exp. Med 1996;184:549–555. [PubMed: 8760808]
54. Darmanin S, et al. *All-trans* retinoic acid enhances murine dendritic cell migration to draining lymph nodes via the balance of matrix metalloproteinases and their inhibitors. J. Immunol 2007;179:4616–4625. [PubMed: 17878359]
55. Geissmann F, et al. Retinoids regulate survival and antigen presentation by immature dendritic cells. J. Exp. Med 2003;198:623–634. [PubMed: 12925678] This paper shows that retinoic acid can also act on DCs, modulating their antigen-presenting capacity.
56. Wiedermann U, Hanson LA, Kahu H, Dahlgren UI. Aberrant T-cell function *in vitro* and impaired T-cell dependent antibody response *in vivo* in vitamin A-deficient rats. Immunology 1993;80:581–586. [PubMed: 8307607]
57. Iwata M, Eshima Y, Kagechika H. Retinoic acids exert direct effects on T cells to suppress Th1 development and enhance Th2 development via retinoic acid receptors. Int. Immunol 2003;15:1017–1025. [PubMed: 12882839]
58. Nikawa T, et al. Vitamin A prevents the decline in immunoglobulin A and Th2 cytokine levels in small intestinal mucosa of protein-malnourished mice. J. Nutr 1999;129:934–941. [PubMed: 10222382]
59. Lovett-Racke AE, Racke MK. Retinoic acid promotes the development of Th2-like human myelin basic protein-reactive T cells. Cell. Immunol 2002;215:54–60. [PubMed: 12142036]
60. Dawson HD, et al. Direct and indirect effects of retinoic acid on human Th2 cytokine and chemokine expression by human T lymphocytes. BMC Immunol 2006;7:27. [PubMed: 17118196]
61. Schuster GU, Kenyon NJ, Stephensen CB. Vitamin A deficiency decreases and high dietary vitamin A increases disease severity in the mouse model of asthma. J. Immunol 2008;180:1834–1842. [PubMed: 18209081]
62. Hoag KA, Nashold FE, Goverman J, Hayes CE. Retinoic acid enhances the T helper 2 cell development that is essential for robust antibody responses through its action on antigen-presenting cells. J. Nutr 2002;132:3736–3739. [PubMed: 12468615]

63. Belkaid Y. Regulatory T cells and infection: a dangerous necessity. Nature Rev. Immunol 2007;7:875–888. [PubMed: 17948021]
64. Mucida D, et al. Reciprocal $T_H17$ and regulatory T cell differentiation mediated by retinoic acid. Science 2007;317:256–260. [PubMed: 17569825] This paper shows that retinoic acid enhances $T_{Reg}$-cell differentiation while blocking differentiation towards $T_H17$ cells.
65. Benson MJ, Pino-Lagos K, Rosemblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. J. Exp. Med 2007;204:1775–1785. [PubMed: 17620362] This paper shows that retinoic acid enhances $T_{Reg}$ -cell differentiation and imprints $T_{Reg}$ cells with gut tropism.
66. Sun CM, et al. Small intestine lamina propria dendritic cells promote *de novo* generation of Foxp3 T reg cells via retinoic acid. J. Exp. Med 2007;204:1775–1785. [PubMed: 17620362] This paper demonstrates that lamina propria DCs induce *de novo* $T_{Reg}$ -cell differentiation in a retinoic acid-dependent manner.
67. Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. Nature Immunol 2007;8:1086–1094. [PubMed: 17873879]
68. Uematsu S, et al. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. Nature Immunol 2008;9:769–776. [PubMed: 18516037]
69. Bettelli E, et al. Reciprocal developmental pathways for the generation of pathogenic effector $T_H17$ and regulatory T cells. Nature 2006;441:235–238. [PubMed: 16648838]
70. Manel N, Unutmaz D, Littman DR. The differentiation of human $T_H$-17 cells requires transforming growth factor-b and induction of the nuclear receptor RORγt. Nature Immunol 2008;9:641–649. [PubMed: 18454151] This paper re-assesses the role of TGFβ in human $T_H17$-cell differentiation, showing that, similar to mouse $T_H17$ cells, human $T_H17$ cells also require this cytokine for their differentiation.
71. Ivanov II, et al. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-$17^+$ T helper cells. Cell 2006;126:1121–1133. [PubMed: 16990136] This paper demonstrates that the transcription factor RORγt is necessary and sufficient for $T_H17$-cell differentiation.
72. Snapper CM, Marcu KB, Zelazowski P. The immunoglobulin class switch: beyond "accessibility". Immunity 1997;6:217–223. [PubMed: 9075922]
73. Mora JR. Homing imprinting and immunomodulation in the gut: role of dendritic cells and retinoids. Inflamm. Bowel Dis 2008;14:275–289. [PubMed: 17924560]
74. Iwasaki A, Kelsall BL. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. J. Exp. Med 1999;190:229–239. [PubMed: 10432286]
75. Sato A, et al. $CD11b^+$ Peyer's patch dendritic cells secrete IL-6 and induce IgA secretion from naive B cells. J. Immunol 2003;171:3684–3690. [PubMed: 14500666]
76. Mora JR, et al. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. Science 2006;314:1157–1160. [PubMed: 17110582] This paper shows that retinoic acid is a mechanistic link between gut-homing imprinting of B cells and differentiation of IgA-secreting cells.
77. Travis MA, et al. Loss of integrin $\alpha_v$ $\beta_8$ on dendritic cells causes autoimmunity and colitis in mice. Nature 2007;449:361–365. [PubMed: 17694047]
78. He B, et al. Intestinal bacteria trigger T cell-independent immunoglobulin $A_2$ class switching by inducing epithelial-cell secretion of the cytokine APRIL. Immunity 2007;26:812–826. [PubMed: 17570691]
79. Tezuka H, et al. Regulation of IgA production by naturally occurring TNF/iNOS-producing dendritic cells. Nature 2007;448:929–933. [PubMed: 17713535] This paper shows that iNOS is required for IgA production in all mucosal compartments. It is also reported that gut-associated DCs express iNOS in a TLR-dependent manner.
80. Tokuyama H, Tokuyama Y. The regulatory effects of all-trans-retinoic acid on isotype switching: retinoic acid induces IgA switch rearrangement in cooperation with IL-5 and inhibits IgG1 switching. Cell. Immunol 1999;192:41–47. [PubMed: 10066345]

81. Nikawa T, et al. Impaired vitamin A-mediated mucosal IgA response in IL-5 receptor-knockout mice. Biochem. Biophys. Res. Commun 2001;285:546–549. [PubMed: 11444878]

82. Ramsay AJ, et al. The role of interleukin-6 in mucosal IgA antibody responses *in vivo*. Science 1994;264:561–563. [PubMed: 8160012]

83. Bjersing JL, Telemo E, Dahlgren U, Hanson LA. Loss of ileal $IgA^+$ plasma cells and of $CD4^+$ lymphocytes in ileal Peyer's patches of vitamin A deficient rats. Clin. Exp. Immunol 2002;130:404–408. [PubMed: 12452829] This paper shows that vitamin A depletion markedly decreases the number of IgA-antibody-secreting cells in the rat ileum.

84. Kuwabara K, Shudo K, Hori Y. Novel synthetic retinoic acid inhibits rat collagen arthritis and differentially affects serum immunoglobulin subclass levels. FEBS Lett 1996;378:153–156. [PubMed: 8549823]

85. Zou F, et al. Retinoic acid activates human inducible nitric oxide synthase gene through binding of RARα/RXRα heterodimer to a novel retinoic acid response element in the promoter. Biochem. Biophys. Res. Commun 2007;355:494–500. [PubMed: 17306764]

86. Seguin-Devaux C, et al. Enhancement of the inducible NO synthase activation by retinoic acid is mimicked by RARα agonist *in vivo*. Am. J. Physiol. Endocrinol. Metab 2002;283:E525–E535. [PubMed: 12169446]

87. Bergqvist P, Gardby E, Stensson A, Bemark M, Lycke NY. Gut IgA class switch recombination in the absence of CD40 does not occur in the lamina propria and is independent of germinal centers. J. Immunol 2006;177:7772–7783. [PubMed: 17114448]

88. Sirisinha S, Darip MD, Moongkarndi P, Ongsakul M, Lamb AJ. Impaired local immune response in vitamin A-deficient rats. Clin. Exp. Immunol 1980;40:127–135. [PubMed: 7389210]

89. Mora JR, von Andrian UH. Differentiation and homing of IgA-secreting cells. Mucosal Immunology 2008;1:96–109. [PubMed: 19079167]

90. Campbell DJ, Butcher EC. Rapid acquisition of tissue-specific homing phenotypes by $CD4^+$ T cells activated in cutaneous or mucosal lymphoid tissues. J. Exp. Med 2002;195:135–141. [PubMed: 11781372]

91. Jung S, et al. *In vivo* depletion of $CD11c^+$ dendritic cells abrogates priming of $CD8^+$ T cells by exogenous cell-associated antigens. Immunity 2002;17:211–220. [PubMed: 12196292]

92. Stagg AJ, Kamm MA, Knight SC. Intestinal dendritic cells increase T cell expression of α4β7 integrin. Eur. J. Immunol 2002;32:1445–1454. [PubMed: 11981833]

93. Mora JR, et al. Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. Nature 2003;424:88–93. [PubMed: 12840763]

94. Johansson-Lindbom B, et al. Selective generation of gut tropic T cells in gut-associated lymphoid tissue (GALT): requirement for GALT dendritic cells and adjuvant. J. Exp. Med 2003;198:963–969. [PubMed: 12963696]

95. Mora JR, et al. Reciprocal and dynamic control of CD8 T cell homing by dendritic cells from skin- and gut-associated lymphoid tissues. J. Exp. Med 2005;201:303–316. [PubMed: 15642741]

96. Dudda JC, et al. Dendritic cells govern induction and reprogramming of polarized tissue-selective homing receptor patterns of T cells: important roles for soluble factors and tissue microenvironments. Eur J. Immunol 2005;35:1056–1065. [PubMed: 15739162]

97. McDermott MR, et al. Impaired intestinal localization of mesenteric lymphoblasts associated with vitamin A deficiency and protein-calorie malnutrition. Immunology 1982;45:1–5. [PubMed: 6799390] This paper shows that vitamin A depletion decreases the migration of mesenteric lymph node lymphoblasts to the small bowel (but not the colon).

98. Chang SY, et al. Colonic patches direct the cross-talk between systemic compartments and large intestine independently of innate immunity. J. Immunol 2008;180:1609–1618. [PubMed: 18209057]

99. Yamanaka KI, et al. Vitamins A and D are potent inhibitors of cutaneous lymphocyte-associated antigen expression. J. Allergy Clin. Immunol 2007;121:148–157. [PubMed: 17910894] This paper shows that $1,25(OH)_2VD_3$ decreases the expression of ligands for E- and P-selectin, inhibits T-cell homing to the inflamed skin and decreases the expression of gut-homing receptors.

100. Mora JR, von Andrian UH. Retinoic acid: an educational "vitamin elixir" for gut-seeking T cells. Immunity 2004;21:458–460. [PubMed: 15485623]

101. Reichrath J. Vitamin D and the skin: an ancient friend, revisited. Exp. Dermatol 2007;16:618–625. [PubMed: 17576242]
102. Maly P, et al. The α1,3 fucosyltransferase Fuc-TVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis. Cell 1996;86:643–653. [PubMed: 8752218]
103. Pedersen LB, Nashold FE, Spach KM, Hayes CE. 1,25-dihydroxyvitamin $D_3$ reverses experimental autoimmune encephalomyelitis by inhibiting chemokine synthesis and monocyte trafficking. J. Neurosci. Res 2007;85:2480–2490. [PubMed: 17600374]
104. Giarratana N, et al. A vitamin D analog down-regulates proinflammatory chemokine production by pancreatic islets inhibiting T cell recruitment and type 1 diabetes development. J. Immunol 2004;173:2280–2287. [PubMed: 15294940]
105. Singh U, Devaraj S. Vitamin E: inflammation and atherosclerosis. Vitam. Horm 2007;76:519–549. [PubMed: 17628188]
106. Jialal I, Devaraj S, Kaul N. The effect of α-tocopherol on monocyte proatherogenic activity. J. Nutr 2001;131:389S–394S. [PubMed: 11160567]
107. Devaraj S, Jialal I. α-tocopherol decreases interleukin-1 β release from activated human monocytes by inhibition of 5-lipoxygenase. Arterioscler. Thromb. Vasc. Biol 1999;19:1125–1133. [PubMed: 10195945]
108. Munteanu A, Zingg JM. Cellular, molecular and clinical aspects of vitamin E on atherosclerosis prevention. Mol. Aspects Med 2007;28:538–590. [PubMed: 17825403]
109. Cominacini L, et al. Antioxidants inhibit the expression of intercellular cell adhesion molecule-1 and vascular cell adhesion molecule-1 induced by oxidized LDL on human umbilical vein endothelial cells. Free Radic. Biol. Med 1997;22:117–127. [PubMed: 8958136]
110. Yoshikawa T, et al. α-tocopherol protects against expression of adhesion molecules on neutrophils and endothelial cells. Biofactors 1998;7:15–19. [PubMed: 9523024]
111. Winkler C, Schroecksnadel K, Schennach H, Fuchs D. Vitamin C and E suppress mitogen-stimulated peripheral blood mononuclear cells *in vitro*. Int. Arch. Allergy Immunol 2007;142:127–132. [PubMed: 17057410]
112. Li-Weber M, et al. Vitamin E inhibits CD95 ligand expression and protects T cells from activation-induced cell death. J. Clin. Invest 2002;110:681–690. [PubMed: 12208869]
113. Pratico D, Tangirala RK, Rader DJ, Rokach J, FitzGerald GA. Vitamin E suppresses isoprostane generation *in vivo* and reduces atherosclerosis in ApoE-deficient mice. Nature Med 1998;4:1189–1192. [PubMed: 9771755]
114. Ichikawa H, et al. A novel vitamin E derivative (TMG) protects against gastric mucosal damage induced by ischemia and reperfusion in rats. Dig. Dis. Sci 2003;48:54–58. [PubMed: 12645790]
115. Onem G, et al. Neuroprotective effects of L-carnitine and vitamin E alone or in combination against ischemia-reperfusion injury in rats. J. Surg. Res 2006;131:124–130. [PubMed: 16457849]
116. Terasawa Y, et al. Increased atherosclerosis in hyperlipidemic mice deficient in α -tocopherol transfer protein and vitamin E. Proc. Natl Acad. Sci. USA 2000;97:13830–13834. [PubMed: 11095717]
117. Ozcan AV, et al. The effects of iloprost and vitamin C on kidney as a remote organ after ischemia/reperfusion of lower extremities. J. Surg. Res 2007;140:20–26. [PubMed: 17397869]
118. Lehr HA, Frei B, Arfors KE. Vitamin C prevents cigarette smoke-induced leukocyte aggregation and adhesion to endothelium *in vivo*. Proc. Natl Acad. Sci. USA 1994;91:7688–7692. [PubMed: 7519784]
119. Lehr HA, Frei B, Olofsson AM, Carew TE, Arfors KE. Protection from oxidized LDL-induced leukocyte adhesion to microvascular and macrovascular endothelium *in vivo* by vitamin C but not by vitamin E. Circulation 1995;91:1525–1532. [PubMed: 7867194]
120. Endo N, et al. Antioxidant activity of vitamin B6 delays homocysteine-induced atherosclerosis in rats. Br. J. Nutr 2006;95:1088–1093. [PubMed: 16768830]
121. Thomson MJ, Puntmann V, Kaski JC. Atherosclerosis and oxidant stress: the end of the road for antioxidant vitamin treatment? Cardiovasc. Drugs Ther 2007;21:195–210. [PubMed: 17484034]

122. Lentz SR, Piegors DJ, Malinow MR, Heistad DD. Supplementation of atherogenic diet with B vitamins does not prevent atherosclerosis or vascular dysfunction in monkeys. Circulation 2001;103:1006–1011. [PubMed: 11181477]

123. Littorin B, et al. Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS). Diabetologia 2006;49:2847–2852. [PubMed: 17072585]

124. Muller K, et al. Vitamin $D_3$ metabolism in patients with rheumatic diseases: low serum levels of 25-hydroxyvitamin $D_3$ in patients with systemic lupus erythematosus. Clin. Rheumatol 1995;14:397–400. [PubMed: 7586974]

125. Cutolo M, Otsa K, Uprus M, Paolino S, Seriolo B. Vitamin D in rheumatoid arthritis. Autoimmun. Rev 2007;7:59–64. [PubMed: 17967727]

126. Cantorna MT, Munsick C, Bemiss C, Mahon BD. 1,25-dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. J. Nutr 2000;130:2648–2652. [PubMed: 11053501]

127. Mathieu C, Gysemans C, Giulietti A, Bouillon R. Vitamin D and diabetes. Diabetologia 2005;48:1247–1257. [PubMed: 15971062]

128. Yener E, Coker C, Cura A, Keskinoglu A, Mir S. Lymphocyte subpopulations in children with vitamin D deficient rickets. Acta Paediatr. Jpn 1995;37:500–502. [PubMed: 7572152]

129. Abe J, et al. Prevention of immunological disorders in MRL/l mice by a new synthetic analogue of vitamin $D_3$, 22-oxa-1α, 25-dihydroxyvitamin $D_3$. J. Nutr. Sci. Vitaminol. (Tokyo) 1990;36:21–31. [PubMed: 2163440]

130. Lemire JM, Ince A, Takashima M. 1,25-dihydroxyvitamin $D_3$ attenuates the expression of experimental murine lupus of MRL/l mice. Autoimmunity 1992;12:143–148. [PubMed: 1617111]

131. Lemire JM, Archer DC. 1,25-dihydroxyvitamin $D_3$ prevents the *in vivo* induction of murine experimental autoimmune encephalomyelitis. J. Clin. Invest 1991;87:1103–1107. [PubMed: 1705564]

132. Spach KM, Nashold FE, Dittel BN, Hayes CE. IL-10 signaling is essential for 1,25-dihydroxyvitamin $D_3$-mediated inhibition of experimental autoimmune encephalomyelitis. J. Immunol 2006;177:6030–6037. [PubMed: 17056528]

133. Barrat FJ, et al. *In vitro* generation of interleukin 10-producing regulatory $CD4^+$ T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. J. Exp. Med 2002;195:603–616. [PubMed: 11877483]

134. Cantorna MT, Hayes CE, DeLuca HF. 1,25-dihydroxycholecalciferol inhibits the progression of arthritis in murine models of human arthritis. J. Nutr 1998;128:68–72. [PubMed: 9430604]

135. Barna M, Bos JD, Kapsenberg ML, Snijdewint FG. Effect of calcitriol on the production of T-cell-derived cytokines in psoriasis. Br. J. Dermatol 1997;136:536–541. [PubMed: 9155954]

136. Durakovic C, Ray S, Holick MF. Topical paricalcitol (19-nor-1α, 25-dihydroxyvitamin $D_2$) is a novel, safe and effective treatment for plaque psoriasis: a pilot study. Br. J. Dermatol 2004;151:190–195. [PubMed: 15270890]

137. Li M, et al. Topical vitamin $D_3$ and low-calcemic analogs induce thymic stromal lymphopoietin in mouse keratinocytes and trigger an atopic dermatitis. Proc. Natl Acad. Sci. USA 2006;103:11736–11741. [PubMed: 16880407]

138. Lemire JM, et al. Prolongation of the survival of murine cardiac allografts by the vitamin $D_3$ analogue 1,25-dihydroxy-δ16-cholecalciferol. Transplantation 1992;54:762–763. [PubMed: 1412777]

139. Amuchastegui S, Daniel KC, Adorini L. Inhibition of acute and chronic allograft rejection in mouse models by BXL-628, a nonhypercalcemic vitamin D receptor agonist. Transplantation 2005;80:81–87. [PubMed: 16003237]

140. Zhang AB, Zheng SS, Jia CK, Wang Y. Effect of 1,25-dihydroxyvitamin $D_3$ on preventing allograft from acute rejection following rat orthotopic liver transplantation. World J. Gastroenterol 2003;9:1067–1071. [PubMed: 12717858]

141. Gregori S, et al. Regulatory T cells induced by 1α, 25-dihydroxyvitamin $D_3$ and mycophenolate mofetil treatment mediate transplantation tolerance. J. Immunol 2001;167:1945–1953. [PubMed: 11489974]

142. Pakkala I, Taskinen E, Pakkala S, Raisanen-Sokolowski A. MC1288, a vitamin D analog, prevents acute graft-versus-host disease in rat bone marrow transplantation. Bone Marrow Transplant 2001;27:863–867. [PubMed: 11477445]

143. Hullett DA, et al. Prevention of chronic allograft nephropathy with vitamin D. Transpl. Int 2005;18:1175–1186. [PubMed: 16162105]

144. Middleton PG, et al. Vitamin D receptor gene polymorphism associates with graft-versus-host disease and survival in HLA-matched sibling allogeneic bone marrow transplantation. Bone Marrow Transplant 2002;30:223–228. [PubMed: 12203138]

145. Sezer S, Uyar M, Arat Z, Ozdemir FN, Haberal M. Potential effects of 1,25-dihydroxyvitamin $D_3$ in renal transplant recipients. Transplant. Proc 2005;37:3109–3111. [PubMed: 16213322]

146. Caravaca F, et al. Are plasma 1,25-dihydroxyvitamin $D_3$ concentrations appropriate after successful kidney transplantation? Nephrol. Dial. Transplant 1998;13 Suppl. 3:91–93. [PubMed: 9568829]

147. Fleseriu M, Licata AA. Failure of successful renal transplant to produce appropriate levels of 1,25-dihydroxyvitamin D. Osteoporos. Int 2007;18:363–368. [PubMed: 17061149]

148. Cantorna MT, et al. 1,25-dihydroxyvitamin $D_3$ prolongs graft survival without compromising host resistance to infection or bone mineral density. Transplantation 1998;66:828–831. [PubMed: 9798689]

149. Smith EA, et al. Effects of long-term administration of vitamin $D_3$ analogs to mice. J. Endocrinol 2000;165:163–172. [PubMed: 10750047]

150. Sommer A, Tarwotjo I, Hussaini G, Susanto D. Increased mortality in children with mild vitamin A deficiency. Lancet 1983;2:585–588. [PubMed: 6136744] References 149 and 150 provide epidemiological evidence that indicate a role for vitamin A in preventing child mortality in developing countries.

151. Sommer A, et al. Impact of vitamin A supplementation on childhood mortality. A randomised controlled community trial. Lancet 1986;1:1169–1173. [PubMed: 2871418]

152. Semba RD, et al. Effect of periodic vitamin A supplementation on mortality and morbidity of human immunodeficiency virus-infected children in Uganda: a controlled clinical trial. Nutrition 2005;21:25–31. [PubMed: 15661475]

153. Racke MK, et al. Retinoid treatment of experimental allergic encephalomyelitis. IL-4 production correlates with improved disease course. J. Immunol 1995;154:450–458. [PubMed: 7527821]

154. Wang T, et al. The effect of Am-80, one of retinoids derivatives on experimental allergic encephalomyelitis in rats. Life Sci 2000;67:1869–1879. [PubMed: 11043609]

155. Brinckerhoff CE, Coffey JW, Sullivan AC. Inflammation and collagenase production in rats with adjuvant arthritis reduced with 13-cis-retinoic acid. Science 1983;221:756–758. [PubMed: 6308759]

156. Nagai H, et al. Effect of Am-80, a synthetic derivative of retinoid, on experimental arthritis in mice. Pharmacology 1999;58:101–112. [PubMed: 9873234]

157. Escribese MM, et al. Therapeutic effect of *all-trans*-retinoic acid (at-RA) on an autoimmune nephritis experimental model: role of the VLA-4 integrin. BMC Nephrol 2007;8:3. [PubMed: 17250768]

158. van de Kerkhof PC. Update on retinoid therapy of psoriasis in: an update on the use of retinoids in dermatology. Dermatol. Ther 2006;19:252–263. [PubMed: 17014480]

159. Ruzicka T, et al. Oral alitretinoin (9-cis-retinoic acid) therapy for chronic hand dermatitis in patients refractory to standard therapy: results of a randomized, double-blind, placebo-controlled, multicenter trial. Arch. Dermatol 2004;140:1453–1459. [PubMed: 15611422]

160. Niwa S, et al. Effect of Am-80, a retinoid derivative, on 2, 4-dinitrofluorobenzene-induced contact dermatitis in mice. Pharmacology 2000;60:208–214. [PubMed: 10828746]

161. Stephensen CB, Moldoveanu Z, Gangopadhyay NN. Vitamin A deficiency diminishes the salivary immunoglobulin A response and enhances the serum immunoglobulin G. response to influenza A virus infection in BALB/c mice. J. Nutr 1996;126:94–102. [PubMed: 8558330]

162. Siewert C, et al. Induction of organ-selective $CD4^+$ regulatory T cell homing. Eur. J. Immunol 2007;37:978–989. [PubMed: 17345581]

163. Buck J, et al. Anhydroretinol: a naturally occurring inhibitor of lymphocyte physiology. J. Exp. Med 1993;178:675–680. [PubMed: 8340762]

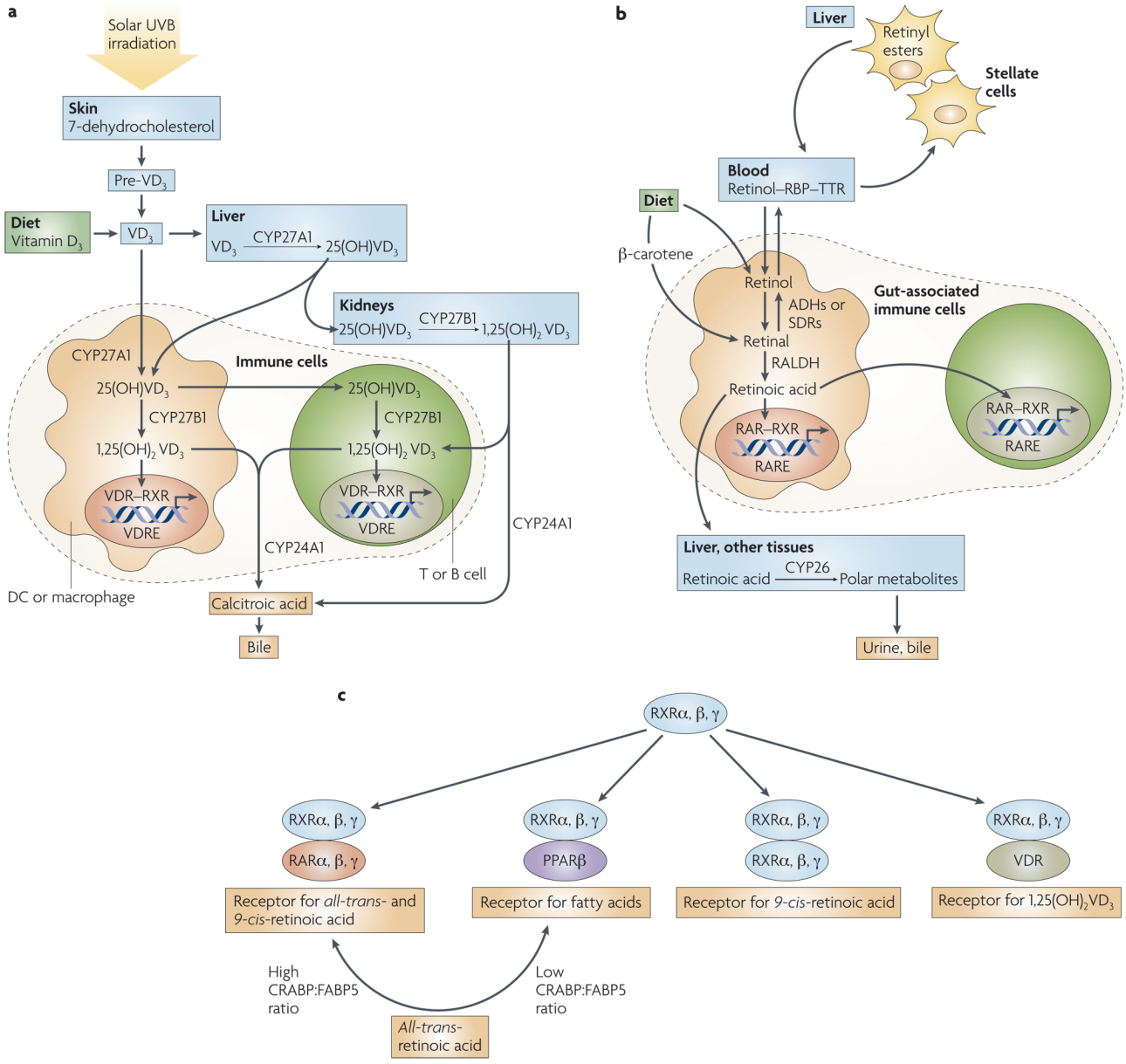


**Figure 1. Overview of vitamin A and D metabolism**

**a** | Vitamin $D_3$ ($VD_3$) is acquired in the diet or synthesized in the skin and hydroxylated in the liver to 25(OH)$VD_3$, the main circulating form. 25(OH)$VD_3$ is then hydroxylated in the kidneys by the cytochrome P450 protein CYP27B1 to become 1,25$(OH)_2VD_3$, 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)$VD_3$ to 1,25$(OH)_2VD_3$. 1,25$(OH)_2VD_3$ acts on immune cells in an autocrine or paracrine manner by binding to the vitamin D receptor (VDR). 24-hydroxylase (CYP24A1) catabolizes 1,25 $(OH)_2VD_3$ to its inactive metabolite, calcitroic 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 $(OH)_2VD_3$) 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.

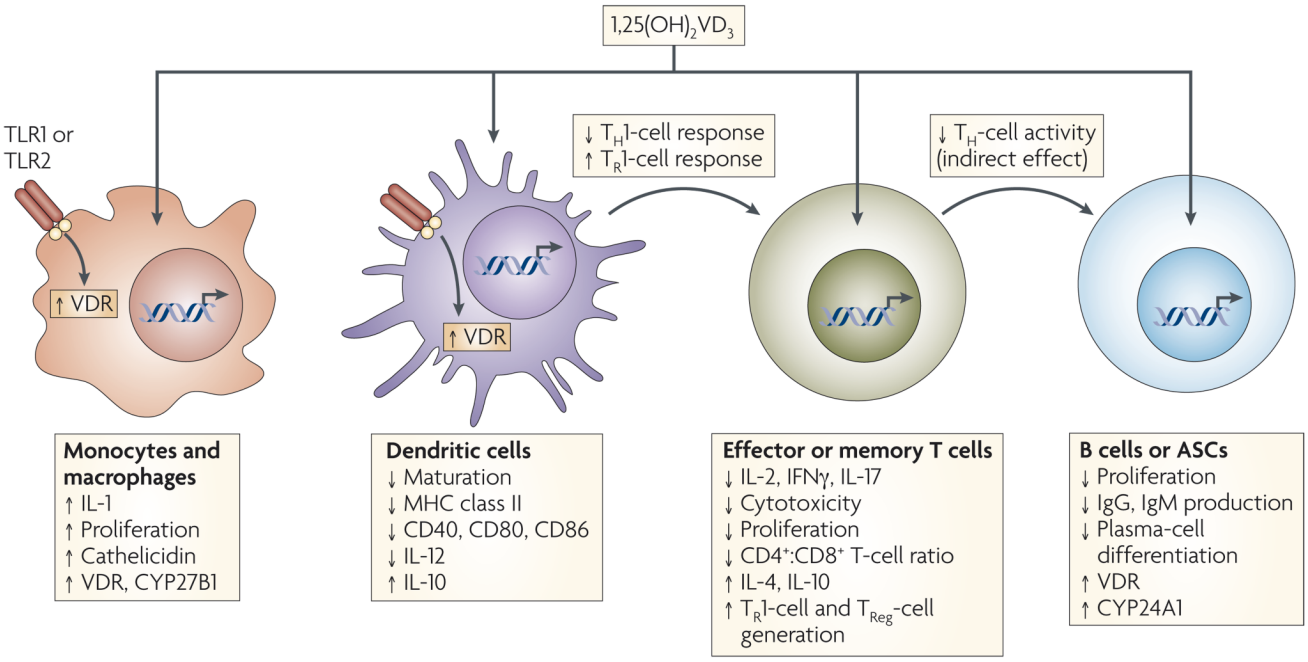


**Figure 2. Mechanisms of vitamin D immunomodulation**

Systemic or locally produced $1,25(OH)_2VD_3$ 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, $1,25(OH)_2VD_3$ 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. $1,25(OH)_2VD_3$ 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. $1,25(OH)_2VD_3$ 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, $1,25(OH)_2VD_3$ 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. $1,25(OH)_2VD_3$ 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, $1,25(OH)_2VD_3$ blocks B-cell proliferation, plasma-cell differentiation and immunoglobulin production. ASCs, antibody-secreting cells.

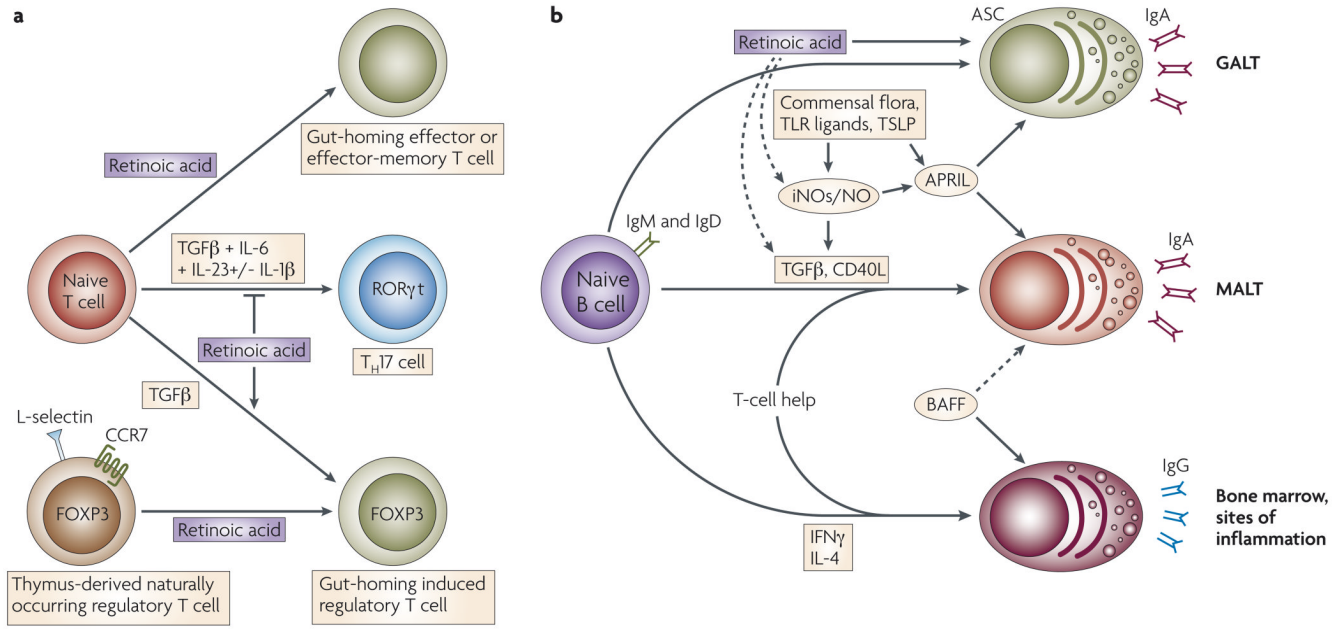


**Figure 3. Effects of vitamin A metabolites on gut mucosal immunity**

**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 (TGFβ) by reciprocally downregulating receptor-related orphan receptor-γt (RORγt) and inducing FOXP3 expression in T cells, respectively. Retinoic acid also enhances the TGFβ-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 TGFβ, interleukin-6 (IL-6), IL-23 and, in humans, IL-1β. **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), TGFβ 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 TGFβ 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.

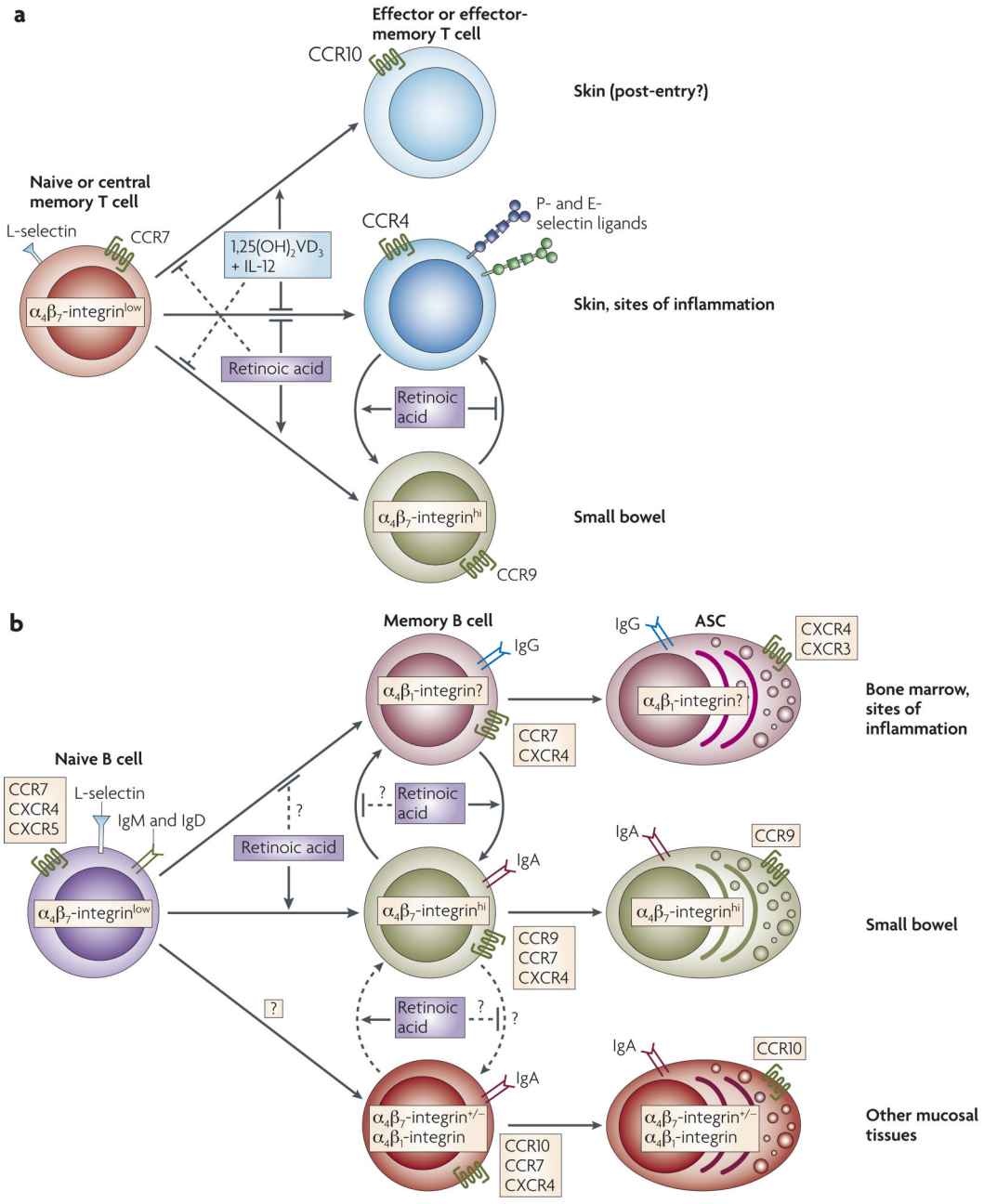


**Figure 4. Roles of retinoic acid and 1,25(OH)$_2$VD$_3$ in tissue-specific lymphocyte homing**

**a** | Retinoic acid produced by gut-associated lymphoid tissue (GALT)-resident dendritic cells and probably by other cells, such as intestinal epithelial cells, potently 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)$_2$VD$_3$ (the main circulating vitamin D$_3$ (VD$_3$) metabolite) induces the expression of skin-associated CCR10 by human (but not mouse)

T cells. However, $1,25(OH)_2VD_3$ blocks the induction of E-selectin ligands and therefore inhibits skin-homing. So, it is possible that $1,25(OH)_2VD_3$ 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). $1,25(OH)_2VD_3$ also antagonizes the upregulation of gut-homing receptors, whereas retinoic acid reciprocally blocks the induction of CCR10 expression by $1,25(OH)_2VD_3$. **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.