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# Mechanisms by which Vitamin A and D may Contribute to (Oral) Tolerance Induction

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Possible relevance for  
inflammatory bowel disease,  
food allergy and asthma

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## Abstract

Vitamin A and D are fat soluble vitamins that have different functions in the body: vitamin A is important for eye sight while vitamin D is necessary for bone maintenance. However, lately has it been demonstrated that both vitamins may have a pivotal role in the immune system, especially in tolerance induction. Diseases as inflammatory bowel disease (IBD), food allergy and asthma have broken tolerance which results in an improperly working immune system, and therefore symptoms of disease. Vitamin A and D however could be a possible treatment or prevention for these diseases.

Vitamin A's metabolite retinoic acid (RA) will bind its intracellular receptor retinoid X receptor (RXR) in the T-cell directly or in a dendritic cell (DC) and subsequently inhibit T helper cell 1 ( $T_{h1}$ ) or  $T_{h17}$  cell differentiation by reducing TGF- $\beta$ , IL-6 and IL-23 expression. Furthermore will RA stimulate forkhead box P3 (FOXP3) expression which results in regulatory T-cell ( $T_{reg}$ ) differentiation. RA can also stimulate a  $T_{h2}$  response by inducing IL-2 and IL-4. Vitamin D metabolites bind the intracellular vitamin D receptor (VDR) which will form a heterodimer with RXR. This complex can bind specific vitamin D response elements (VDRE) on the genes of different cytokines and in this way skew towards a  $T_{reg}$  or a  $T_{h2}$  response. The cytokines that are affected by vitamin D are especially IL-10 (up-regulation), IL-12 (down-regulation) and the most important, vitamin D affects the Treg marker FOXP3 (up-regulation).

Tolerance, in relation to the described diseases, is the stimulation of  $T_{reg}$  cell differentiation, resulting in a balance between  $T_{h1}$  and  $T_{h2}$  cells, inhibition of inflammatory reaction and a maintenance of mucosal homeostasis. Both vitamin A and D will contribute to oral tolerance induction as they stimulate  $T_{reg}$  differentiation. The induction of oral tolerance by vitamin A and D may be important for the treatment of IBD, food allergy and asthma, as tolerance is broken in these diseases.

Different animal and human studies have been performed to research the relation between vitamin A and D and the described diseases. There are no conclusive results, however the supplementation of vitamin D in both IBD and asthma appear to be promising. Furthermore is it striking that vitamin A demonstrates contradicting results in both human and animal studies regarding the diseases of interest, there is not one disease that shows a conclusive picture. Therefore more research is necessary for both vitamins, in both humans and animals for all diseases.

In conclusion, in immune system function vitamin A and D are pivotal as they both stimulate  $T_{reg}$  differentiation and therefore tolerance. However both animal and human studies with regard to the diseases of interest did not demonstrate a conclusive picture which means more research is necessary.

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## List of abbreviations

1,25-(OH) <sub>2</sub> -D <sub>3</sub> :	1,25-dihydroxyvitamin D3 /calcitriol
9cisRA:	9-cis retinoic acid
25-OH-D <sub>3</sub> :	25-hydroxyvitamin D3
ADH:	alcohol dehydrogenase
APC:	antigen presenting cell
atRA:	all trans retinoic acid
BALT:	bronchus associated lymphoid tissue
CCR9:	C-C chemokine receptor type 9
CD:	cluster of differentiation
DC:	dendritic cell
FOXP3:	forkhead box P3
GALT:	gut associated lymphoid tissue
IBD:	inflammatory bowel disease
IDO:	indoleamine 2,3-dioxygenase
IFN:	interferon
IL:	interleukin
ILT:	immunoglobulin like transcript
MS:	multiple sclerosis
MHC:	major histocompatibility complex
NO:	nitric oxide
RA:	retinoic acid
RALDH:	retinaldehyde dehydrogenase
RAR:	retinoic acid receptor
RARE:	retinoic acid response elements
RXR:	retinoid X receptor
RBP:	retinol-binding protein
SDR:	short-chain dehydrogenase/reductase
T <sub>h</sub> :	T helper cell
TLR:	toll like receptor
TNF:	tumor necrosis factor
T <sub>reg</sub> :	regulatory T-cell
TSLP:	thymic stromal lymphopoietin
VDR:	vitamine D receptor
VDRE:	vitamin D response elements

## Introduction

Vitamin A and D are best known for their function in respectively eye sight and bone maintenance, however there is evidence that they also play a pivotal role in immune system functioning. Both vitamins can be gained from food intake and in addition, vitamin D can also be synthesized by the body itself. A deficiency of vitamin A can result in blindness and even though vitamin A deficiencies are rare in the western world, an estimate of 500 000 children in developing countries are blinded by this deficiency. It is suggested that this deficiency originates from malnutrition and possibly fat mal-absorption (1). Besides blindness, vitamin A deficiency is now also connected to auto-immunity and allergic diseases (2–4) (which are both the result of a failing immune system). Vitamin D deficiency on the other hand can lead to, for example, a disorder in bone mineralization, numbness and seizures. This deficiency is hypothesized to arise from a decreased exposure to sunlight, a decreased dietary intake, renal or liver disease or fat mal-absorption (1). It is now also hypothesized that a deficiency of this vitamin is a cause of the development of IBD and allergies (5,6). The relation between both vitamins, auto-immunity and allergies are center of this report.

## Vitamin A

Vitamin A is a fat soluble vitamin that is not synthesized in the body, which means that humans require an intake of this vitamin that ranges from 600 mg/ day (for women) to 700 mg/day (for men). Sources of vitamin A include butter, whole milk, liver/ fish liver oils and some types of vegetable (green, yellow, orange) (1) and vitamin A is derived from these sources in the form of retinyl esters, retinol and provitamin carotenoids (7).

It has been shown that vitamin A is essential for good eye sight, maintenance of epithelial cell lining, reproduction, embryonic growth and development and this reports interest: immune function (8–10). When vitamin A enters the GI tract, it is converted to retinol and taken up by the intestinal cells through carrier mediated process (however, involved proteins are unknown) (8). Subsequently,

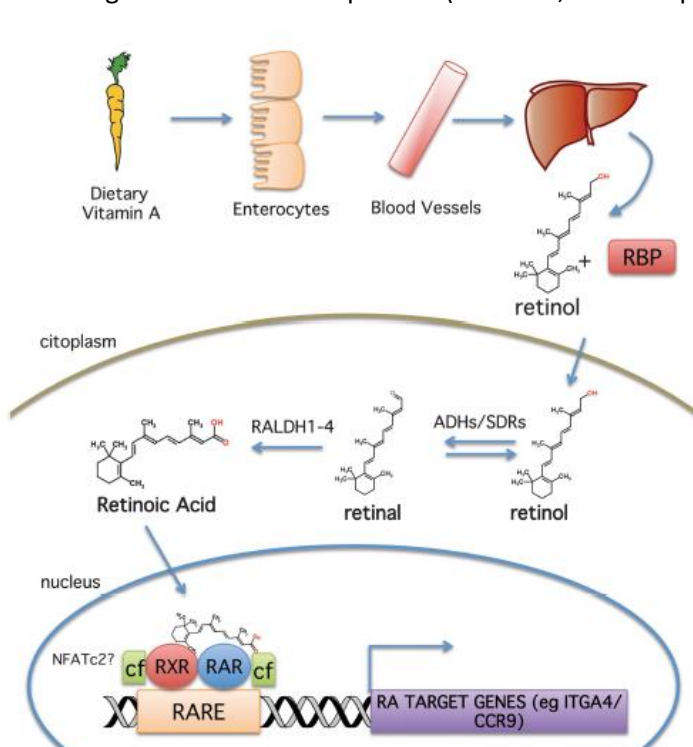


Figure 1. Schematic representation of dietary intake of vitamin A (7). After absorption, vitamin A passes the liver and is metabolized to its active form: RA.

retinol is transported to the liver and here, retinol can be stored (11) or bound to retinol-binding protein (RBP). After this binding, retinol is secreted, circulates and is taken up by most cells of the body through a RBP specific receptor (12). Retinol is intracellularly metabolized to retinal by alcohol dehydrogenase (ADH) or short-chain dehydrogenase/reductase (SDR) and subsequently metabolized to the active metabolite, retinoic acid (RA), by retinaldehyde dehydrogenase enzymes (RALDHs) (7,8,10). RA is the active metabolite of vitamin A and can be present in two forms: all-trans RA (atRA) and 9-cis RA (9cisRA) (13), these can bind to a RA receptor (RAR) or RXR in cells of the body. After RA binding, these receptors form an active RXR-RAR/ RXR-RXR heterodimer complex that recruit and form transcription factors which are called RA response elements (RARE)(7,10). This transcription complex can bind specific DNA sequences and it

is suggested that (for example) the gene for C-C chemokine receptor type 9 (CCR9), a regulator of T-cell maturation and migration, contains RARE (10). Even though vitamin A itself is not active in the body, its metabolites are and they have many important roles in the immune system. The uptake, metabolism and effect of vitamin A is portrayed in figure 1.

### Vitamin D

Vitamin D is also a fat soluble vitamin, however it can be synthesized by the body, which means that it does not require a specific intake amount. Nevertheless, vitamin D is present in fish liver oils (1). The classical effects of vitamin D are focussed on the mineral metabolism and skeletal function, however vitamin D also exerts effects on the immune system. It has been reported that vitamin D has pleiotropic effects on various chronic diseases such as cardiovascular disease, autoimmune disease and diabetes(14). Particularly the effect of the vitamin on diseases of the immune system are of interest.

Vitamin D's most relevant form is vitamin D3, which can be synthesized in the skin from 7-dehydrocholesterol or supplemented via the diet. When the skin is exposed to sunlight, the UV radiation provides a environment where 7-dehydrocholesterol is transferred to vitamin D3. Subsequently, the dietary or sunlight provided vitamin D3 will be metabolized in the liver to 25-hydroxyvitamin D3 (25-OH-D3) catalyzed by the enzymes CYP2R1 and CYP27A1 and finally in the kidneys metabolized to calcitriol (1,25-(OH)<sub>2</sub>-D3) the most active form in the body, catalyzed by CYP27B1 (15–18). Calcitriol exerts its effect through binding to the VDR in cytoplasm. After this binding, the complex enters the nucleus where it heterodimerizes with RXR to VDR-RXR (note that VDR-vitamin D can heterodimerise with bound as well as unbound RXR which means that there is practically no competition with RA). This heterodimer complex will bind VDRE located on DNA (14,19). Subsequently, the activated receptor will recruit co-factors that bind DNA sites to modify the targeted genes (15), which include genes involved in immunity. In addition to the metabolism by the liver and kidneys, vitamin D3 can directly be metabolized by immune cells themselves as these cells contain CYP27B1 (16). This proces ensures that high 1,25-(OH)<sub>2</sub>-D3 concentrations occur in the lymphoid tissues and thereby increasing specific action and decreasing potentially systemic effects (17). Furthermore, there have been at least 38 different tissues identified throughout the body that express the VDR, including intestinal tissue, circulating immune cells, monocytes and macrophages (18). Moreover, the VDR and vitamin D metabolizing enzymes are expressed by key cells of the immune system, making vitamin D important for maintaining a

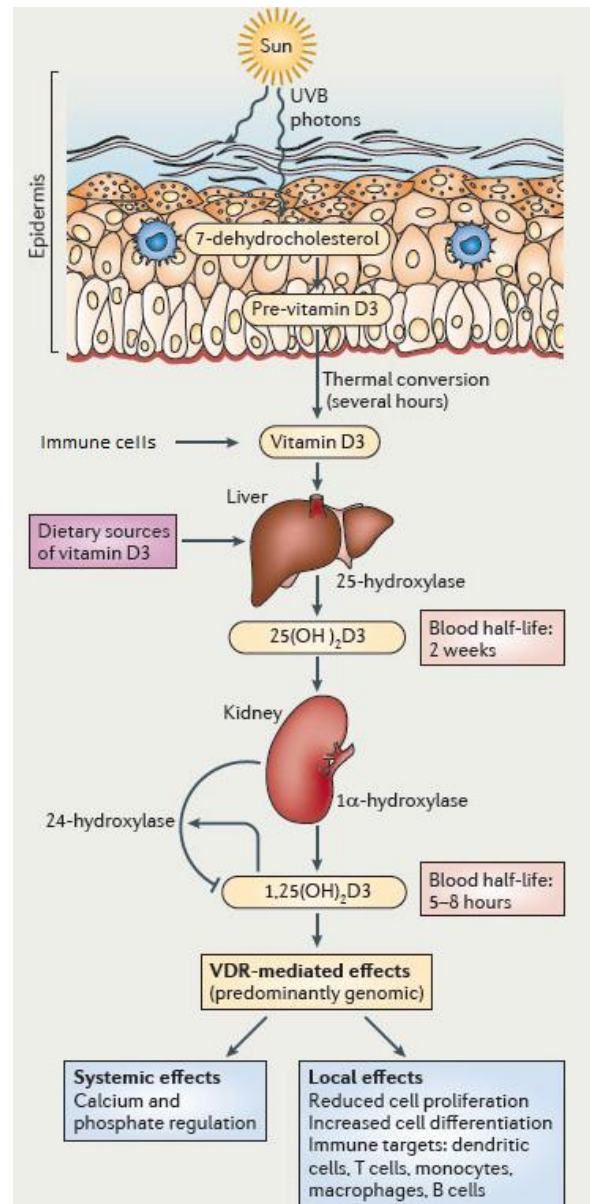


Figure 2. Schematic representation of vitamin D metabolism (20). Vitamin D can enter the body through diet or UV radiation, whereas it is metabolized to its active metabolite.

balanced immune system (14). Figure 2 is a schematic representation of the metabolism of vitamin D3 and its pleiotropic effects.

### *Function of vitamin A and D in immune disorders*

Lately, there has been more interest in the function of both vitamins in autoimmunity and allergic diseases. In this report the diseases of interest are IBD, food allergy and asthma. IBD is a collective noun for chronic, inflammatory, autoimmune diseases that occur in the gastrointestinal (GI) tract, with Crohn's disease and colitis ulcerosa as the two most important forms. In Crohn's disease flare ups can cause transmural inflammation located throughout the entire GI tract and in ulcerative colitis exacerbations cause superficial inflammation of mucosal tissue of the large intestine and/or rectum. Nevertheless these diseases are collectively called IBD because the symptoms can be similar e.g. abdominal pain, diarrhea, and weight loss (18,21). It has been suggested that IBD occurs through the breakdown of mucosal tolerance, and therefore active immune responses against endogenous antigens and bacterial flora in the intestines (22).

While patients who suffer from IBD have immunologic reactions against self-antigens, patients with allergies suffer from reactions against foreign antigens. Food allergy and asthma are both allergic diseases which means that they expose a comparable pathophysiology. Patients that suffer from food allergy develop immune responses to harmless food related antigens: an immune response to food antigens is persuaded, through which food specific IgE molecules from B-cells are produced and a  $T_H2$  response that stimulates IgE class-switch is stimulated (23,24). Though symptoms are expressed in a wide range, a distinction between two types of food allergies can be made. The first type is an immediate reaction to the food antigens, symptoms of this immediate reaction vary from anaphylaxis, skin swelling and allergic skin reaction. Furthermore, the second type is a late reaction of a few hours till a few days after the food intake, in which symptoms as fatigue, headache, paleness, asthma, diarrhea or insomnia occur (25). In the case of asthma is it believed that an allergen will cause a hyper stimulation of  $T_H2$  cells which will result in an allergic reaction (asthma attack). The  $T_H2$  cytokines play a pivotal role in the induction of asthma as for example IL-4 and IL-13 will stimulate IgE production, IL-5 stimulates eosinophils and IL-9 and IL-13 will stimulate hyper secretion of the mucus and airway hyper reactivity (26,27). This results in inflammation, hyper responsiveness and obstruction of the airflow due to specific triggers. Symptoms of this disease include wheezing, cough, dyspnea (shortness of breath) and a feeling of tightness in the chest (28,29).

### *Hypothesis*

Vitamin A and D are capable of inducing tolerance and are a potential treatment or prevention method for IBD, food allergy and asthma.

## Vitamin A and D status in western world in relation to auto-immunity and allergic disease

As mentioned in the introduction, the diseases of interest are IBD, food allergy and asthma: these diseases exist due to a disturbed immune system. IBD is an autoimmune disease where inflammation occurs through attacks of the immune system to cells of the body while food allergy and asthma are diseases whereby allergic inflammation against in principle harmless antigens from outside the body arise. This chapter will give a global outline about vitamin A and D, their status in western countries and how this is related to auto-immune diseases (with focus on IBD) and allergic diseases (with focus on food allergy and asthma).

### *Vitamin A*

It is obvious that vitamin A metabolites have a big impact on the immune system: they stimulate T<sub>reg</sub> differentiation (9), they play a role in effector T-cell differentiation and affect immunoglobulin types (17). Though the role of vitamin A in autoimmune diseases such as IBD is not completely clear, there has been research that suggests that RA inhibits nitric oxide (NO): a molecule present in inflammations and possibly important in IBD pathogenesis (30). Furthermore it has been suggested that vitamin A plays a beneficial role in the treatment of multiple sclerosis (MS), another autoimmune disease (31). Nevertheless, these studies do not mention vitamin A deficiency as a direct cause of autoimmunity, but only as a possible therapy as it is known that vitamin A has a pivotal part in the immune system.

Vitamin A and its relation to allergic diseases is also immune function focused. RA can for example stimulate the IgA switch of B lymphocytes and in this way promote immunity. Furthermore, it has been shown in rats that reduction of vitamin A results in a decrease of IgA producing plasma cells and a disturbed mucosal immune defense (32). It has also been shown that vitamin A plays a key role in immune and oral tolerance induction by stimulating FOXP3 T<sub>reg</sub> cells by DC which are important in the development of oral tolerance (33). However, there are studies that claim there is a direct connection between vitamin A supplementation and food allergy incidence (24). Furthermore, the relation between vitamin A and asthma seems to be more towards the antioxidant functions of vitamin A (in combination with other antioxidants) instead of its immuno-modulatory functions (34). Vitamin A has an obvious function in immune functioning and seems to be related to allergic diseases, however in which way is not clear.

### *Vitamin D*

The last years there have been more theories about how vitamin D deficiency has a pivotal role in the pathogenesis of autoimmune diseases as IBD and multiple sclerosis (MS) (6,35). Though it is possible that vitamin D deficiency is a consequence instead of a cause of these diseases (as the patients are more indoors and vitamin intake is limited due to the inflammations (6,35)), the prevalence of, for example of IBD, is increased in northern countries (such as Iceland, Norway, Denmark, Netherlands). These findings suggest that the lack of UV light and thus vitamin D metabolism may be a cause of IBD. Moreover, vitamin D deficiency may be due to industrialization, urbanization and pollution: the time spent outdoors is reduced by the industrialization, tall buildings may block sunlight and pollution may absorb the UV light (6,18,21). Another more general status of vitamin D is its dependence of the seasons. It has been demonstrated that the lowest levels are at the end of winter while the highest are at the end of summer (16).

Similar to the connection between vitamin D deficiency and autoimmunity, there is also a relation between vitamin D deficiency and allergic diseases as food allergy and asthma. Indirect evidence for this link is once again the lack of UV light: there have been studies that showed that patients with



food allergy are more likely to be born in autumn/ winter (24). Furthermore, there have been studies that showed direct links between vitamin D deficiency and food allergy, these studies demonstrated that vitamin D deficiency increased the risk for peanut and egg allergy. However, there is also indirect and direct evidence that is applicable for a hypothesis where increased vitamin D levels in fact cause a food allergy (24,36,37). The findings that a low and a high vitamin D level can cause food allergy could suggest that vitamin D acts through a U-shaped association, nevertheless it is not mentioned which concentrations are optimal (24). Additionally, it also seems that vitamin D may have a role in the pathophysiology of asthma. Decreased vitamin D levels associate with impaired lung function and hyper responsiveness. Furthermore another study concluded that the administration of vitamin D in combination with calcium decreases airway obstruction (28,38). Both auto-immunity and allergy are associated with vitamin D deficiency however, its effectiveness could be influenced through an U-shaped association.

## General effects of vitamin A and D on the immune system

Before addressing the specific function of vitamin A and D on DC and T-cells, the general function of the vitamins on the immune system will be covered. Furthermore is it necessary to mention that the  $T_{reg}$  that are referred to in this part of the review are adaptive/ induced  $T_{reg}$ . This type of  $T_{reg}$  does not originate in the thymus but develops in inflamed tissue and is induced by different cytokines. Auto-immunity is mediated by  $T_h1$  or  $T_h17$  cells while allergies are mediated by  $T_h2$  cells (9).

### *Vitamin A and the immune system*

After vitamin A is metabolized to RA, RA will affect the adaptive immune response. RA can inhibit  $T_h1$  and  $T_h17$  cells by down regulating IFN- $\gamma$  and IL-17 secretion. Furthermore can it stimulate cytotoxicity, reduce antigen-presenting cell (APC) activation and enhance  $T_h2$  cell growth and differentiation by stimulating IL-2 and IL-4 production in T-cells (11,17). Additionally, RA can inhibit B-cell apoptosis through binding the nuclear RAR receptor and subsequently influence the correct genes. RA can also directly affect DC function as it stimulates DC maturation and antigen presenting capacity through binding the RXR receptor, with the help of other cytokines (17).

The vitamin A metabolites also have part in maintaining a proper inflammatory/ anti-inflammatory balance by affecting more specific aspects of the immune response. Vitamin A metabolites promote a  $T_h2$  cell response and inhibit a  $T_h1$  cell response while maintaining a balance between these two  $T_h$  cell subsets by stimulating  $T_{reg}$  differentiation (10,11,17).

Besides the  $T_h1$  and  $T_h2$  subsets, there is another subset of  $T_h$  cells that is influenced by RA:  $T_h17$  cells.  $T_h17$  cells are CD4+ T cells that play a part in autoimmunity and allergies by maintaining inflammations. These type of  $T_h$  cells are induced by TGF- $\beta$  and IL-6 and will produce IL-17, IL-23, IL-22, IL-6, TNF- $\alpha$  and chemokines (9). Immature T cells (CD4+ cells) can be differentiated to  $T_{reg}$  or  $T_h17$  cells, dependent on the cytokine signal. CD4+ cells that are activated by TGF- $\beta$  alone will differentiate to  $T_{reg}$  cells, however as mentioned, a combination of TGF- $\beta$  and IL-6 (and possible IL-1 $\beta$  and IL-23/IL-21) will enhance a  $T_h17$  differentiation by blocking FOXP3 induction. It has now been demonstrated that RA will stimulate a TGF- $\beta$  dependent  $T_{reg}$  response while inhibiting a  $T_h17$  response (10,17). In addition, RA may enhance CCR9 expression (receptor on T- and B-cells) (11,39) and inhibit signals from APCs and memory T-cells that would normally inhibit  $T_{reg}$  induction (10). RA can also block the retinoic-acid-receptor-related-orphan-receptor- $\gamma$  (ROR): one of the necessary transcription factors needed for  $T_h17$  differentiation. Nevertheless, the concentration of RA is of great importance: research has demonstrated that low concentrations will in fact enhance  $T_h17$  differentiation (17).

RA can also bind the RXR receptor on T-cells directly and inhibit the NF- $\kappa$ B pathway (33). NF- $\kappa$ B is a complex of proteins that control the DNA transcription. This pathway is also concerned by inflammation which means that inhibition of this pathway will result in a lesser inflammation (40).

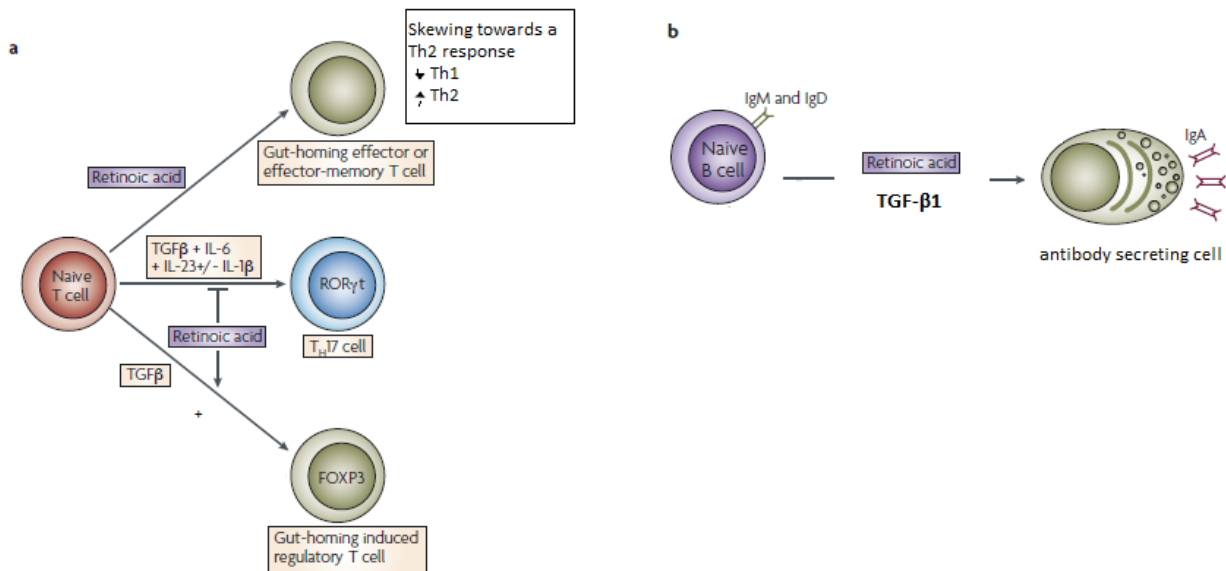


Figure 3. Schematic representation of the vitamin A effects on the immune system. Adapted from (17).

- a. A naïve T-cell will differentiate to a  $T_H2$  by IL-2, RA will stimulate this  $T_H2$  response. Furthermore will RA inhibit the cytokines that are necessary for  $T_H17$  differentiation, skewing the immune response towards a  $T_{reg}$  response.
- b. RA will, in combination with TGF-β1, stimulate a class switch from IgM/ IgD on the B-cells to IgA on the antibody secreting cell.

As mentioned, B-cell apoptosis is inhibited by RA, which is an important function as one type of B-cells is responsible for Ig production. After binding to effector antibody secreting cells, the antibodies can go through class switching dependent on the signal from the T-cell. A naïve B-cell will produce only IgM and IgD, but cytokines can enhance a class switch to IgA, IgG and IgE (41). IgA is the immunoglobulin that is important in protecting the mucosa (lungs, intestines, skin etc.) and will develop after stimulation by TGF-β1 (10,17,39). However, it is not understood how IgA is predominant in mucosal tissues because TGF-β1 also stimulates towards a IgG class switch (39). Because of these findings, it is more and more hypothesized and researched that RA has a specific action in class switch to IgA (10,17,39). Research has found that RA is certainly involved in IgA class switching and more selective than TGF-β1, which suggest that these two molecules have a cooperative relation to enhance IgA production in the mucosa (39).

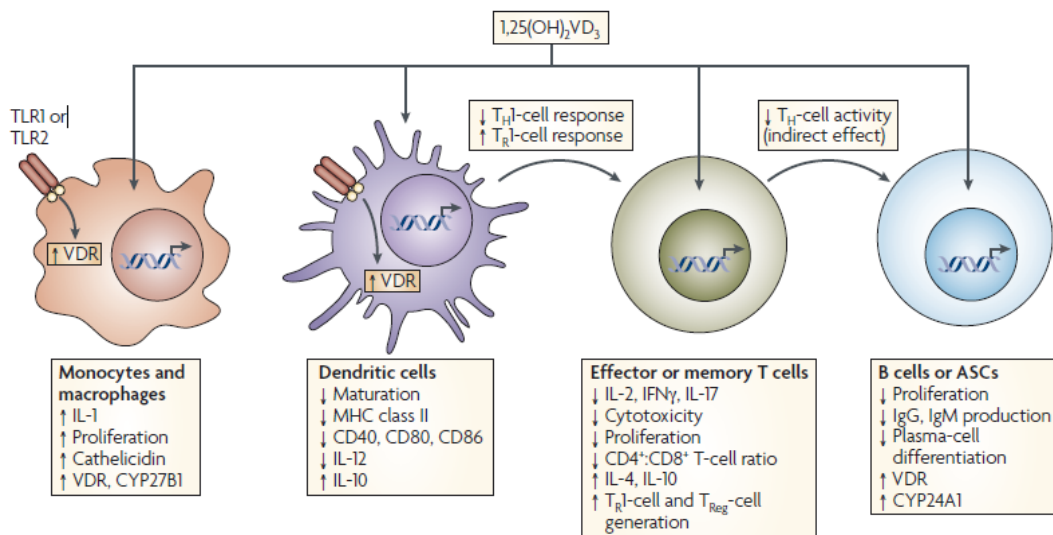
The effects of the vitamin A metabolites on the immune system are represented in Figure 3.

### Vitamin D and the immune system

The vitamin D metabolites also influence the immune system: they can inhibit T-cell proliferation, interleukin (IL)-2 and interferon (IFN)  $\gamma$  mRNA and protein expression in T-cells and CD8 T-cell mediated cytotoxicity (17). This inhibition and the vitamin D mediated up-regulation of IL-4, IL-5 and IL-10 concentrations, will create a immune response skewing towards a  $T_H2$  cell response. Besides the direct inhibition of T-cell proliferation, the metabolites also inhibit IL-12: a promoter of a  $T_H1$ -cell response. Additionally, the metabolites inhibit  $T_H17$  cell response by inhibiting the expression of IL-6 (stimulator of  $T_H17$  cells) and stimulate  $T_{reg}$  differentiation. It is also important to mention that  $T_{reg}$  cells will also secrete TGF-β themselves, which means that TGF-β also acts through a positive feedback loop (14,16,17). This positive feedback loop is present in both vitamin functions

(additionally, RA will as well act through a positive feedback loop, this will be explained later). The vitamin D metabolites can also induce the differentiation of forkhead box P3 (FOXP3)+ T<sub>reg</sub> cells (14,17). FOXP3 acts as a transcription factor and regulator in the function of T<sub>reg</sub> cells and vitamin D can stimulate these cells, acting as a tolerance inducer (17). Further, vitamin D metabolites have a direct effect on B cells and Ig production as they inhibit these processes (14,16,17). T-cells also express VDR which means they can be direct targets of vitamin D. Vitamin D can therefore down-regulate the NF-κB pathway in both DC and T-cells (42). This will have the same result as vitamin A: a reduced susceptibility of excessive inflammatory responses.

The effects of vitamin D described above are actions of the adaptive immune system, however vitamin D also exerts effects on the innate immune system. The most important task of the innate immune system is to destroy unknown organisms. These organisms are recognized by specific molecular patterns such as flagellin or single stranded RNA through specific pattern recognition receptors. Toll like receptors (TLR) are a sub-class of these specific receptors that are displayed on the cell membranes or on endosomes. When the TLR recognize foreign material, they trigger the production of antimicrobial peptides, cytokines and apoptosis of the host cell (14) and examples of antimicrobial peptides are defensin β2 (DEFB) and cathelicidin (hCAP18). In humans, hCAP18 is cleaved to 37-residue active cationic peptide (LL-37) which causes destabilization of the microbial membrane. Neutrophils, macrophages, cells of the skin, respiratory tract and GI tract produce cathelicidin as a reaction to infections. It is now suggested that the vitamin D-VDR-RXR complex up-regulates this production as the genes of cathelicidin contain a VDRE (14,16). Furthermore, it appears that vitamin D and calcitriol can induce a tolerogenic action in DC as these cells contain the CYP27B1 enzyme. Through this mechanism a locally high concentration of vitamin D metabolites, and therefore immunomodulatory effect is achieved (16). The effects of vitamin D on the innate and adaptive immune system are depicted in Figure 4.



*Figure 4. Schematic representation of vitamin D effects on the innate and adaptive immune system (17). Vitamin D will inhibit the stimulatory molecules on the DC cells causing a blockade for T-cell binding resulting in a tolerogenic DC. Furthermore will the T-cell differentiation skew towards a T<sub>H2</sub> or a T<sub>H17</sub> response by inhibiting the cytokines necessary for T<sub>H1</sub> or T<sub>H17</sub> response. B-cells will also be affected by vitamin D as IgG and IgM production and B-cell proliferation is inhibited. This will cause a better regulated tolerance system.*

## Vitamin A and D effects on dendritic cell function and the induction of regulatory T-cells

The immune system is there to protect the body against harmful invaders. DC's ingest the invader and present antigenic parts of it through the MHC class II molecules. After this presentation, a naïve T-cell that has specific recognition for this antigen will bind the MHC II molecule with its T-cell receptor (TCR) and other co-stimulatory molecules (cluster of differentiation (CD) CD28 – CD80/86; CD40-CD40L). Subsequently, this binding will cause cytokine release and dependent of the cytokines, the naïve T-cell will differentiate into a T<sub>h</sub>1 or a T<sub>h</sub>2 cell (43). However, this reaction can also occur against endogenous molecules, causing an auto-immune inflammatory reaction, and exogenous antigens, resulting in allergies. To prevent this reaction, T<sub>reg</sub> cells are stimulated, which will inhibit the T<sub>h</sub> cells and maintain peripheral tolerance (44). Even though a rapid protection against exogenous antigens is important, T<sub>h</sub>1 and T<sub>h</sub>17 cells can cause great damage to tissue (9). It is now suggested that vitamin A and D may affect the development of tolerogenic CD103+ gut DC and T<sub>reg</sub> cells.

### Vitamin A

The vitamin A metabolites can influence the differentiation of the DC's as well as its function. DC's have three isoforms of RAR (RAR $\alpha$ , RAR $\beta$ , and RAR $\gamma$ ) and are in this way capable to directly respond to RA. Though it was shown that RA and the differentiation of the DC's are related, the mechanism behind it is not completely known. It is suggested that RA affects the Notch-2-pathway, necessary for DC differentiation, however it is not demonstrated through which mechanism (13). Furthermore, the function of DC's is shaped in a way that its migratory function is enhanced as RA induces the expression of matrix metalloproteinases (MMP) MMP-9 and MMP-14 (13,45). Additionally, RA also inhibits their inhibitors: tissue inhibitor of matrix metalloproteinase (TIMP). Even though it is hypothesized that the expression of MMP-9 is most likely enhanced through RA binding the RAR $\alpha$ , this mechanism needs to be further researched (13). RA can also influence the DC pro-inflammatory function by inhibiting or stimulating MHC class II and co-stimulatory molecules, dependent on the cytokines present (13,45). Moreover, RA may induce the DC secretion of IL-6 and TGF- $\beta$ , which contribute to gut-homing receptor expression enhancement and IgA responses(13,46).

The capacity of RA to induce its own metabolism in DC through a positive feedback loop, may be its most important function. RA does not have a large impact on other DC characteristics, however since DC secrete RA while instructing T-cells it has a major effect on the T-cells. Through this positive feedback loop the function of T-cells can be affected better. There are two main types of DC: gut related DC and peripheral DC. However, mainly gut related DC contain RALDH1 and RALDH2 and it has been shown that RA primarily induces RALDH2 expression (10,13,46). By up-regulating RALDH2 expression, there will be a higher expression of RA in DC. The gene coding for RALDH2 is *aldh1a2* which contains RARE, RA can stimulate RARE and through this mechanism it can enhance RALDH2 expression (7). Besides the positive feedback of RA on the RA metabolism in DC, other molecules can up-regulate RALDH2 expression and thereby increasing RA levels: GM-CSF, IL-4, IL-13, MAPK, p38a and toll like receptor (TLR) activation. Furthermore has it been shown that specific activation of the TLR2 receptor induces the strongest increase of RA metabolism (7,10,13). Now that RA levels are increased it can stimulate naïve T-cells to differentiate into T<sub>reg</sub> (with the help of TGF- $\beta$ ) and inhibit T<sub>h</sub>17 cells. Furthermore, T<sub>reg</sub> differentiation can be blocked by a population of CD4-CD44<sup>high</sup> memory T-cells which secrete IL-4, IL-21 and IFN- $\gamma$ . When RA is secreted by the induced DC, it can inhibit this specific CD population and in this way promote a T<sub>reg</sub> differentiation (13).

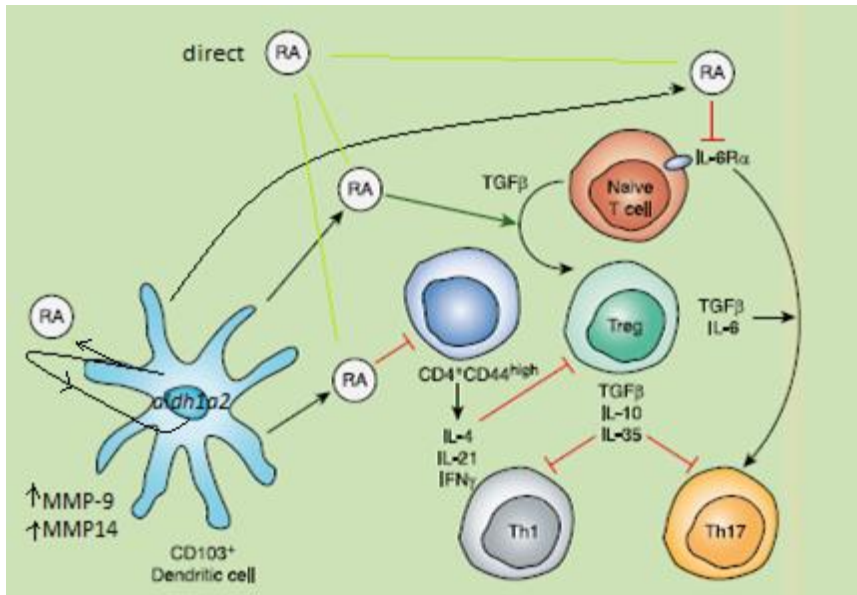


Figure 6. Schematic representation of retinoic acid's function on DC and  $T_{reg}$  cells, adjusted from (13). RA can be metabolized in tolerogenic DC or directly from the liver and blood influence the T-cells. RA will stimulate its own metabolism in the DC and will stimulate migrational factors on the DC. Furthermore can it stimulate naïve T-cells to differentiate into  $T_{reg}$  (with help from TGF- $\beta$ ) and it can inhibit a specific population of  $CD4^+CD44^{high}$  cells. Additionally, RA can inhibit  $T_{h17}$  differentiation.

Besides that DC's are stimulated by RA and will provide for increased RA levels, RA can also directly affect T-cells. As mentioned before, dependent on the cytokine signals a  $T_{h17}$  or a  $T_{reg}$  cell will be differentiated. The most important regulators of the  $T_{h17}$  cells are the ROR $\gamma$ t and the ROR $\alpha$  receptor and it has been demonstrated that FOXP3 can directly bind these regulators and in this way inhibit transcriptional activity. This means that an increase in  $T_{reg}$  cells will cumulatively reduce the expression of  $T_{h17}$  cell through FOXP3 in addition to the direct inhibitory effect of RA on ROR $\gamma$  (9,13). Furthermore, RA suppresses  $T_{h17}$  development through mediating an increase in TGF- $\beta$  signaling and a reduction of the IL-6 receptor expression (9). Besides enhancing the  $T_{reg}$  cells, the vitamin A metabolites will induce its function by increasing gut-homing receptors  $\alpha 4\beta 7$  and CCR9 (13,47). Though the mechanism by which RA will induces  $T_{reg}$  cells and their regulatory function is unclear, RA appears to be the determinant in  $T_{reg}$  differentiation. Since both  $T_{h17}$  and  $T_{reg}$  cells require TGF- $\beta$  and RA also inhibits IL-6, this concept seems logical (13). Figure 6 is a schematic representation of the RA effect on DC and  $T_{reg}$  cells.

### Vitamin D

Vitamin D metabolites will affect the DC's through binding the VDR and, as mentioned in the introduction, subsequently construct a heterodimer with RXR (42,45,48,49). This complex will enroll different co-repressor and co-activator proteins which subsequently stimulate chromatin remodeling. They achieve this by modifying intrinsic histone actions and recruiting transcription initiation components (48). Hence, the complex will act as a transcription factor that can bind vitamin D responsive DNA sequences and consequently influence the RNA polymerase II transcription. In this way, vitamin D can affect gene expression of different cytokines and binding factors important for immunologic cells (42,48,49).

DC are important targets of vitamin D metabolites as they express the VDR. There are several types of DC, however immunogenic and tolerogenic DC are the most important in this report. The immunogenic DC will stimulate the effector  $T_h$  cells while tolerogenic DC will stimulate  $T_{reg}$  cells (49,50). The difference between an immunogenic DC and a tolerogenic DC is the expression of co-stimulatory molecules, the expression of inhibitory molecules and the expression of cytokines. It has been researched that tolerogenic DC express low surface major histocompatibility complex (MHC) class II molecules, low CD40, CD80 and CD86, and have high expression of immunoglobulin like transcript (ILT) 3. Furthermore is there a decreased production of IL-12, an increased production of programmed cell death 1 ligand (PDL-1) and an increased production of CCL22 and IL-10 (45,49). CD40, CD80 and CD86 molecules that are present on the DC and will bind to naïve T-cells in addition to the TCR-MHCII complex resulting in a co stimulatory signal that will subsequently result in differentiation of the naïve T-cell into a  $T_h1$  or  $T_h2$  cell (51). Furthermore, ILT molecules are inhibitory receptors that mediate the inhibition of cell activation through recruiting tyrosine phosphatase SHP-1 and PDL-1 is a molecule that will suppress T-cell activation and thus the immune system (52). The cytokine IL-12 will stimulate a  $T_h1$  differentiation, IL-10 will inhibit  $T_h1$  differentiation and CCL22 is a chemokine that will attract CCR4+  $T_{reg}$  and  $T_h2$  cells (43). Vitamin D metabolites will provide these effects and induce tolerogenic DC (45).

Another important molecule may be indoleamine 2,3-dioxygenase (IDO), a tryptophan degrading enzyme. Metabolites of tryptophan can inhibit T-cell response and thereby stimulating immunosuppression and tolerance induction. Immunogenic DC's are the main cells that express IDO, making them an important part in tolerance induction. An in vivo study in rats concluded that vitamin D will induce IDO+ DC's resulting in an enhancement of  $T_{reg}$  differentiation. Furthermore is it suggested that the absence of tryptophan will stimulate direct toxicity and auto-reactive T-cell apoptosis (53), making IDO+ DC's an important target of vitamin D.

In vivo research has demonstrated that vitamin D metabolites will adjust DC phenotype and function through the VDR, skewing towards a tolerogenic DC (42,45,48–50). Furthermore, DC themselves are capable of synthesizing the active  $1,25-(OH)_2-D_3$  metabolite from its precursor 25-OH- $D_3$ , as a result of increased  $1\alpha$ -hydroxylase expression. This could also be a possible mechanism to stimulate the  $T_{reg}$  cells (42,48,49). Nevertheless, T-cells can also be directly affected by vitamin D metabolites, without the interference of DC. Vitamin D metabolites suppress TCR mediated T-cell proliferation and adjust the cytokine expression. IFN- $\gamma$  and IL-2 production will be inhibited while IL-5 and IL-10 will be stimulated. This will result in skewing towards a more tolerogenic  $T_h2$  type reaction. Furthermore, the metabolites will induce FOXP3+  $T_{reg}$  cells by stimulating the genes and inhibiting IL-6 and IL-17 ( $T_h17$  stimulating cytokines). This results in a skewing towards a  $T_{reg}$  instead of a  $T_h17$  reaction (14). The skew towards a more  $T_h2$  response could be harmful for allergic diseases as they arise from  $T_h2$  responses. Nevertheless,  $T_{reg}$  cells are also stimulated, maintaining the  $T_h1/ T_h2$  balance. The process of vitamin D metabolites on DC and  $T_{reg}$  cells is demonstrated in figure 5.

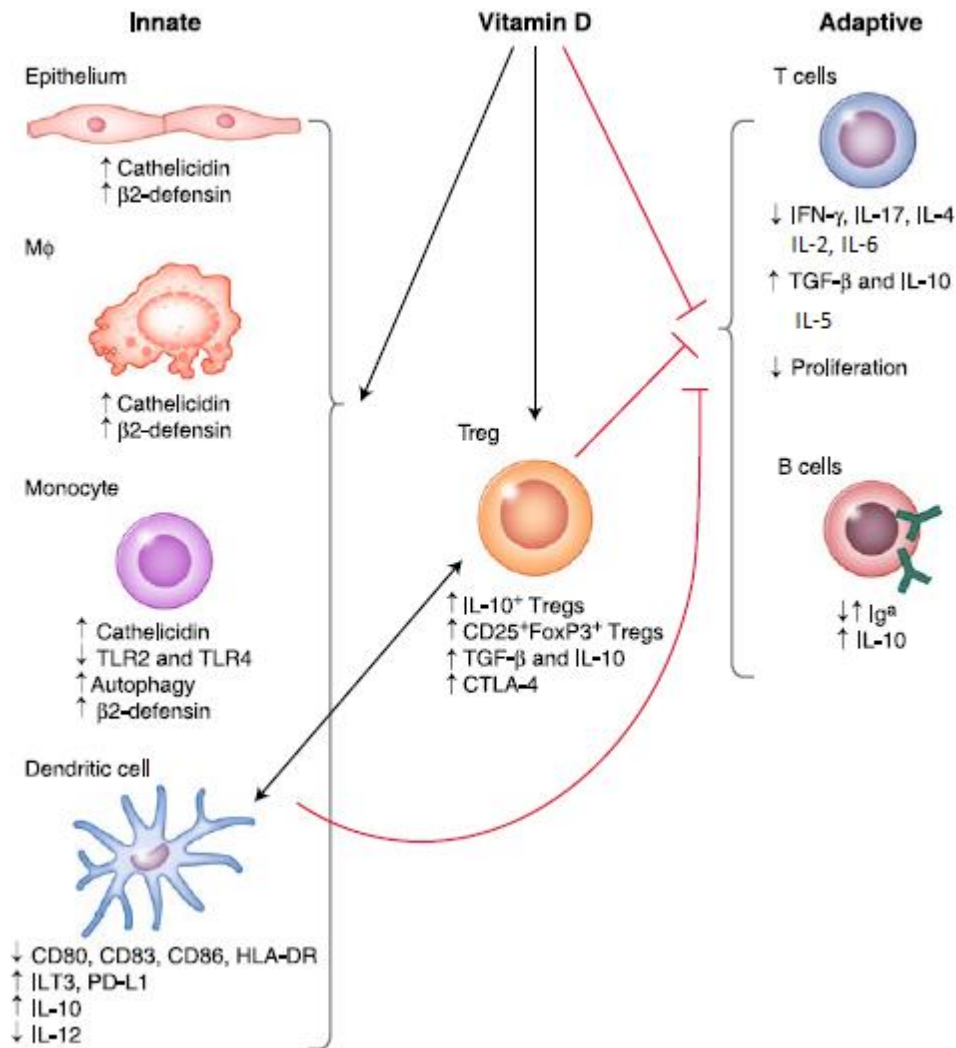


Figure 5. Schematic representation of the effects of vitamin D on DC's and  $T_{reg}$ 's (44). This picture makes it clear that there is a interaction between DC's and  $T_{reg}$ 's, and that they are both stimulated by vitamin D metabolites. IL-10 and IL-12 will be up-regulated under the influence of vitamin D while IL-17 and IL-6 will be inhibited.

### Vitamin A and D in (oral) tolerance induction in the gut and lung

The previous chapters described what specific functions vitamin A and D have in the immune system and what place they have in the Western world. Vitamin A is a pivotal vitamin in immune regulation because of its co-stimulatory capacities on tolerogenic  $T_{reg}$  differentiation, in combination with TGF-β. Without RA, a  $T_{reg}$  will most likely not differentiate in a tolerogenic  $T_{reg}$  resulting in an inflammation. Furthermore, it will inhibit pro-inflammatory  $T_{h17}$  and  $T_{h1}$  cell differentiation. Vitamin D also has an important role in immune system functioning as its metabolites will stimulate tolerogenic DC's through different stimulations and inhibitions (for example inhibiting CD40, CD80 and CD82 molecules, increased production of CCL22 and IL-10). Subsequently, these up-regulated tolerogenic DC's will stimulate  $T_{reg}$  cells through increasing IL-10, TGF-β and FOXP3 for example. Furthermore, vitamin D metabolites can directly stimulate  $T_{reg}$  differentiation. These effects of both vitamins indicate a pivotal function in tolerance induction.

## *Tolerance*

Tolerance is a process in the mucosa of the intestine, the gut associated lymphoid tissue (GALT), and possibly also the lungs, the bronchus associated lymphoid tissue (BALT). The mucosal antigens in GALT are food antigens while the mucosal antigens in BALT are airborne. The process of tolerance induction is defined as the suppression of an immune response to an harmless antigen as well as the non-responsiveness of the immune system (54–56). It was thought that mechanisms behind tolerance were either anergy (an immune cell does not have enough signals to induce an immune response) or apoptosis/ deletion. However, the function of antigen specific T<sub>reg</sub> cells in tolerance induction is now well accepted (47,54,55). Furthermore it is suggested that low dose presentation of the antigen will induce a T<sub>reg</sub> reaction while when the presentation of the antigen is high in dose, the tolerance reaction will be more of anergy/ deletion type (but these mechanisms are not exclusive) (47,56,57).

The mechanism of tolerance is applicable for both oral antigens and inhaled antigens (26). An antigen will cross the GALT or MALT and is taken up by DC or macrophages. When this antigen is harmful an effector T-cell response will be initiated. However, when the antigen is not harmful, tolerance is induced via one of the mechanisms described above (43). As described, the T<sub>reg</sub> will inhibit an immune reaction and keep a good homeostasis by secreting down-regulatory cytokines TGF- $\beta$  and IL-10 (and also suppressing by-stander cells) (22,57). However, when the mechanism of anergy/ deletion is started, the complete T-cell will be destroyed. Though the DC and the naïve T-cell will bind, there are signals missing, causing the T-cell to become anergic (or directly deleted). The TCR will now be blocked by molecules of the ubiquitin pathway and subsequently the T-cell will undergo apoptosis (43). Additionally, DC also play an important role in the induction of tolerance as they will produce regulatory cytokines (e.g. TGF- $\beta$ ) and stimulate antigen specific T<sub>reg</sub> cells (55). Another important molecule that stimulates DC to mature into mucosal DC is a cytokine secreted from the intestinal epithelial cells: thymic stromal lymphopoietin (TSLP). This cytokine will bind its receptor (TSLPR) and IL-7R $\alpha$ . Subsequently, STAT-5 and other unidentified molecules will be activated causing the development of T- and B-cells and the activation of for example DC. In other words, TSLP will help skewing towards a more regulatory or T<sub>H</sub>2 type reaction. Nevertheless, TSLP is also linked to allergy development which suggests that low TSLP levels result in tolerance while high levels cause allergy (58). The tolerance process is portrayed in Figure 7.

## *Tolerance induction by vitamin A and D*

The mechanism of tolerance induction and the supplementation of vitamin A and D are most likely connected. As described in the previous chapters, both vitamin A and D induce tolerogenic DC and T<sub>reg</sub>, which suggests that both vitamins are only helpful with low dose antigens (as high dose initiates anergy or deletion). Furthermore, they will both inhibit a T<sub>H</sub>1 or a T<sub>H</sub>17 reaction, prohibiting an inflammatory reaction in mucosal tissues. The function of RA in the immune system is better understood than the vitamin D function. For example it is known that RA is necessary for T<sub>reg</sub> cells to differentiate (in combination with TGF- $\beta$ ), however the vitamin D metabolites have a more general function as they induce different molecules in different immune cells (e.g. IL-10 up-regulation, FOXP3 induction). It is suggested that to create a tolerogenic environment, factors as IL-10, RA and TGF- $\beta$  need to be present. Furthermore it has been known that the induction of CD103+ DC is important in the enhancement of oral tolerance (47).

The role of vitamin A metabolites in oral tolerance is better understood than the role of vitamin D in this process. Vitamin A metabolites are abundantly present in the gut environment. In a normal situation they will stimulate a T<sub>reg</sub> response (as mentioned in the previous chapter) however instead of a Treg response they CD103+ DC can also stimulate a T<sub>H</sub>17 response under inflammatory circumstances. During inflammation RA will still stimulate the DC, however it is now accompanied with TLR-ligands and the cytokine GM-CSF. This results in an up-regulation of RA metabolism in the



DC, causing a high concentration of RA and the addition of other cytokines (such as IL-6, IL-12, IL-23 and IL-27). The mixture of these cytokines and RA will stimulate a  $T_H17$  response causing inflammation and tissue damage (59). This suggests that RA is present in the induction of tolerance as well as inflammation, however its function is dependent of its concentration and the cytokine environment.

Vitamin D however is more unknown. It is mentioned before that the T-cells and DC express the VDR, making it an important target for vitamin D. Nevertheless, the specific function of the vitamin is not known. Furthermore, as mentioned before, it has been proven that both deficiency as well as excess of vitamin D will cause a break in tolerance (24).

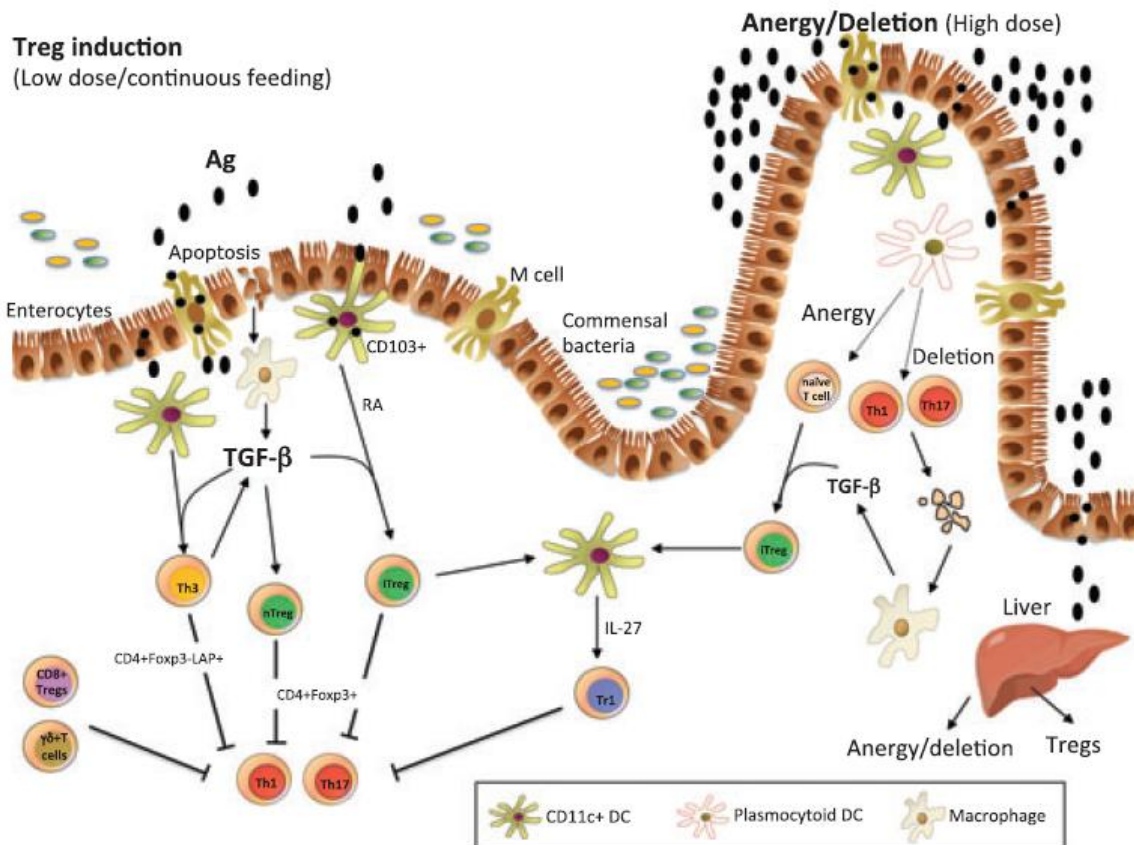


Figure 7. Schematic representation of the tolerance process (47). A low exposure to the antigen will cause a  $T_{reg}$  response while a high dose exposure will cause anergy or deletion.

### Relevance for inflammatory bowel disease, food allergy and asthma

The function of vitamin A and D in the immune system is clear but it is important to clarify their relevance for the diseases of interest. This chapter will cover known defects in tolerance induction and  $T_{reg}$  function in the diseases of interest. Furthermore results from animal and human studies using vitamin A and D in these type diseases will be discussed.

### *Tolerance defects in relation to IBD, food allergy and asthma*

As mentioned in the introduction, IBD manifests itself by inflammations throughout the GI tract. It is claimed that intestinal homeostasis is maintained through the interaction between epithelial cells and DC and like the previous chapter mentioned, TSLP is an important stimulator of tolerogenic DC. A study with human derived DC and TSLP concluded that TSLP is a mediator in the maintenance of this homeostasis. They also suggest that in Crohn's disease, TSLP is undetectable as 70% of their subjects did not have detectable TSLP, resulting in a Th1 response (note that healthy subjects were not mentioned) (60). Additionally, another cause for IBD could be a defect in TGF- $\beta$  secretion/ expression as this cytokine plays a pivotal role in T<sub>reg</sub> differentiation (which inhibits T<sub>h</sub>1/T<sub>h</sub>17 differentiation) (57). Moreover, IBD patients have an increased expression of TLR2, TLR4 and CD40 when compared with healthy subjects. This suggests that IBD patients may have an increased recognition of bacterial products and therefore an increased reaction leading to inflammation (61). Both vitamin A and D will stimulate T<sub>reg</sub> differentiation, causing an increased secretion of TGF- $\beta$ . Furthermore will they stimulate tolerogenic DC where for example CD40 expression is decreased and moreover, it is demonstrated in the lungs that vitamin D will induce TSLP (62). These findings might be applicable for IBD as well. Though these are three examples of what might be defect in the tolerance induction in patients with IBD, the exact defects and pathology are still unclear which makes it difficult to rationalize the supplementation of vitamin A and D .

Patients with food allergy develop an immune response to food antigens through which food specific IgE molecules from B-cells are produced via an initial T<sub>h</sub>2 response that stimulates IgE class-switch (23). It suggested that oral tolerance may be broken by an adjuvant. This was researched with cholera toxin, serving as a surrogate adjuvant known to break tolerance, that was supplemented with an antigen and exposure to this combination results in an increase in antigen specific IgE molecules (63). Other studies suggest that an modification in the physiologic mucosal barrier (for example change in pH, enzymes and bile salts) can lead to an enhanced IgE production in both children and adults (64). These processes suggest that the susceptibility to develop food allergy is dependent on the environment and physical-chemical properties of the antigen when coming in contact with the GALT. Another example of this is the fact that peanut allergy occurs in higher rates in westernized countries, where the consumed peanuts are roasted while in China (where peanut allergy prevalence rates are lower) the peanuts are boiled or fried (64). This suggest that roasted peanuts have another adjuvant capacities than the boiled/ fried peanuts and this peanut more easily causes a escape from tolerance. As the case in IBD, TSLP seems to have a pivotal role in the development food allergy only now its abundance can directly instruct CD4+ to intensify the Th2 response in the intestines, causing an allergy (63). In food allergy is it important that the IgE molecules are inhibited and especially vitamin D will perform this action as it inhibits B-cells. In the case of TSLP and vitamin D would vitamin D actually worsen the allergy as it induces TSLP (this was in the lungs, however it could also be applied for GALT).

Asthma is comparable with food allergy as this disease displays an abundant T<sub>h</sub>2 response. Furthermore, the function of T<sub>reg</sub> is also of importance as these cells will inhibit a T<sub>h</sub>2 mediated inflammation and moreover, the commensal bacteria may have a part as they enhance IL-10 production (26). It is suggested that asthma is a consequence of dysregulation of T<sub>reg</sub> development, loss of tolerance due to environmental allergens or the overdevelopment of T<sub>h</sub>2 cells. This could be a result from corticosteroid supplementation and the non-responding action of the mucosa to the commensal bacteria (65). Furthermore is it demonstrated that in asthma TSLP expression is increased which leads to attracting Th2 cytokines and an enhancement in disease severity (58). Although it is clear that there is a defect in T<sub>reg</sub> cells and the communication between the mucosal bacteria and the immune cells in asthma pathogenesis, the exact defects are not known. Nevertheless, to stimulate the T<sub>reg</sub> cell differentiation both vitamin A and D could be administered. On the other hand it is shown in lungs that vitamin D will increase TSLP which will result in an increase of the severity of

asthma (62). Further animal and human studies should be performed to determine if vitamin A and D could be administered in asthma treatment.

TSLP is a recurring cytokine in diseases of the immune system. However, in IBD it is beneficial that its expression is increased while in both allergic diseases it would be favorable if its expression is decreased. These results suggest that there is an optimal TSLP concentration and that a low TSLP will break tolerance while a high TSLP will cause inflammation.

### *Animal studies with vitamin A and D*

There have been different animal studies performed to examine the relation of both vitamin A and D and the diseases of interest. Nevertheless, not all studies can be included in this review, only three or four per vitamin, per disease are enclosed. This means that in the research databases there greatly more investigations. Additionally, there seem to be no animal studies towards the relation between food allergy and vitamin D and therefore only research in animals and the relation between food allergy and vitamin A are included.

### *IBD*

The pathogenesis of IBD is not completely understood, however treatments for IBD have been widely researched, especially vitamin D and IBD. Nevertheless, Reiven *et al.* (2002) performed an in vivo study with Crohn's disease induced rats administered these rats with different quantities of vitamin A (non, 1200 µg/kg diet or 1200 µg/kg diet + 300 µg retinyl palmitate in 0.25 mL of 1% glycerol) for seven weeks. The study concluded that a deficiency will cause inflammation, Crohn's disease is degenerates through this deficiency and vitamin A supplementation improved the disease symptoms (2). Bai *et al.* (2009) researched the effect of vitamin A on colitis induced mice and supplemented them with different diets: medium, RA (20 µg, 100 µg, or 300 µg), or 100 µg LE135 (antagonist RARα). The experiment with the animals continued for 7 days. The conclusion of this experiment was that RA down-regulated the production of TNF-α (a pro-inflammatory cytokines that stimulates the progression of IBD) while LE135 stimulated this cytokine (66). While these findings suggest that RA will inhibit an inflammatory response and improve IBD, other studies found contradicting results. Oehlers *et al.* (2012) examined enterocolitis (one form of ulcerative colitis) induced zebrafish, administered vitamin A in different concentrations (not specified). The researchers concluded that supplemented RA disrupted the mucus causing an increased mortality and inflammation mediated by neutrofiles and furthermore, they also suggest that RA could trigger ulcerative colitis (in combination genetic and environmental factors) (67). Nevertheless, this research was performed in zebrafish and it is questionable if these findings can be extrapolated to human. Another animal study with mice was performed to explore in what way the gut induces T<sub>reg</sub> and how this was relevant for IBD. In this study the researchers also researched the function of RA in the induction of T<sub>reg</sub> cells and they concluded that T<sub>reg</sub> differentiation is not dependent of RA (68). The supplementation of vitamin A in IBD demonstrates some contradictions, which means that further research to this relation is necessary.

Vitamin D is also related to IBD treatment. It has been researched if vitamin D will affect a T<sub>h</sub>17 response in mice affected with colitis. This colitis was induced by a specific strand of bacteria: *Citrobacter Rodentium* which causes colitis and strong damage through a T<sub>h</sub>1/T<sub>h</sub>17 response. The mice were treated with buffer (control) or 10 ng 1,25-(OH)<sub>2</sub>-D<sub>3</sub> up to 11 days. The researchers concluded that the mice treated with vitamin D were protected against chemically induced colitis, selectively inhibited the T<sub>h</sub>17 response in the gut and induced host susceptibility for a *Citrobacter Rodentium* (69). These findings suggest that vitamin D will improve tolerance as it protects the body against a reaction to commensal bacteria. Another study with IBD affected mice administered vitamin D sufficient mice with 5 µg cholecalciferol/day, 0,005 µg/ day 1,25-(OH)<sub>2</sub>-D<sub>3</sub> to 'normal' IBD mice or held the mice vitamin D deficient (no supplementation). They concluded that the mice

supplemented with 1,25-(OH)<sub>2</sub>-D<sub>3</sub> were protected to and demonstrated improved symptoms of IBD (70). A third study with colitis impaired mice compared the effectiveness of a vitamin D analogue and 1,25-(OH)<sub>2</sub>-D<sub>3</sub>. The researchers administered 0.1–2.0 µg/kg body weight of the vitamin D analogue and 0.2 µg/kg body weight 1,25-(OH)<sub>2</sub>-D<sub>3</sub> for 5 days. It was concluded that both forms of vitamin D reduced the symptoms of the ulcerative colitis, however neither of the forms was more potent (71). Furthermore, a fourth study with acute induced colitis mice supplemented the mice with 0.2 µg/day for 14 days. This study also concluded with the statement that vitamin D has a protective role in IBD (72). The role of vitamin D in IBD is more conclusive as vitamin A (in animal studies) as all the studies concluded that vitamin D has a protective role in IBD.

### Food allergy

Rühl *et al.* (2007) examined the role of vitamin A in the sensitization of allergies while the animals were lactating. The researchers used first generation mice that were sensitized for food allergy and subsequently administered them with either no diet (control), with basal quantities of retinol equivalents (4,5 mg), with a retinol elimination diet (no retinol equivalents, vitamin A free vitamin mix) or a retinol supplementation diet (216 mg retinyl palmitaat (one form of vitamin A)). The diets were supplemented for 21 days. The conclusion of this experiment was that the second generation mice who were injected once with an allergen during the postnatal period, induced an allergic sensitization which was enhanced after vitamin A supplementation while the basal and elimination diet did not. This means that vitamin A supplementation, according to this experiment, is not desirable in the postnatal period (73). Worm *et al.* (2001) investigated whether vitamin A was capable of neutralizing IgE molecules. OVA sensitized mice were divided in three groups. One group served as a control, one group was supplemented with RA (20 mg/kg) and the third group was supplemented with CD336 (a high selective retinoid for RAR $\alpha$ , better IgE production inhibiting capacities). This study demonstrated that retinoids (both studied forms) were not capable of neutralizing IgE, which means that in allergies (food allergy as well as asthma) vitamin A cannot inhibit IgE (74). Both experiments are somewhat negative as vitamin A seems not to work in food allergy. However, much more experiments have to be performed before vitamin A supplementation in food allergy can be excluded.

### Asthma

A mouse model was used to research the relation between asthma and the supplementation of vitamin A. The mice were sensitized with OVA to mimic asthma and subsequently administered with 400 µg RA or corn oil (control) on different days. This study showed that the T<sub>h</sub>2 and T<sub>h</sub>17 cytokines (that cause the symptoms in asthma) were significantly decreased which suggests that vitamin A supplementation will decrease asthma symptoms. The researchers concluded with the remark that vitamin A may be a treatment option for asthma (75). A second study also used sensitized mice and researched the relation between MMP-7 and RA and the function of RA in asthma. The researchers supplemented the mice with RA (quantity not defined) or with nothing (control). The conclusion of this study was that MMP-7 is activated in asthma and that this protein will down-regulate RALDH (76). Furthermore, a third study sensitized vitamin A deficient rats and subsequently administered them with RA 25 µg on different days. There were also rats who were not administered with RA. The conclusion of this study was that RA increased the airway elastic fibers which results in a decrease in airway hyper responsiveness (77). These study demonstrate that there is a function for vitamin A in asthma treatment, however more research has to be performed. Chen *et al.* (2014) researched whether vitamin A deficiency in early life was related with airway hyper responsiveness later on in life. The researchers administered the mice with a standard diet of 15 IU vitamin A/g, subsequently the mice were randomly assigned to a vitamin A supplementation diet (not defined) or a vitamin A deficient diet (<0.2 IU of vitamin A/g of diet). This study demonstrated that it is possible that a period of fetal vitamin A deficiency can lead to chronic airway hyper responsiveness (78).

To investigate the relation between vitamin D and asthma, asthma sensitized mice were fed with 1,574 g vitamin D (the mice were not vitamin D deficient) for 11 days. The conclusion of this study is somewhat contradictory as the researchers state vitamin D should be considered as a supplement to the diet of infants because it decreases airway eosinophilia development, however they also state that it enhances IgE production (79). Another study with vitamin D and asthma administered sensitized mice with special vitamin D-deficient or vitamin D-sufficient (2000 IU/kg) or vitamin D-supplemented (10 000 IU/kg) diet for 13 weeks. The conclusion of this research was that vitamin D deficiency causes an impairment of asthma, however supplementation of vitamin D could be beneficial (80). A third study also sensitized mice and supplemented with 100 ng of 1,25-(OH)<sub>2</sub>-D<sub>3</sub> or with nothing (control) for nine weeks. This research demonstrated that that vitamin D could reduce alterations in the airways which suggests that it could be used a treatment for chronic asthma (81). Another study with mice and vitamin D, sensitized the mice and then supplemented the mice with 2000 IU/kg or no supplementation for 13 weeks. When the mice are vitamin D deficient, VDR and prohibitin (a vitamin D target gene) expression are decreased and TNF- $\alpha$  expression is increased. These findings suggest that supplementation of vitamin D will reverse these actions and reduce asthma symptoms (82). As the relation between vitamin D and IBD, the relation between vitamin D and asthma is rather conclusive. The different studies conclude that vitamin D supplementation could reduce asthma symptoms.

### *Human studies with vitamin A and D*

As with the animal studies, not all researches are enclosed. There are three or four studies per vitamin, per disease included but at the search databases there are much more studies. Studies to investigate the relation between food allergy and vitamin A in humans seems not to be performed, or by all means not findable. Therefore, only studies in humans towards the relation between vitamin D and food allergy are included.

### **IBD**

To research the relation between IBD and vitamin A in humans, one study collected DNA of patients with IBD (Crohn's disease or ulcerative colitis) and researched the sequence coding for RALDH. They concluded that there is most likely a polymorphism in this gene and that the resulting different enzyme could be an increased risk for IBD. Nevertheless, the researchers did not find significant difference for this polymorphism between IBD patients and controls. Even though, it is not known if this polymorphism has a function in IBD, this data suggests that it does (83). Bai *et al.* (2009) researched the effect of vitamin A in 10 ulcerative colitis patients. Biopsies were collected and prepared with different types of antibiotics. Subsequently, the biopsies were divided in three groups: a control group, a group cultured with RA (100 nM) and a group cultured with LE135 (RAR $\alpha$  antagonist, 1 $\mu$ M). It was demonstrated that RA enhanced the expression of FOXP3 and inhibited IL-17 in the biopsies, compared with the controls, while LE135 achieved the opposite of RA. The conclusion of this research was that RA inhibits colon inflammatory responses in IBD patients (in vitro) which makes vitamin A a potential therapeutic for IBD patients (66). Bernardo *et al.* (2013) collected monocyte derived DC through blood isolation of healthy volunteers (no auto-inflammatory or allergy reactions). The researchers isolated Peripheral blood mononuclear cells (PBMC) from the donated blood and then monocytes were obtained. Monocytes were cultured to monocyte derived DC by different incubation steps (described in the article) and subsequently these were incubated with different quantities of RA (10<sup>-6</sup> M, 10<sup>-7</sup> M, 10<sup>-8</sup> M). The obtained monocyte derived DC were also exposed to inflamed tissue of ulcerative colitis patients. The conclusion of this study was that even though ulcerative colitis patients are not able to induce the regulatory properties of monocyte derived DC, the supplementation of RA to these specific DC could induce and maintain these properties. This suggest that RA supplementation could be a potential therapy for ulcerative colitis patients (84)

Jørgensen *et al.* (2010) researched the supplementation of vitamin D in Crohn's disease patients. 46 patients received 1200 IU vitamin D while 48 other patients received a placebo once per day for 12 months. The conclusion of this study was that the serum vitamin D concentrations were significantly increased with respect to the controls. Furthermore was it demonstrated that the relapses in the vitamin D group were decreased more than in the control group, however this was not significantly (85). Pappa *et al.* (2012) compared three different procedures of vitamin D in IBD patients. The patients were divided in three groups: group one received 2000 IU vitamin D<sub>2</sub> per day (control), group two received 2000 IU vitamin D<sub>3</sub> per day and group three received 50000 IU vitamin D<sub>2</sub> per week, for six weeks. Furthermore received all participants calcium supplementation, dependent of their age. The researchers found that group two and three were both superior to group one, however they do not recommend supplementation of vitamin D in vitamin D deficient IBD patients but advise further research. Fu *et al.* (2012) researched whether there is a difference in serum vitamin D concentrations and different ethnicities. The researchers included 60 ulcerative colitis and 40 Crohn's disease patients, of these groups 65 % were Caucasians and 29 % were South Asians. Vitamin D serum concentrations were measured at random time points. The conclusion of this study was that there was no difference between the ethnicities and that vitamin D supplementation can be available for all IBD patients (86).

### Food allergy

Zakariaeeabkoo *et al.* (2014) researched what the vitamin D levels in infants were who suffered from an allergy to peanut, egg, sesame, cow's milk or shrimp. The conclusion of this study was that children who endured from one of these allergies did have a vitamin D deficiency (24). Allen *et al.* (2013) studied the effect of vitamin D on food allergy in children. The children underwent skin prick testing for different food allergies and vitamin D levels were measured and subsequently it was concluded that vitamin D sufficiency could have a protective function in food allergy (in the first life year of the infants) (87). Weisse *et al.* (2013) investigated if the vitamin D concentration in maternal and cord blood are related to atopic diseases later on in the infants. After the blood was assessed for the vitamin D levels, the conclusion of this study was that high vitamin D levels in pregnancy was related to an increased risk for food allergy (88). Furthermore, Norizoe *et al.* (2014) researched if vitamin D supplementation while mother were lactating could improve eczema. The mother received either vitamin D supplementation (800 IU/day) or a placebo for six weeks. They concluded that the vitamin D did not decrease the eczema and moreover, the risk of a food allergy development was increased (89). The supplementation of vitamin D in food allergy is somewhat conflicted and further research is necessary.

### Asthma

For asthma, a study with vitamin A was performed in children. These children suffered from respiratory infections by viruses which caused episodes of asthma. The researchers assessed the lungs and found that vitamin A deficiency is related with airway function protecting during the recovery of an infection (3). This study was not specifically focused on asthmatic patients, however the focus was on inflammations in the airways and that makes these results applicable for vitamin A supplementation in asthma. These results suggest that vitamin A supplementation could stimulate airway recovery after an asthma attack. Luo *et al.* (2010) researched the relation between vitamin A deficiency and wheezing (a symptom of asthma). The conclusion of this study was that vitamin A deficiency occurs significantly ( $P < 0,01$ ) in patients with wheezing, nevertheless the degree of wheezing is related to the severity of the deficiency (4). Checkley *et al.* (2011) researched what effect supplementation of vitamin A in children would have on the risk of asthma throughout life. A double-blind, placebo-controlled, cluster randomised trial supplemented children with vitamin A, doses dependent on their age. Children aged older than 12 months were administered with either a placebo or 200000 IU of vitamin A (60000 mg retinol equivalents (RE)), children aged between 1-11 months received either a placebo or 30000 mg RE and newborns (aged less than a month) were

administered with a placebo or 15000 mg RE. The conclusion of this study was that vitamin A supplementation in infants was not related to a decreased prevalence of asthma (90).

Baris *et al.* (2013) combined vitamin D, as an adjunct, with allergen specific immunotherapy to study which effect it would have on the immune system. They administered this combination in asthmatic children sensitized to house dust mite. The children were divided in three groups: subcutaneous immunotherapy (SCIT) along with vitamin D supplementation(650 U/day), SCIT alone, and pharmacotherapy alone. They demonstrated that there were small positive outcomes, however it was not significant and the researchers concluded that studies with higher dosages needed to be performed. Furthermore did the researchers demonstrate that a significant amount of children suffering from asthma showed low levels of vitamin D which could be negatively correlated to asthma attacks (91). Wu *et al.* (2012) researched what the vitamin D levels were of patients who inhaled corticosteroids and what the effects of this levels were on for example bronchodilator response. The levels of children with continual asthma were measured and based on these levels the children were placed in one of three groups: vitamin D sufficiency (>30 ng/ml), insufficiency (20–30 ng/ml) and deficiency (<20 ng/ml) groups. The conclusion of this study was that children who were treated with inhaled corticosteroids and vitamin D deficient had poorer lung function than the children who were vitamin D insufficient of sufficient (92). Sutherland *et al.* (2010) also researched vitamin D levels and the relationship with the lung function, only now in adults. The conclusion of this was that reduced vitamin D levels are related to a poorer lung function (93). These studies suggest that vitamin D deficiency in both children and adults are related to impaired lung function and that vitamin D supplementation could reduce symptoms. Furthermore is it demonstrated that vitamin D is deficient in asthmatic children, which suggests that vitamin D supplementation could also serve as a prevention for asthma.

## Summarizing discussion and conclusion

Vitamin A and D are both vitamins with increasing interest. Even though they are best known for respectively eyesight and bone maintenance, they both have an essential function in the immune system.

The position of vitamin D in auto-immunity and allergic diseases is more clear than vitamin A's position as it has been shown that vitamin D deficiency is related with autoimmunity and allergic disease (6,24,35). Furthermore, it has been shown that northern countries from the westernized world have a higher prevalence of these diseases. The most important reasons of vitamin D deficiency are the lack of sunlight, urbanization and pollution resulting in reduced sunlight exposure. However, for vitamin A it is known that its metabolite is mandatory for  $T_{reg}$  differentiation. The administration of these vitamins nevertheless, are somewhat contradictory. Most studies focus on either vitamin A or vitamin D, however it may be more effective to supplement them together. The vitamins seem to share function in inflammation and tolerance and moreover, calcitriol and RA display a synergistic effect on  $T_H17$  cell regulation. Based on these facts, a possible productive therapy for  $T_H17$  related diseases could be a combination of both vitamins (31). However, it has been demonstrated that abundant RA concentrations will confiscate the RXR receptors as homodimers, which means that heterodimers can not be formed. This will result in a neutralization of vitamin D actions by vitamin A (19). These results indicate that a combination administration of vitamin A and D could be efficient however it needs to be further researched before they can be supplemented together.

The effects of vitamin A and D on the immune system are numerous. Vitamin A can bind the RXR receptor on the T-cells which then is stimulated to differentiate into either a  $T_{reg}$  or a  $T_H2$  cell. Furthermore, it will inhibit the cytokines that are needed for  $T_H1$  or  $T_H17$  differentiation. Additionally, RA will inhibit cytokines that would normally inhibit a  $T_{reg}$  differentiation. Another important function of RA could be the class switch towards a IgA molecule. Because of the great prevalence of IgA in mucosal tissues, it is suggested that RA is a dominant factor for this switch. A contradiction in the function of RA is the fact that it will inhibit the  $T_H17$  differentiation, but at the same time stimulate monocyte-derived DC to convert into a mucosal type DC which can secrete TGF- $\beta$  and IL-6 (13). The cytokine combination of TGF- $\beta$  and IL-6 will also stimulate  $T_H17$  differentiation which makes the statement that it will inhibit  $T_H17$  differentiation somewhat paradoxical. On the other hand, this could support the suggestion that RA predominates IL-6 and will skew the differentiation towards  $T_{reg}$  cells (9). Another point that seems conflicting is the concentration in which RA manifests itself. One study suggests that a low concentration will cause a  $T_H17$  differentiation (17), while another study states that a high RA concentration causes  $T_H17$  differentiation (59) and the following inflammation. These findings suggest that the body will retain an optimum RA concentration to support a most advantageous  $T_H17$ -Treg balance, however this needs to be further investigated.

The function of vitamin D metabolites in the immune system are not studied as well as vitamin A and therefore its function is more unclear. Even so, it is known that the vitamin D metabolites will bind the VDR, which is present on the immune cells, and subsequently form a heterodimer with RXR. This complex can bind the genes of different cytokines and in this way inhibit, or stimulate, different processes. Vitamin D metabolites will stimulate IL-10 expression while inhibiting IL-12 levels and furthermore will they inhibit IL-17. One of the most important functions of vitamin D is the stimulation of FOXP3 which is an essential transcription factor for  $T_{reg}$  cells. These actions will cause a stimulation of a  $T_{reg}$  or a  $T_H2$  response while inhibiting a  $T_H1$  or a  $T_H17$  response. Vitamin D metabolites can also affect the innate immune system as it up-regulates the production of the antimicrobial peptide cathelicidin. Vitamin D has a pivotal function in the immune system, however further research about the exact function is necessary.



With regard to the diseases of interest (IBD, food allergy and asthma), vitamin A and D play an important role. These diseases occur due to a loss of tolerance, which gives the immune system the opportunity to initiate an immune response to harmless endogenous and exogenous antigens. Even though a deficiency of the vitamins is the most logical explanation as they both stimulate a  $T_{reg}$  response, it has been demonstrated that an excess of both vitamins can also cause loss of tolerance (24,59). Nevertheless, it seems inconsequential that vitamin A and D have a functional effect on asthma, as asthma is the consequence of an excess  $T_H2$  response. Both vitamins can skew the effector T-cell reaction towards a  $T_H2$  response, which suggests that the vitamins should worsen the asthma. Therefore it is important to research whether  $T_{reg}$  differentiation dominates  $T_H2$  differentiation. A possible hypothesis is that  $T_{reg}$  differentiation is predominant because the cytokine environment provides for more  $T_{reg}$  differentiation. Both vitamin A and D will stimulate TGF- $\beta$  for example. However research is necessary, for now allergic patients could theoretically be supplemented with both vitamin A and D.

There have been different researches performed in which vitamin A and D and the relation to IBD, food allergy and asthma are of interest. The relation between vitamin A and IBD has some inconsistencies as one study concludes that vitamin A has a positive effect on the symptoms of IBD while another study (in zebrafish) concludes that vitamin A may induce IBD. These contradictions make it necessary to have more research towards this relation. The association between vitamin D and IBD is also researched in animals. However, for vitamin D the studies are more conclusive as the four studies included in this report all conclude that vitamin D has a protective function in IBD. Food allergy research in animals is very meager and in this report only research with vitamin A is included. Both studies concluded that vitamin A supplementation in food allergy (in animals) did not have a positive effect and that vitamin A even could induce food allergy. However, this also needs to be researched further. Research towards the relation between vitamin A and asthma in animals demonstrated that vitamin A deficiency leads to asthma symptoms and that supplementation of this vitamin could have a positive effect. The relation between vitamin D and asthma is quite conclusive as all the described studies conclude with the remark that vitamin D supplementation could reduce asthma symptoms. Furthermore, research with vitamin A has demonstrated that a deficiency of this vitamin can cause inflammation and that patients with Crohn's disease could own a polymorphism of the RALDH enzyme. The studies with vitamin A in human IBD patients could have a positive function and that supplementation is a suggestion. Vitamin D in IBD patients seems to have a protective function however the researchers reserved and they all recommend further research. As with the animal studies, human studies towards food allergy and the relation with the vitamins have not been researched enough, especially with vitamin A. However, vitamin D and food allergy have been researched and the results are conflicting. Two studies conclude that the vitamin has a protective function in food allergy, however two other studies conclude that the vitamin could induce food allergy. These conflicting outcomes make it evident that more research is required. Studies with vitamin A are not conclusive, there has been research which suggests that vitamin A supplementation has a protective function on asthma however, another study concludes that vitamin A deficiency is not related to asthma which makes supplementation meaningless. Research with vitamin D in both children and adults however demonstrated that vitamin D probably has a protective function in asthma.

Nevertheless, for all diseases and vitamin supplementations it is necessary to perform more researches, in both animal and humans.

In conclusion, the effects of vitamin A and D on the immune system are pleiotropic and they will skew the immune reaction towards a  $T_{reg}/T_H2$  response. In theory, vitamin A and D should induce tolerance and improve the progress of autoimmune and allergic diseases as IBD, food allergy and asthma. Dietary intervention studies with both animals and humans did demonstrate some minor effects, however more research is necessary. Vitamin A and D could be the future treatment (or even prevention) for IBD, food allergy and asthma, but for now more research is necessary.

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