Modulation of Th17 Cell Populations by Vitamin D: Exploring Therapeutic use in Rheumatoid Arthritis

Wendy Dankers

Modulation of Th17 Cell Populations by Vitamin D: Exploring Therapeutic Use in Rheumatoid Arthritis

Wendy Dankers

The work presented in this thesis was performed at the department of Rheumatology and the department of Immunology, Erasmus MC, Rotterdam, The Netherlands. Financial support was provided by the Dutch Arthritis Foundation. Publication of this thesis was financially supported by Erasmus Postgraduate School Molecular Medicine and the Erasmus University Rotterdam.

Modulation of Th17 cell populations by vitamin D: exploring therapeutic use in rheumatoid arthritis.

Layout: Thomas van der Vlis - www.persoonlijkproefschrift.nl
Printing: Ridderprint BV - www.ridderprint.nl
Cover: paint by Tera Bakker, photograph by UitjedakFotografie

ISBN: 978-94-6299-959-6

Modulation of Th17 Cell Populations by Vitamin D:
Exploring Therapeutic Use in Rheumatoid Arthritis

Modulatie van Th17 celpopulaties door vitamine D:
verkenning van therapeutisch gebruik in reumatoïde artritis

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof. Dr. R.C.M.E. Engels

en volgens het besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

dinsdag 26 juni om 11.30 uur

door

Wendy Dankers
geboren te Etten-Leur

Erasmus University Rotterdam

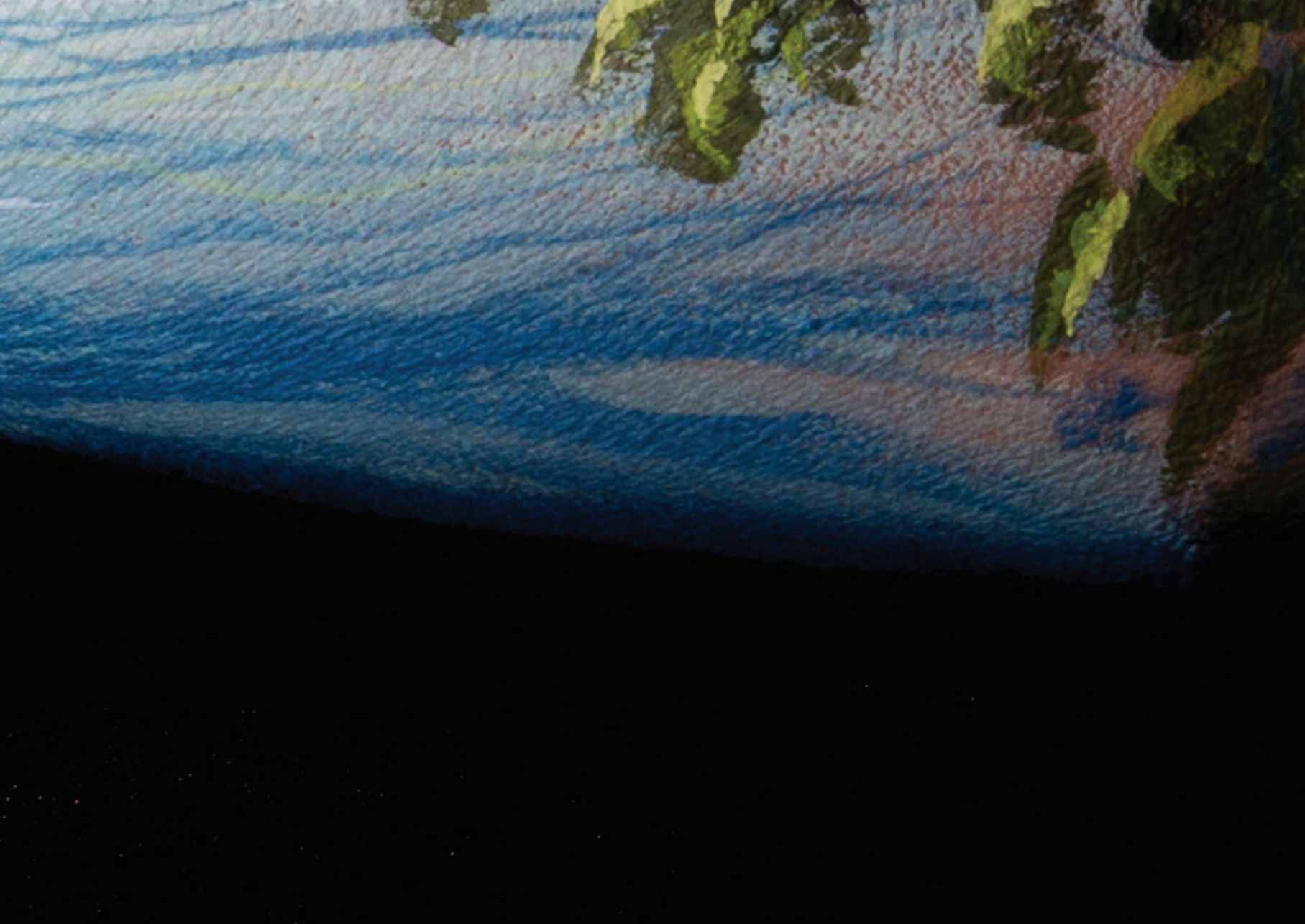
Promotiecommissie:

Promotor: Prof. dr. J.M.W. Hazes

Overige leden: Prof. dr. P. Katsikis
Dr. J.N. Samsom
Prof. dr. P.P. Tak

Copromotor: Dr. E. Lubberts

Table of contents



Chapter 1

Introduction

1 RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease, which mainly affects the synovial joints. About 0.5 to 1% of adults suffer from this debilitating disease in western countries and about three times more women than men are affected.[1] RA is characterized by the influx of immune cells into the joint, which leads to synovial hyperplasia, vascularization and in later stages damaged cartilage and bone. Patients suffer from swollen and painful joints and the progressive joint damage leads to functional decline over time. Furthermore, patients have an increased risk for co-morbidities such as cardiovascular diseases, certain types of cancer and depression.[2] Since there is currently no cure for RA, early detection and good treatment are essential to minimize the disease burden in patients. To achieve these goals, it is crucial to understand the development and underlying immunopathogenesis. Therefore, this section provides an overview of the current knowledge in these two areas.

1.1 Development of RA

The diagnosis of RA is given to a patient at the moment they present to the rheumatologist with inflammatory arthritis in more than one joint and, depending on the number and type of joints affected, the presence of auto-antibodies, symptom duration longer than 6 weeks and/or an abnormal acute-phase response.[3] However, it is currently incompletely understood which processes precede this stage of clinically defined RA, although it is believed that multiple factors play a role in the development of RA. Based on these factors and studies in patients at risk for developing RA, five different phases leading up to RA diagnosis have been distinguished, although it should be noted that these phases do not necessarily occur in all patients or in this sequence, and can also occur simultaneously.[4]

The first phase is the presence of genetic risk factors. Considering the high heritability rate of RA, which is estimated to be 50% for seropositive RA and 20% for seronegative RA[5], genetic factors play an important role in the susceptibility to develop RA. The strongest genetic association is currently found in the HLA genes, where 5 amino acids explain 12,7% of the heritability of RA.[6]

The second phase entails the exposure to environmental risk factors. These comprise a large number of factors, such as education level, weight, breastfeeding, air pollution, periodontal disease and ultraviolet light exposure.[7] The latter is mainly related to the vitamin D serum level, which is inversely correlated with RA disease activity and may affect the risk of developing RA. However, the environmental risk factor that is most

strongly correlated with an increased risk of developing RA is smoking, where the number of cigarettes and the duration of smoking both contribute to the increased risk up to 20 years after cessation.[8]

Together, the genetic and environmental risk factors may lead to the third phase of the disease; systemic autoimmunity. This phase, which can already be identified years before disease onset, markers of autoimmune inflammation can be detected in the peripheral blood. The most extensively described markers are autoantibodies. Various types of autoantibodies have been described to be present predating RA diagnosis, including anti-carbamylated peptide antibodies (ACarPs), anti-PAD4-antibodies and anti-IgG4 hinge antibodies, but the best known are rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA).[7] Rheumatoid factor is directed against the Fc part of IgG and is found in 50-80% of patients and 10% of healthy individuals.[9-10] ACPA are directed against citrullinated peptides, such as α-enolase, fibrinogen, vimentin, filaggrin and collagen type I and II, and are highly specific for RA.[10] Citrullination is the process where an arginine in the protein is changed into a citrulline by peptidylarginine deiminases (PADs) and which occurs mainly during apoptosis.[11] However, certain environmental risk factors can increase the level of citrullinated proteins and thereby increase the likelihood of citrullinated peptides to be detected by the immune system. For example, the amount of citrullinated proteins is increased in the lungs in response to smoking or other air pollutants.[12-13] Furthermore, citrullination is increased in neutrophils in response to *A. actinomycetemcomitans*, one of the bacteria associated with periodontitis.[14] Of note, during apoptosis PAD enzymes may not only citrullinate proteins inside the apoptotic cells. They may also leak out and citrullinate proteins in their environment, thereby providing even more sources for an immune response in RA.[15]

Next to the presence of autoantibodies, the systemic autoimmunity phase is also characterized by elevated levels of cytokines and chemokines, such as monocyte chemotactic protein (MCP)-1, tumor necrosis factor (TNF)-α, interleukin (IL)-6, soluble TNF receptor II, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1β and IL-10.[16-20] Interestingly, the concentration of cytokines increases towards the diagnosis of RA.[8, 16, 21] Additionally, there is epitope spreading and altered glycosylation of the autoantibodies, of which the latter recently was shown to be essential for pathogenicity of autoantibodies.[21-23]

In the next phases there is a conversion from systemic autoimmunity to local joint inflammation, which is usually the start of clinical presentation. In the fourth phase, defined as symptoms without clinical arthritis, patients have complaints such as pain and morning stiffness but without evidence of synovitis on imaging and clinical evaluation. In the fifth phase, which is unclassified arthritis, there is clinical evidence of synovitis, but the patient does not (yet) meet the criteria set for the RA diagnosis.[4]

In 60% of the unclassified arthritis patients, the joint inflammation will resolve. However, in the remaining 40% the inflammation becomes chronic and they will be diagnosed with RA within three years after the first symptoms. Notably, 90% of ACPA positive unclassified arthritis patients will progress to RA, making ACPA positivity a clear risk factor for developing RA.[24]

1.2 The immunopathogenesis of RA

In recent years, research focus has shifted from the established phase of RA towards the earlier phases. It is thought that there is a 'window of opportunity' in which treatment is most effective, since symptom duration is associated with drug-free sustained remission and radiographic progression.[25] Therefore, early detection may limit the risks of chronic synovitis and may even provide a cure for the disease. In order to do that, it is important to understand the underlying immunological processes. Due to extensive research, a picture is beginning to emerge in which both cells from the immune system and joint stromal cells play an important role in disease pathogenesis. Through cell-cell interaction and production of pro-inflammatory cytokines, these cells activate each other and thereby fuel a chronic inflammation. Although anti-inflammatory factors, such as IL-10 and soluble TNF receptors, are produced by the RA pannus and somewhat limit the inflammation[26-27], they are not sufficient to completely control the immune system and restore joint homeostasis.

Since there is still debate on the order in which cells are activated[28], we here provide an overview of the various cell types that can be found in the pannus of RA joints and the roles they may play in the onset, development and chronicity of RA. An overview of their interactions is displayed in figure 1.

Antigen-presenting cells

The normal adaptive immune response starts with antigen-presenting cells (APCs), such as dendritic cells (DCs) but also macrophages and B cells. These cells scavenge the body

for foreign intruders and can present specific parts of the intruders as peptides on their major histocompatibility complex (MHC) molecules. The antigens can be sensed by highly specific T cells, which will initiate an immune cascade with the goal to eradicate the intruder.

In the context of RA, APCs can initiate the autoimmune response by presenting self-antigens instead of foreign antigens, such as citrullinated or carbamylated peptides. Interestingly, the HLA-DR variant that is associated with increased RA susceptibility encodes an MHC class II molecule with higher avidity for citrullinated peptides than for native peptides.[29] This may explain the increased RA susceptibility for individuals carrying this specific HLA-DR allele, and could also explain the further increase in susceptibility when these patients also smoke and hence have higher levels of citrullinated proteins in their lungs.[30]

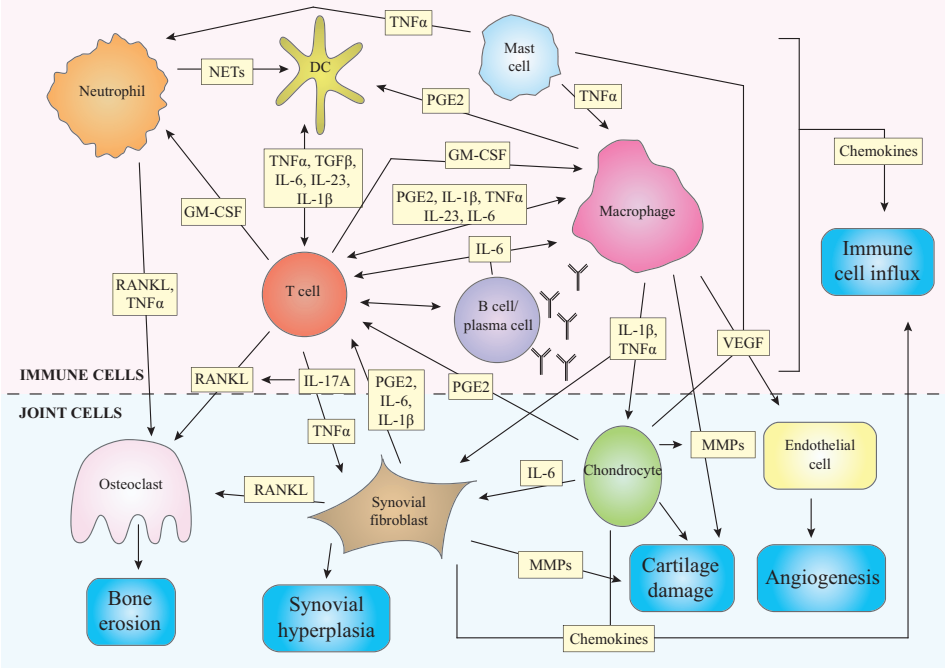


Figure 1. Schematic overview of the cellular interactions mediated by different proinflammatory cytokines in RA. The interaction between immune cells and joint-residing cells in RA is shown. TNFα is produced by almost all immune cells and affects many immune cells and joint cells. During the inflammatory process a counter reaction of regulatory and anti-inflammatory factors will also be induced (not depicted in this figure).

APCs are also efficient producers of pro-inflammatory cytokines such as IL-6, IL-23 and TNFα, to drive differentiation of the antigen-specific T cells and to further activate other APCs, but also synovial fibroblasts. Chemokines such as IL-8 are produced to attract more immune cells, such as monocytes, thereby further activating inflammation. Macrophages and DCs are found in high levels in the synovium and synovial fluid of RA patients.[31-32] Notably, DCs in the synovial fluid are distinct from DCs in the blood and are also called inflammatory DCs.[33] Furthermore, the levels of macrophages in the synovium are correlated with disease activity and decrease during successful antirheumatic treatment.[34-35] These data suggest that APCs such as DCs and macrophages not only play a role in the initiation of the inflammatory response, but they also contribute to the perpetuation of the inflammation.

T cells

T cells can roughly be divided into three groups; (1) cells with a T cell receptor (TCR) consisting of an α and β chain (TCRαβ) and the coreceptor CD4, (2) cells with TCRαβ and the coreceptor CD8 and (3) cells with a TCR consisting of a γ and δ chain.

CD4+ T cells, also called T-helper (Th) cells, are activated in the normal immune response once their highly specific TCR recognizes an antigen presented on MHC class II molecules by an APC presenting antigens on their MHC class II molecules. A second signal via interaction between CD28 on T cell and CD80 and CD86 on the APC ensures survival of the T cell. Depending on the cytokines secreted by the APC, the Th cells then differentiate towards several types of effector cells. IL-12 leads to the differentiation of a Th1 cell, whereas IL-4 directs towards Th2 cells and the combination of transforming growth factor (TGF)-β or IL-1β, IL-6 and IL-23 induces a Th17 phenotype. These Th cells can then propagate the immune response through activation of B cells and production of pro-inflammatory chemokines and cytokines to activate and attracts other immune cells. Of note, when there is only TGFβ in the environment during antigen-presentation, the Th cells will become a regulatory T cell (Treg) and suppress inflammation. After the infection is cleared, most Th cells will die but some will become memory Th cells in order to clear the intruder faster during the next infection.

CD8+ T cells are known for their cytotoxic activity and are activated when their TCR recognizes the antigen presenting on an MHC class I molecules of an APC. These cells then start producing lytic enzymes, such as granzymes and perforin, which will kill the infected cells. Furthermore, they will also produce pro-inflammatory cytokines like

interferon (IFN)-γ and TNFα. Similar to Th cells, also CD8+ T cells will form memory cells after the infection is cleared.

The γδ T cells are somewhat different than the Th and C8+ T cells, in that they do not require stimulation from an APC to get activated. Instead, they are activated by small nonpeptide phosphoantigens. Upon activation, γδ T cells can induce DC maturation, produce pro-inflammatory cytokines and help B cells.

Since the APCs in RA present self-antigens instead of foreign antigens, the reaction mounted by T cells is directed against self-tissue. This is a reaction that should normally be suppressed by deletion of autoreactive T cells during thymic development. However, in RA patients this tolerogenic mechanism is defective and autoreactive T cells are able to initiate the autoimmune response.

Although γδ T and CD8+ T cells are both found in the synovium of RA patients and their normal functions provide clues that they may play a role in synovial inflammation[36-37], it is thought that Th cells play the major role in RA pathogenesis. This is partly due to the correlation between HLA-DR haplotypes and RA susceptibility, since HLA-DR is an MHC class II molecule and hence activates the Th cells. Although all Th cells can activate B cells to further induce the immunological cascade, it seems that not all Th cells are equally important in the development of RA. Classically it was thought that Th1 cells were the most pathogenic cells in autoimmune diseases such as RA, but the discovery of IL-23 has placed the Th17 cells at the center of attention.[38] Several studies in experimental arthritis models have suggested a role for Th17 cells in the development of arthritis, such decreased severity of collagen-induced arthritis upon deletion of IL-17A, the signature cytokine of Th17 cells.[39] Furthermore, mice deficient in IL-23 or IL-17R are protected against developing arthritis.[40-41] In human RA, Th17 cells and IL-17A are elevated compared to healthy controls and are correlated with disease activity.[42-43]

Functionally, the difference in pathogenicity between Th17 cells and other Th subsets could lie in the activation of synovial fibroblasts. CCR6+ memory Th (memTh) cells, which include Th17 cells but not Th1 or Th2 cells, are specifically able to activate synovial fibroblasts from RA patients. This interaction creates a pro-inflammatory feedback loop that promotes the production of pro-inflammatory cytokines such as IL-17A, IL-6 and IL-8, but also tissue-destructive matrix metalloproteases.[43] Another important mechanism by which Th17 cells contribute to RA disease pathogenesis is through modulation of

plasma cells. A recent study has shown that specifically IL-23-activated Th17 cells reduce the expression of St6gal1, a sialyltransferase, in plasma cells via IL-21 and IL-22. As a result, autoantibodies produced by these plasma cells have reduced sialic acid residues and become pathogenic, resulting in autoimmune synovial inflammation.[23] These data may also explain the disappointing results in clinical trials targeting IL-17A or its receptor in established RA patients, since the role for Th17 cells may lie predominantly in disease onset.[38]

All the Th cells discussed so far have a pro-inflammatory role and could thereby contribute to synovial inflammation. However, there may also be a role for the regulatory T cells (Tregs). These cells normally inhibit inflammation via production of the anti-inflammatory cytokine IL-10 and inhibiting proliferation of other Th cells. Their importance in RA is demonstrated in murine experimental arthritis, where Treg-depletion aggravates arthritis and administration of antigen-specific regulatory cells cures and protects from arthritis.[44-45]

Interestingly, Tregs are specifically decreased during active disease in RA patients and patients have a higher Th17/Treg ratio than healthy controls.[46] This immunological imbalance may play an important role in the chronic inflammation and normalization is an important therapeutic challenge.

B cells and antibody production

B cells are activated through interaction with Th cells via their antigen-specific B cell receptor (BCR). Upon activation they will proliferate and undergo class switch recombination and somatic hypermutation of their BCR. This results in highly specific memory B cells and most importantly; large amounts of plasma cells which secrete antibodies towards the antigen. In addition, activated B cells secrete cytokines such as IL-6 to stimulate other immune cells.

Similar to T cells, B cells should normally not be activated by self-antigens because the autoreactive B cells are mostly removed during their development. In RA patients, for an unknown reason there is a break of tolerance leading to an increased amount of autoreactive B cells in the peripheral blood of RA patients.[47] These autoreactive B cells can contribute to RA development by the production of high-affinity autoantibodies, such as ACPA or RF which are associated with the risk of developing RA. There are various mechanisms through which these auto-antibodies can contribute to the disease progress

in RA. First, it has been shown that ACPAs specific for citrullinated vimentin activate osteoclastogenesis, which starts bone loss but also induces pain and the production of IL-8.[48] IL-8 acts as a chemokine and can attract immune cells such as neutrophils and macrophages, which may start the pro-inflammatory cascade leading up to chronic synovitis. Furthermore, autoantibodies can bind autoantigens to form immune complexes, which attracts complement and thereby drives the inflammation.[49]

Next to their antibody production, B cells can also contribute to inflammation through antigen-presentation, as described in the APC section, and through cytokine production. IL-6 is one of the cytokines that is produced by B cells upon activation and which is important for the development of Th17 cells and activation of other immune cells.[50] Importantly, synovial fluid B cells from RA patients also produce RANKL, which is important for osteoclast differentiation and thus contributes to the bone loss observed in patients.[51]

Innate immune cells

Although the adaptive immune response appears to play a major role in starting RA pathogenesis, also immune cells of innate origin contribute to the ongoing synovial inflammation.

During a normal immune response, innate immune cells form the first line of defense. They fight pathogens through phagocytosis, complement activation and lytic cell death. Furthermore, they activate the adaptive immune system via antigen-presentation as discussed previously.

In RA synovial inflammation, especially macrophages play a significant role. Next to their function as APC, as described earlier in this chapter, macrophages are potent producers of pro-inflammatory cytokines such as TNFα, IL-1 and IL-6. Furthermore, they produce matrix metalloproteases (MMPs) that mediate tissue destruction, reactive oxygen species and chemokines to recruit other immune cells.[52] Finally, the macrophages from RA synovial tissues are capable of promoting neovascularization, an important process in the formation of the inflammatory pannus in RA.[53] It is hypothesized that these pro-inflammatory macrophages differentiate from the monocytes that invaded the RA tissue, rather than representing aberrantly activated tissue-resident macrophages.[54]

Mast cells and neutrophils are two other innate immune cell types which can be found in the RA synovial tissue. They are thought to contribute to RA pathogenesis through the production of pro-inflammatory cytokines, prostaglandins, chemokines and reactive oxygen species.[52] However, recently a new role has been hypothesized for neutrophils in RA pathogenesis. Neutrophils can create neutrophil extracellular traps (NETs) via a process called NETosis, which is normally used to trap and kill microbes. However, these NETs also contain high amounts of citrullinated histones and could therefore provide a source of autoantigens to which other immune cells react.[55]

Synovial fibroblasts

Next to the immune cells, also the local joint cells play an important role in RA pathogenesis. In a synovial joint, the non-articular surfaces are lined with a 1 to 3 cells thick layer called the synovium. The synovium consists of fibroblast-like synoviocytes, or synovial fibroblasts, and tissue-resident macrophages. In RA patients, the synovial cells start hyperproliferating, producing pro-inflammatory cytokines, chemokines and tissue-destructive enzymes and there is vascular growth in the synovium. Furthermore, the fibroblasts start presenting antigens that further aggravate synovial inflammation.[56]

Already in 1996 it was demonstrated that the synovial fibroblasts from RA patients (RASF) have an activated invasive and destructive phenotype which they maintain over longer periods of time after isolation from the arthritic joint.[57] New studies suggest that this change in phenotype already occurs very early in the disease; RASF from early RA patients do not exhibit the suppressive functions anymore that RASF from healthy individuals or patients with resolving arthritis have. Later, in the established disease, the RASF even stimulate inflammation by activating synovial endothelium to attract immune cells.[58] Interestingly, it was recently shown that synovial fibroblasts differ in their phenotype depending on the joint they originate from. For example, synovial fibroblasts from lower extremities such as knees and ankles respond stronger to TNFα than those from upper extremities such as shoulders and hands. Also, hand synovial fibroblasts produce more MMP13 than shoulder and knee synovial fibroblasts.[59] Thereby, these cells could contribute to the symmetric joint inflammation that is observed in RA, further demonstrating their importance in disease pathogenesis. Since gene expression patterns that discern these groups of fibroblasts are similar between knees of RA patients and healthy controls, it is postulated that the differences are intrinsic and not due to disease.[59] This phenomenon could be related to the aberrant epigenetic signatures in RASF, as described for DNA methylation and histone modifications.[60]

Due to the increased recognition for the role of synovial fibroblasts in RA, they have also become interesting therapeutic targets.[61] This emerging field will expand over the coming years and more studies will further increase our knowledge on the role and the potential therapeutic targeting of RASF.

Chrondrocytes and osteoclasts

Two important features of RA are damaged cartilage and bone erosions, which are largely mediated by chondrocytes and osteoclasts, respectively.

Although synovial fibroblasts and macrophages are potent producers of MMPs that mediate cartilage destruction, this cannot explain the cartilage damage in the deep zones of the cartilage not directly adjacent to the pannus.[62-63] Therefore, it is thought that chondrocytes may also play a crucial role in joint damage. In a healthy joint, chondrocytes reside in the cartilage and maintain a balance between cartilage degradation and synthesis of cartilage components. However, in RA they are stimulated by pro-inflammatory cytokines such as IL-1 and TNFα.[64] In response, they release MMPs and other proteases that tip the balance towards cartilage degradation. Furthermore, chondrocytes start producing IL-6, PGE2 and chemokines to further aggravate the ongoing inflammation.[64]

While chondrocytes are mediators of cartilage damage in RA, osteoclasts are responsible for the bone erosions in this disease. Under homeostatic conditions, the bone-resorbing osteoclasts are in equilibrium with the bone-forming osteoblasts. During inflammation, osteoclasts are highly activated through the increased abundance of RANKL expressed by immune cells and synovial fibroblasts, leading to an increased RANKL/OPG balance. Furthermore, TNFα is also capable of directly promoting osteoclastogenesis independent of RANKL. This increased activation leads to increased bone resorption at the site of the pannus.[65]

2 TREATMENT OF RHEUMATOID ARTHRITIS

Before the development of the modern RA medication, patients were treated with non-steroidal anti-inflammatory drugs (NSAIDs), hydroxychloroquine, gold or steroids and bed rest, which were not very effective or quite toxic. Luckily, that situation has greatly improved by the introduction of methotrexate and later by the biological disease-modifying antirheumatic drugs (bDMARDs), or 'biologicals'. Here we will give an overview of the current treatment protocols, how they intervene in the immune pathogenesis as described above, and the opportunities to improve the current therapies.

2.1 Current treatment

The goal of all RA treatment is to achieve sustained disease remission or low disease activity if remission is not possible due to longstanding disease. In order to reach remission as early as possible, it is important that an RA patient is treated as soon as the diagnosis is made.[66] According to the most recent EULAR recommendations, patients are first treated with methotrexate (MTX) or another conventional DMARD (cDMARD) such as sulfasalazine when patients have a contraindication for MTX.[66] When there is no improvement in disease activity within three months after the start of the treatment, or when the treatment goal is not reached after six months, other drugs can be added to the MTX therapy. Usually patients are started on a bDMARD which targets the cytokine TNFα. Although this is only one of the cytokines in the immunological processes described above, the success rate of this therapy indicates that TNFα plays a central role in the inflammatory cascade. This has fueled the hypothesis that there is a hierarchical cytokine structure in RA, with certain cytokines being central in driving the joint inflammation.[67]

If TNFα-targeting treatment is not effective in bringing down disease activity, other bDMARDs are available that inhibit IL-6-mediated signaling, another key pathway in the hierarchical cytokine structure, via inhibition of IL-6R, suppress T cell activation through mimicking CTLA4 or deplete B cells by targeting CD20. Recently, also so-called targeted, or non-biological, DMARDs have been approved which interfere in the Janus Kinase (JAK) pathways. The JAKs are important in the downstream signaling of many cytokines, including IL-6, IL-23 and IFNγ, and thereby target multiple pro-inflammatory pathways simultaneously. Although they are currently not in the first line of treatment, that may change in the future since their route of administration may be preferred by patients (oral versus injection) and there are indications that they work equally well in monotherapy as in combination with MTX.[68] Finally, patients are treated with glucocorticoids (GC), such as prednisone and dexamethasone, as a bridging therapy when a new treatment is started or a patient switches to a different treatment. These GCs quickly resolve inflammation, but due to their side effects they should be tapered and preferably stopped as soon as clinically possible. Of note, these GCs are often combined with vitamin D supplements to prevent the osteoporotic side effects of the GCs.

2.2 Improving RA treatment: restore the immunological imbalance

Although the current treatment protocol has improved the quality of life of many RA patients, there is also a large group of patients who do not respond sufficiently to these

therapies or who become resistant over time. Also, there is still no cure for the disease. Therefore, it is important to keep working on better treatments and preferably a way to cure or prevent RA.

One way to achieve this goal is to normalize the immunological imbalance that is present in autoimmune diseases such as RA. An important sign of the immunological imbalance in RA is the increased level and activity of the Th17 cells, combined with the decreased level and functionality of Tregs. Therefore, inhibiting the activity of Th17 cells could be an important step towards normalizing the immunological balance in early RA patients.

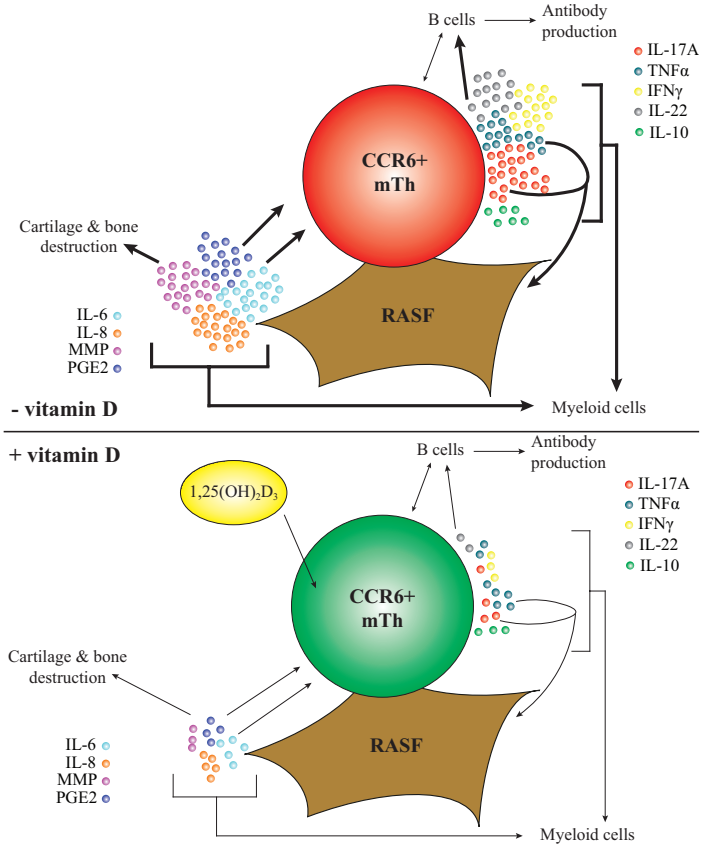


Figure 2. The pathogenic role of CCR6+ memTh cells in RA synovial inflammation. (Upper panel) Current knowledge on the potential pathogenic effects of CCR6+ memTh cells, including RASF activation, direct and indirect macrophage activation, B cell modulation and indirect cartilage and bone destruction. (Lower panel) The effects of $1,25(OH)_2D_3$ on this pro-inflammatory system, by inhibiting CCR6+ memTh pathogenicity through unknown mechanisms. Thickness of the arrows indicate strength of the stimulation.

Interestingly, we have previously shown that the pro-inflammatory activity of CCR6+ memTh cells, which include Th17 cells and several other IL-17A-producing subpopulations, can be efficiently inhibited by the active vitamin D metabolite $1,25(OH)_2D_3$.[69] Furthermore, $1,25(OH)_2D_3$ can also inhibit the pro-inflammatory loop between CCR6+ memTh cells and synovial fibroblasts, which could prevent the establishment of chronic synovial inflammation (figure 2).[70] In line with these findings, it has been shown that $1,25(OH)_2D_3$ can suppress both the incidence and progression of experimental arthritis and other autoimmune diseases.[71-73]

Combining these data with the correlation between serum vitamin D levels and the incidence and severity of RA,[74-75] vitamin D seems like an interesting tool to modulate the unbalanced immune system through targeting Th17 cells. However, supplementing high doses of $1,25(OH)_2D_3$ is clinically limited due to the high risk of severe side effects due to hypercalcemia.

Therefore, it is of great interest to understand how $1,25(OH)_2D_3$ modulates CCR6+ memTh cells, including Th17 cells, and how it can contribute to inhibiting arthritis. Using this knowledge, we can circumvent the need for high levels of vitamin D supplementation and directly target the relevant pathways.

3 AIMS OF THE THESIS

In this thesis we aim to explore the mechanisms by which vitamin D can modulate CCR6+ memTh cells, including Th17 cells, and how this could contribute to alleviating RA disease activity.

Therefore, we first provide an overview of the current knowledge concerning vitamin D in autoimmune diseases (**Chapter 2**), including an overview of the clinical trials performed in this context and the immunomodulatory effects that have been described.

Since $1,25(OH)_2D_3$-treatment can prevent onset and progression of experimental arthritis, we then examined how $1,25(OH)_2D_3$ modulated Th17 differentiation in the context of this disease model in **Chapter 3**.

For investigating the effects of $1,25(OH)_2D_3$ on Th17 cells in human RA, CCR6+ memTh cells are used. However, CCR6+ Th cells are a heterogeneous group of cells, which consist of various subpopulations. Currently it is unknown whether these subpopulations are

separate cell types and if they are all equally susceptible to modulation by $1,25(OH)_2D_3$. These issues are addressed in **Chapter 4**.

Previously, it has been shown that $1,25(OH)_2D_3$ inhibits the pro-inflammatory activity of the CCR6+ memTh cells. In **Chapter 5** we further explored the phenotypical changes in CCR6+ memTh cells from healthy controls and RA patients that are induced by $1,25(OH)_2D_3$. In order for these phenotypical changes to be useful in clinical practice, the effects need to be maintained in a pro-inflammatory environment even in the absence of $1,25(OH)_2D_3$. **Chapter 6** describes this functional stability of the effect of $1,25(OH)_2D_3$ under various stimuli.

Given the immunomodulatory properties of $1,25(OH)_2D_3$, it is of interest to find direct clinical uses for vitamin D supplementation. **Chapter 7** describes the immunomodulatory potential of $1,25(OH)_2D_3$ in combination with dexamethasone in the pro-inflammatory loop between CCR6+ memTh cells and RASF and how this combination could increase effectiveness of TNFα-blocking therapies.

Since all previous studies were performed in vitro and these do not always reflect the complexity of the human body, in **Chapter 8** we investigated the clinical correlation between serum vitamin D levels and the treatment response on etanercept, a widely-used TNFα-blocking agent.

REFERENCES

1. Scott, D. L.; Wolfe, F.; Huizinga, T. W., Rheumatoid arthritis. *Lancet* **2010,** *376* (9746), 1094-108.
2. Dougados, M., Comorbidities in rheumatoid arthritis. *Curr Opin Rheumatol* **2016,** *28* (3), 282-8.
3. Aletaha, D.; Neogi, T.; Silman, A. J.; Funovits, J.; Felson, D. T.; Bingham, C. O., 3rd; Birnbaum, N. S.; Burmester, G. R.; Bykerk, V. P.; Cohen, M. D.; Combe, B.; Costenbader, K. H.; Dougados, M.; Emery, P.; Ferraccioli, G.; Hazes, J. M.; Hobbs, K.; Huizinga, T. W.; Kavanaugh, A.; Kay, J.; Kvien, T. K.; Laing, T.; Mease, P.; Menard, H. A.; Moreland, L. W.; Naden, R. L.; Pincus, T.; Smolen, J. S.; Stanislawska-Biernat, E.; Symmons, D.; Tak, P. P.; Upchurch, K. S.; Vencovsky, J.; Wolfe, F.; Hawker, G., 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* **2010,** *69* (9), 1580-8.
4. Gerlag, D. M.; Raza, K.; van Baarsen, L. G.; Brouwer, E.; Buckley, C. D.; Burmester, G. R.; Gabay, C.; Catrina, A. I.; Cope, A. P.; Cornelis, F.; Dahlqvist, S. R.; Emery, P.; Eyre, S.; Finckh, A.; Gay, S.; Hazes, J. M.; van der Helm-van Mil, A.; Huizinga, T. W.; Klareskog, L.; Kvien, T. K.; Lewis, C.; Machold, K. P.; Ronnelid, J.; van Schaardenburg, D.; Schett, G.; Smolen, J. S.; Thomas, S.; Worthington, J.; Tak, P. P., EULAR recommendations for terminology and research in individuals at risk of rheumatoid arthritis: report from the Study Group for Risk Factors for Rheumatoid Arthritis. *Ann Rheum Dis* **2012,** *71* (5), 638-41.
5. Frisell, T.; Holmqvist, M.; Kallberg, H.; Klareskog, L.; Alfredsson, L.; Askling, J., Familial risks and heritability of rheumatoid arthritis: role of rheumatoid factor/anti-citrullinated protein antibody status, number and type of affected relatives, sex, and age. *Arthritis Rheum* **2013,** *65* (11), 2773-82.
6. Raychaudhuri, S.; Sandor, C.; Stahl, E. A.; Freudenberg, J.; Lee, H. S.; Jia, X.; Alfredsson, L.; Padyukov, L.; Klareskog, L.; Worthington, J.; Siminovitch, K. A.; Bae, S. C.; Plenge, R. M.; Gregersen, P. K.; de Bakker, P. I., Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. *Nat Genet* **2012,** *44* (3), 291-6.
7. Karlson, E. W.; van Schaardenburg, D.; van der Helm-van Mil, A. H., Strategies to predict rheumatoid arthritis development in at-risk populations. *Rheumatology (Oxford)* **2016,** *55* (1), 6-15.
8. Costenbader, K. H.; Feskanich, D.; Mandl, L. A.; Karlson, E. W., Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. *Am J Med* **2006,** *119* (6), 503 e1-9.

9. Mannik, M.; Nardella, F. A.; Sasso, E. H., Rheumatoid factors in immune complexes of patients with rheumatoid arthritis. *Springer Semin Immunopathol* **1988,** *10* (2-3), 215-30.
10. Conigliaro, P.; Chimenti, M. S.; Triggianese, P.; Sunzini, F.; Novelli, L.; Perricone, C.; Perricone, R., Autoantibodies in inflammatory arthritis. *Autoimmun Rev* **2016,** *15* (7), 673-83.
11. Witalison, E. E.; Thompson, P. R.; Hofseth, L. J., Protein Arginine Deiminases and Associated Citrullination: Physiological Functions and Diseases Associated with Dysregulation. *Curr Drug Targets* **2015,** *16* (7), 700-10.
12. Catrina, A. I.; Ytterberg, A. J.; Reynisdottir, G.; Malmstrom, V.; Klareskog, L., Lungs, joints and immunity against citrullinated proteins in rheumatoid arthritis. *Nat Rev Rheumatol* **2014,** *10* (11), 645-53.
13. Makrygiannakis, D.; Hermansson, M.; Ulfgren, A. K.; Nicholas, A. P.; Zendman, A. J.; Eklund, A.; Grunewald, J.; Skold, C. M.; Klareskog, L.; Catrina, A. I., Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. *Ann Rheum Dis* **2008,** *67* (10), 1488-92.
14. Konig, M. F.; Abusleme, L.; Reinholdt, J.; Palmer, R. J.; Teles, R. P.; Sampson, K.; Rosen, A.; Nigrovic, P. A.; Sokolove, J.; Giles, J. T.; Moutsopoulos, N. M.; Andrade, F., Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. *Sci Transl Med* **2016,** *8*,[369] 369ra176.
15. Vossenaar, E. R.; Radstake, T. R.; van der Heijden, A.; van Mansum, M. A.; Dieteren, C.; de Rooij, D. J.; Barrera, P.; Zendman, A. J.; van Venrooij, W. J., Expression and activity of citrullinating peptidylarginine deiminase enzymes in monocytes and macrophages. *Ann Rheum Dis* **2004,** *63* (4), 373-81.
16. Deane, K. D.; O'Donnell, C. I.; Hueber, W.; Majka, D. S.; Lazar, A. A.; Derber, L. A.; Gilliland, W. R.; Edison, J. D.; Norris, J. M.; Robinson, W. H.; Holers, V. M., The number of elevated cytokines and chemokines in preclinical seropositive rheumatoid arthritis predicts time to diagnosis in an age-dependent manner. *Arthritis Rheum* **2010,** *62* (11), 3161-72.
17. Rantapaa-Dahlqvist, S.; Boman, K.; Tarkowski, A.; Hallmans, G., Up regulation of monocyte chemoattractant protein-1 expression in anti-citrulline antibody and immunoglobulin M rheumatoid factor positive subjects precedes onset of inflammatory response and development of overt rheumatoid arthritis. *Ann Rheum Dis* **2007,** *66* (1), 121-3.
18. Jorgensen, K. T.; Wiik, A.; Pedersen, M.; Hedegaard, C. J.; Vestergaard, B. F.; Gislefoss, R. E.; Kvien, T. K.; Wohlfahrt, J.; Bendtzen, K.; Frisch, M., Cytokines, autoantibodies and viral antibodies in premorbid and postdiagnostic sera from patients with rheumatoid arthritis: case-control study nested in a cohort of Norwegian blood donors. *Ann Rheum Dis* **2008,** *67* (6), 860-6.

19. Karlson, E. W.; Chibnik, L. B.; Tworoger, S. S.; Lee, I. M.; Buring, J. E.; Shadick, N. A.; Manson, J. E.; Costenbader, K. H., Biomarkers of inflammation and development of rheumatoid arthritis in women from two prospective cohort studies. *Arthritis Rheum* **2009,** *60* (3), 641-52.
20. Kokkonen, H.; Soderstrom, I.; Rocklov, J.; Hallmans, G.; Lejon, K.; Rantapaa Dahlqvist, S., Up-regulation of cytokines and chemokines predates the onset of rheumatoid arthritis. *Arthritis Rheum* **2010,** *62* (2), 383-91.
21. Sokolove, J.; Bromberg, R.; Deane, K. D.; Lahey, L. J.; Derber, L. A.; Chandra, P. E.; Edison, J. D.; Gilliland, W. R.; Tibshirani, R. J.; Norris, J. M.; Holers, V. M.; Robinson, W. H., Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. *PLoS One* **2012,** *7* (5), e35296.
22. Rombouts, Y.; Ewing, E.; van de Stadt, L. A.; Selman, M. H.; Trouw, L. A.; Deelder, A. M.; Huizinga, T. W.; Wuhrer, M.; van Schaardenburg, D.; Toes, R. E.; Scherer, H. U., Anti-citrullinated protein antibodies acquire a pro-inflammatory Fc glycosylation phenotype prior to the onset of rheumatoid arthritis. *Ann Rheum Dis* **2015,** *74* (1), 234-41.
23. Pfeifle, R.; Rothe, T.; Ipseiz, N.; Scherer, H. U.; Culemann, S.; Harre, U.; Ackermann, J. A.; Seefried, M.; Kleyer, A.; Uderhardt, S.; Haugg, B.; Hueber, A. J.; Daum, P.; Heidkamp, G. F.; Ge, C.; Bohm, S.; Lux, A.; Schuh, W.; Magorivska, I.; Nandakumar, K. S.; Lonnblom, E.; Becker, C.; Dudziak, D.; Wuhrer, M.; Rombouts, Y.; Koeleman, C. A.; Toes, R.; Winkler, T. H.; Holmdahl, R.; Herrmann, M.; Bluml, S.; Nimmerjahn, F.; Schett, G.; Kronke, G., Regulation of autoantibody activity by the IL-23-TH17 axis determines the onset of autoimmune disease. *Nat Immunol* **2017,** *18* (1), 104-113.
24. van Gaalen, F. A.; Linn-Rasker, S. P.; van Venrooij, W. J.; de Jong, B. A.; Breedveld, F. C.; Verweij, C. L.; Toes, R. E.; Huizinga, T. W., Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. *Arthritis Rheum* **2004,** *50* (3), 709-15.
25. van Nies, J. A.; Krabben, A.; Schoones, J. W.; Huizinga, T. W.; Kloppenburg, M.; van der Helm-van Mil, A. H., What is the evidence for the presence of a therapeutic window of opportunity in rheumatoid arthritis? A systematic literature review. *Ann Rheum Dis* **2014,** *73* (5), 861-70.
26. Katsikis, P. D.; Chu, C. Q.; Brennan, F. M.; Maini, R. N.; Feldmann, M., Immunoregulatory role of interleukin 10 in rheumatoid arthritis. *J Exp Med* **1994,** *179* (5), 1517-27.
27. Brennan, F. M.; Gibbons, D. L.; Cope, A. P.; Katsikis, P.; Maini, R. N.; Feldmann, M., TNF inhibitors are produced spontaneously by rheumatoid and osteoarthritic synovial joint cell cultures: evidence of feedback control of TNF action. *Scandinavian journal of immunology* **1995,** *42* (1), 158-65.

28. Firestein, G. S.; McInnes, I. B., Immunopathogenesis of Rheumatoid Arthritis. *Immunity* **2017,** *46* (2), 183-196.
29. Hill, J. A.; Southwood, S.; Sette, A.; Jevnikar, A. M.; Bell, D. A.; Cairns, E., Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. *J Immunol* **2003,** *171* (2), 538-41.
30. Klareskog, L.; Stolt, P.; Lundberg, K.; Kallberg, H.; Bengtsson, C.; Grunewald, J.; Ronnelid, J.; Harris, H. E.; Ulfgren, A. K.; Rantapaa-Dahlqvist, S.; Eklund, A.; Padyukov, L.; Alfredsson, L., A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. *Arthritis Rheum* **2006,** *54* (1),.[38-46]
31. Harding, B.; Knight, S. C., The distribution of dendritic cells in the synovial fluids of patients with arthritis. *Clin Exp Immunol* **1986,** *63* (3), 594-600.
32. Smeets, T. J.; Kraan, M. C.; Galjaard, S.; Youssef, P. P.; Smith, M. D.; Tak, P. P., Analysis of the cell infiltrate and expression of matrix metalloproteinases and granzyme B in paired synovial biopsy specimens from the cartilage-pannus junction in patients with RA. *Ann Rheum Dis* **2001,** *60* (6), 561-5.
33. Segura, E.; Touzot, M.; Bohineust, A.; Cappuccio, A.; Chiocchia, G.; Hosmalin, A.; Dalod, M.; Soumelis, V.; Amigorena, S., Human inflammatory dendritic cells induce Th17 cell differentiation. *Immunity* **2013,** *38* (2), 336-48.
34. Haringman, J. J.; Gerlag, D. M.; Zwinderman, A. H.; Smeets, T. J.; Kraan, M. C.; Baeten, D.; McInnes, I. B.; Bresnihan, B.; Tak, P. P., Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* **2005,** *64* (6), 834-8.
35. Tak, P. P.; Smeets, T. J.; Daha, M. R.; Kluin, P. M.; Meijers, K. A.; Brand, R.; Meinders, A. E.; Breedveld, F. C., Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. *Arthritis Rheum* **1997,** *40* (2), 217-25.
36. Su, D.; Shen, M.; Li, X.; Sun, L., Roles of gammadelta T cells in the pathogenesis of autoimmune diseases. *Clin Dev Immunol* **2013,** *2013*, 985753.
37. Carvalheiro, H.; da Silva, J. A.; Souto-Carneiro, M. M., Potential roles for CD8(+) T cells in rheumatoid arthritis. *Autoimmun Rev* **2013,** *12* (3), 401-9.
38. Lubberts, E., The IL-23-IL-17 axis in inflammatory arthritis. *Nat Rev Rheumatol* **2015,** *11* (7), 415-29.
39. Nakae, S.; Nambu, A.; Sudo, K.; Iwakura, Y., Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J Immunol* **2003,** *171* (11), 6173-7.

40. Murphy, C. A.; Langrish, C. L.; Chen, Y.; Blumenschein, W.; McClanahan, T.; Kastelein, R. A.; Sedgwick, J. D.; Cua, D. J., Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. *J Exp Med* **2003,** *198* (12), 1951-7.
41. Corneth, O. B.; Mus, A. M.; Asmawidjaja, P. S.; Klein Wolterink, R. G.; van Nimwegen, M.; Brem, M. D.; Hofman, Y.; Hendriks, R. W.; Lubberts, E., Absence of interleukin-17 receptor a signaling prevents autoimmune inflammation of the joint and leads to a Th2-like phenotype in collagen-induced arthritis. *Arthritis Rheumatol* **2014,** *66* (2), 340-9.
42. Leipe, J.; Grunke, M.; Dechant, C.; Reindl, C.; Kerzendorf, U.; Schulze-Koops, H.; Skapenko, A., Role of Th17 cells in human autoimmune arthritis. *Arthritis Rheum* **2010,** *62* (10), 2876-85.
43. van Hamburg, J. P.; Asmawidjaja, P. S.; Davelaar, N.; Mus, A. M.; Colin, E. M.; Hazes, J. M.; Dolhain, R. J.; Lubberts, E., Th17 cells, but not Th1 cells, from patients with early rheumatoid arthritis are potent inducers of matrix metalloproteinases and proinflammatory cytokines upon synovial fibroblast interaction, including autocrine interleukin-17A production. *Arthritis Rheum* **2011,** *63* (1),.[73-83]
44. Frey, O.; Petrow, P. K.; Gajda, M.; Siegmund, K.; Huehn, J.; Scheffold, A.; Hamann, A.; Radbruch, A.; Brauer, R., The role of regulatory T cells in antigen-induced arthritis: aggravation of arthritis after depletion and amelioration after transfer of CD4+CD25+ T cells. *Arthritis Res Ther* **2005,** *7* (2), R291-301.
45. Asnagli, H.; Martire, D.; Belmonte, N.; Quentin, J.; Bastian, H.; Boucard-Jourdin, M.; Fall, P. B.; Mausset-Bonnefont, A. L.; Mantello-Moreau, A.; Rouquier, S.; Marchetti, I.; Jorgensen, C.; Foussat, A.; Louis-Plence, P., Type 1 regulatory T cells specific for collagen type II as an efficient cell-based therapy in arthritis. *Arthritis Res Ther* **2014,** *16* (3), R115.
46. Wang, W.; Shao, S.; Jiao, Z.; Guo, M.; Xu, H.; Wang, S., The Th17/Treg imbalance and cytokine environment in peripheral blood of patients with rheumatoid arthritis. *Rheumatol Int* **2012,** *32* (4), 887-93.
47. Samuels, J.; Ng, Y. S.; Coupillaud, C.; Paget, D.; Meffre, E., Impaired early B cell tolerance in patients with rheumatoid arthritis. *J Exp Med* **2005,** *201* (10), 1659-67.
48. Harre, U.; Georgess, D.; Bang, H.; Bozec, A.; Axmann, R.; Ossipova, E.; Jakobsson, P. J.; Baum, W.; Nimmerjahn, F.; Szarka, E.; Sarmay, G.; Krumbholz, G.; Neumann, E.; Toes, R.; Scherer, H. U.; Catrina, A. I.; Klareskog, L.; Jurdic, P.; Schett, G., Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. *J Clin Invest* **2012,** *122* (5), 1791-802.
49. Tan, E. M.; Smolen, J. S., Historical observations contributing insights on etiopathogenesis of rheumatoid arthritis and role of rheumatoid factor. *J Exp Med* **2016,** *213* (10), 1937-50.
50. Bugatti, S.; Vitolo, B.; Caporali, R.; Montecucco, C.; Manzo, A., B cells in rheumatoid arthritis: from pathogenic players to disease biomarkers. *Biomed Res Int* **2014,** *2014*, 681678.

51. Yeo, L.; Toellner, K. M.; Salmon, M.; Filer, A.; Buckley, C. D.; Raza, K.; Scheel-Toellner, D., Cytokine mRNA profiling identifies B cells as a major source of RANKL in rheumatoid arthritis. *Ann Rheum Dis* **2011,** *70* (11), 2022-8.
52. McInnes, I. B.; Schett, G., The pathogenesis of rheumatoid arthritis. *N Engl J Med* **2011,** *365* (23), 2205-19.
53. Koch, A. E.; Polverini, P. J.; Leibovich, S. J., Stimulation of neovascularization by human rheumatoid synovial tissue macrophages. *Arthritis Rheum* **1986,** *29* (4), 471-9.
54. Kurowska-Stolarska, M.; Alivernini, S., Synovial tissue macrophages: friend or foe? *RMD Open* **2017,** *3* (2), e000527.
55. Corsiero, E.; Pratesi, F.; Prediletto, E.; Bombardieri, M.; Migliorini, P., NETosis as Source of Autoantigens in Rheumatoid Arthritis. *Front Immunol* **2016,** *7*, 485.
56. Tran, C. N.; Davis, M. J.; Tesmer, L. A.; Endres, J. L.; Motyl, C. D.; Smuda, C.; Somers, E. C.; Chung, K. C.; Urquhart, A. G.; Lundy, S. K.; Kovats, S.; Fox, D. A., Presentation of arthritogenic peptide to antigen-specific T cells by fibroblast-like synoviocytes. *Arthritis Rheum* **2007,** *56* (5), 1497-506.
57. Muller-Ladner, U.; Kriegsmann, J.; Franklin, B. N.; Matsumoto, S.; Geiler, T.; Gay, R. E.; Gay, S., Synovial fibroblasts of patients with rheumatoid arthritis attach to and invade normal human cartilage when engrafted into SCID mice. *Am J Pathol* **1996,** *149* (5), 1607-15.
58. Filer, A.; Ward, L. S. C.; Kemble, S.; Davies, C. S.; Munir, H.; Rogers, R.; Raza, K.; Buckley, C. D.; Nash, G. B.; McGettrick, H. M., Identification of a transitional fibroblast function in very early rheumatoid arthritis. *Ann Rheum Dis* **2017**.
59. Frank-Bertoncelj, M.; Trenkmann, M.; Klein, K.; Karouzakis, E.; Rehrauer, H.; Bratus, A.; Kolling, C.; Armaka, M.; Filer, A.; Michel, B. A.; Gay, R. E.; Buckley, C. D.; Kollias, G.; Gay, S.; Ospelt, C., Epigenetically-driven anatomical diversity of synovial fibroblasts guides joint-specific fibroblast functions. *Nat Commun* **2017,** *8*, 14852.
60. Ospelt, C.; Gay, S.; Klein, K., Epigenetics in the pathogenesis of RA. *Semin Immunopathol* **2017,** *39* (4), 409-419.
61. Jones, D. S.; Jenney, A. P.; Swantek, J. L.; Burke, J. M.; Lauffenburger, D. A.; Sorger, P. K., Profiling drugs for rheumatoid arthritis that inhibit synovial fibroblast activation. *Nat Chem Biol* **2017,** *13* (1),.[38-45]
62. Mitchell, N. S.; Shepard, N., Changes in proteoglycan and collagen in cartilage in rheumatoid arthritis. *The Journal of bone and joint surgery. American volume* **1978,** *60* (3), 342-8.
63. Dodge, G. R.; Poole, A. R., Immunohistochemical detection and immunochemical analysis of type II collagen degradation in human normal, rheumatoid, and osteoarthritic articular cartilages and in explants of bovine articular cartilage cultured with interleukin 1. *J Clin Invest* **1989,** *83* (2), 647-61.

64. Otero, M.; Goldring, M. B., Cells of the synovium in rheumatoid arthritis. Chondrocytes. *Arthritis Res Ther* **2007,** *9* (5), 220.
65. Jung, S. M.; Kim, K. W.; Yang, C. W.; Park, S. H.; Ju, J. H., Cytokine-mediated bone destruction in rheumatoid arthritis. *Journal of immunology research* **2014,** *2014*, 263625.
66. Smolen, J. S.; Landewe, R.; Bijlsma, J.; Burmester, G.; Chatzidionysiou, K.; Dougados, M.; Nam, J.; Ramiro, S.; Voshaar, M.; van Vollenhoven, R.; Aletaha, D.; Aringer, M.; Boers, M.; Buckley, C. D.; Buttgereit, F.; Bykerk, V.; Cardiel, M.; Combe, B.; Cutolo, M.; van Eijk-Hustings, Y.; Emery, P.; Finckh, A.; Gabay, C.; Gomez-Reino, J.; Gossec, L.; Gottenberg, J. E.; Hazes, J. M. W.; Huizinga, T.; Jani, M.; Karateev, D.; Kouloumas, M.; Kvien, T.; Li, Z.; Mariette, X.; McInnes, I.; Mysler, E.; Nash, P.; Pavelka, K.; Poor, G.; Richez, C.; van Riel, P.; Rubbert-Roth, A.; Saag, K.; da Silva, J.; Stamm, T.; Takeuchi, T.; Westhovens, R.; de Wit, M.; van der Heijde, D., EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis* **2017,** *76* (6), 960-977.
67. Livshits, G.; Kalinkovich, A., Hierarchical, imbalanced pro-inflammatory cytokine networks govern the pathogenesis of chronic arthropathies. *Osteoarthritis Cartilage* **2018,** *26* (1), 7-17.
68. Burmester, G. R.; Pope, J. E., Novel treatment strategies in rheumatoid arthritis. *Lancet* **2017,** *389* (10086), 2338-2348.
69. Colin, E. M.; Asmawidjaja, P. S.; van Hamburg, J. P.; Mus, A. M.; van Driel, M.; Hazes, J. M.; van Leeuwen, J. P.; Lubberts, E., 1,25-dihydroxyvitamin D3 modulates Th17 polarization and interleukin-22 expression by memory T cells from patients with early rheumatoid arthritis. *Arthritis Rheum* **2010,** *62* (1), 132-42.
70. van Hamburg, J. P.; Asmawidjaja, P. S.; Davelaar, N.; Mus, A. M.; Cornelissen, F.; van Leeuwen, J. P.; Hazes, J. M.; Dolhain, R. J.; Bakx, P. A.; Colin, E. M.; Lubberts, E., TNF blockade requires 1,25(OH)2D3 to control human Th17-mediated synovial inflammation. *Ann Rheum Dis* **2012,** *71* (4), 606-12.
71. Lemire, J. M.; Archer, D. C., 1,25-dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. *J Clin Invest* **1991,** *87* (3), 1103-7.
72. Cantorna, M. T.; Hayes, C. E.; DeLuca, H. F., 1,25-Dihydroxycholecalciferol inhibits the progression of arthritis in murine models of human arthritis. *J Nutr* **1998,** *128* (1),.[68-72]
73. Cantorna, M. T.; Munsick, C.; Bemiss, C.; Mahon, B. D., 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. *J Nutr* **2000,** *130* (11), 2648-52.
74. Lin, J.; Liu, J.; Davies, M. L.; Chen, W., Serum Vitamin D Level and Rheumatoid Arthritis Disease Activity: Review and Meta-Analysis. *PLoS One* **2016,** *11* (1), e0146351.

75. Hiraki, L. T.; Arkema, E. V.; Cui, J.; Malspeis, S.; Costenbader, K. H.; Karlson, E. W., Circulating 25-hydroxyvitamin D level and risk of developing rheumatoid arthritis. *Rheumatology (Oxford)* **2014,** *53* (12), 2243-8.

Chapter 2

Vitamin D in Autoimmunity: Molecular Mechanisms and Therapeutic Potential

*Wendy Dankers[1,2], Edgar M. Colin[1,3], Jan Piet van Hamburg[1,2], and Erik Lubberts[1,2]**

Departments of [1]Rheumatology and [2]Immunology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands; [3]Department of Rheumatology, ZGT, Almelo, The Netherlands

Frontiers in Immunology 2017, 7:697

ABSTRACT

Over the last three decades it has become clear that the role of vitamin D goes beyond the regulation of calcium homeostasis and bone health. An important extra-skeletal effect of vitamin D is the modulation of the immune system. In the context of autoimmune diseases, this is illustrated by correlations of vitamin D status and genetic polymorphisms in the vitamin D receptor with the incidence and severity of the disease. These correlations warrant investigation into the potential use of vitamin D in the treatment of patients with autoimmune diseases. In recent years several clinical trials have been performed to investigate the therapeutic value of vitamin D in multiple sclerosis, rheumatoid arthritis, Crohn's disease, type I diabetes and systemic lupus erythematosus. Additionally, a second angle of investigation has focused on unraveling the molecular pathways used by vitamin D in order to find new potential therapeutic targets. This review will not only provide an overview of the clinical trials that have been performed, but also discuss the current knowledge about the molecular mechanisms underlying the immunomodulatory effects of vitamin D and how these advances can be used in the treatment of autoimmune diseases.

1 INTRODUCTION

Autoimmune diseases, including rheumatoid arthritis (RA), multiple sclerosis (MS) and Crohn's disease, result from an aberrant activation of the immune system whereby the immune response is directed against harmless self-antigens. This results in inflammation, tissue damage and loss of function of the affected organs or joints. With the increasing prevalence of autoimmunity in the Western countries [1], also the societal burden of these diseases increases. Although the treatment of autoimmune diseases has improved due to the development of so-called biologics like tumor necrosis factor alpha (TNFα) inhibitors, a large proportion of patients is still not adequately responding to these treatments.[2] Therefore it is still important to improve current therapies or to uncover new treatment options.

In this context, the immunomodulatory effects of vitamin D provide opportunities to enhance the treatment of autoimmune diseases. Firstly, given the high prevalence of vitamin D deficiency in patients suffering from autoimmunity, vitamin D supplementation might decrease disease severity or augment the therapeutic effect of current medication. Secondly, knowing the molecular mechanisms underlying the immunomodulatory effects could lead to the discovery of new potential therapeutic targets. Therefore, this review will explore the advances that have been made in both clinical trials and molecular studies. In addition, it will give an overview of the challenges that still remain before the immunomodulatory effects of vitamin D can be utilized in clinical practice.

2 VITAMIN D METABOLISM, SIGNALING AND FUNCTION

Vitamin D, or cholecalciferol, is a secosteroid hormone that can be obtained from dietary sources, but that is predominantly synthesized in the skin from 7-dehydroxycholesterol in response to UV light (figure 1). Cholecalciferol is bound by vitamin D binding protein (DBP) and transported to the liver. In the liver various cytochrome p450 (Cyp) vitamin D hydroxylases convert cholecalciferol into 25(OH)D_3. Cyp2R1 is considered to be the primary 25-hydroxylase responsible for this process. Subsequently DBP transports 25(OH)D_3 to the kidneys, where the 1α-hydroxylase Cyp27B1 converts 25(OH)D_3 into 1,25(OH)$_2D_3$. 1,25(OH)$_2D_3$, also called calcitriol, is the active vitamin D metabolite. To control calcitriol concentrations, the 24-hydroxylase Cyp24A1 hydroxylates 25(OH) D_3 or 1,25(OH)$_2D_3$ at C-24, yielding the less active metabolites 24,25(OH)$_2D_3$ and 1,24,25(OH)$_3D_3$, respectively.[3] The level of 1,25(OH)$_2D_3$ is therefore mainly determined by the balance between Cyp27B1 and Cyp24A1. Two proteins that are important for

regulating this balance are fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH). FGF23 shifts the balance towards Cyp24A1 and therefore inactivation of vitamin D signaling, and is induced by high concentrations of $1,25(OH)_2D_3$ and low serum phosphate. On the other hand, PTH favors the balance towards Cyp27B1 and activation of vitamin D signaling. PTH is inhibited by high concentrations of $1,25(OH)_2D_3$ and induced by low serum calcium (figure 1).[3]

$1,25(OH)_2D_3$ initiates its signaling cascade by binding to the vitamin D receptor (VDR), which is a nuclear receptor that acts as a transcription factor. VDR binds to vitamin D responsive elements (VDREs) in the DNA, mostly to so-called DR3-type VDREs that are characterized by two hexameric core binding motifs separated by 3 nucleotides. In the absence of ligand, VDR is mostly bound to non-DR3-type VDREs and is associated with co-repressor proteins. When $1,25(OH)_2D_3$ binds to VDR, this induces a conformational change leading to the formation of two new protein interaction surfaces. One is for binding with heterodimeric partners to facilitate specific DNA binding, such as retinoid X receptor (RXR), and the other is for recruitment of co-regulatory complexes that will exert the genomic effects of VDR.[4] Furthermore, there is a shift in binding to primarily DR3-type VDREs.[5] Interestingly, although RXR has multiple binding partners, specifically with VDR it will bind to the DR3-type elements. This indicates that the heterodimerization of VDR and RXR is important for functioning of the VDR.[6] However, research in colorectal cancer cells has shown that 25% of the VDR binding sites are not enriched for RXR.[7] No direct data on colocalization of VDR and RXR in immune cells has been reported, although Handel *et al.* found a significant overlap between VDR in $CD4^+$ T cells and RXR in a promyelocytic leukemia cell line.[8] Therefore it is currently unknown whether the rate of VDR/RXR colocalization differs between cell types. Also, the functional consequence of VDR binding with or without RXR remains to be understood.

The best known function of $1,25(OH)_2D_3$ is the maintenance of calcium homeostasis by facilitating the absorption of calcium in the intestine. However, in the presence of low $1,25(OH)_2D_3$ levels, calcium will be mobilized from the bone rather than the intestine. If these conditions are prolonged, this may lead to osteomalacia and rickets, both well-known clinical signs of vitamin D deficiency. An overview of the current knowledge on the role of vitamin D signaling in calcium homeostasis was recently given by Carmeliet *et al*. and will not be discussed here.[9] The first hint that vitamin D might also be important for extraskeletal health came from mycobacterial infections like tuberculosis, in which vitamin D was used as a treatment before antibiotics were discovered.[10] The discovery

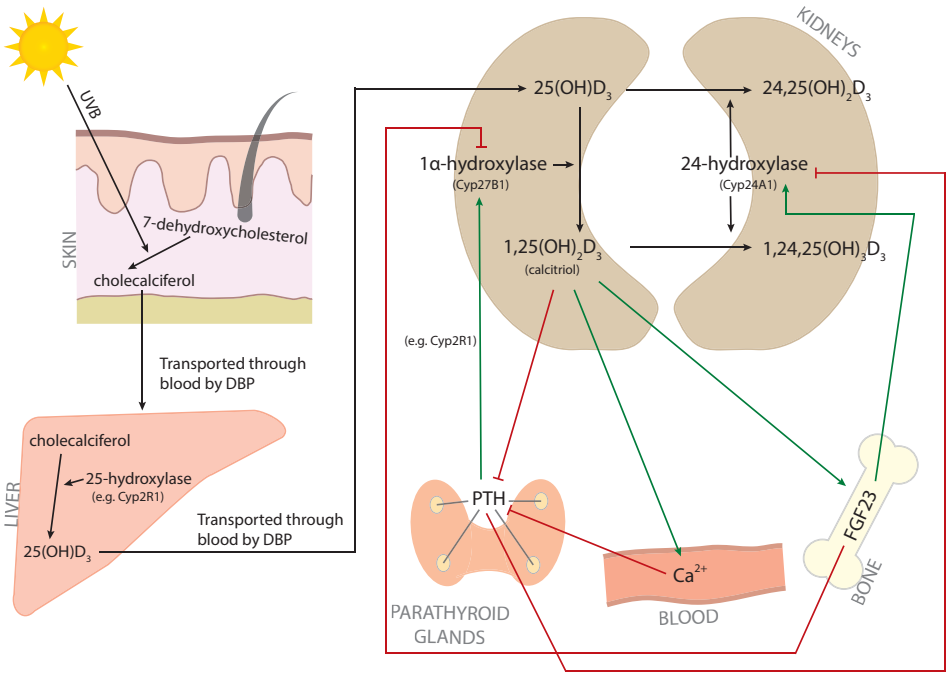


Figure 1. Vitamin D metabolism. The metabolic pathway of vitamin D. Red arrows indicate inhibition, green arrows indicate induction.

that the VDR is expressed in almost all human cells has further increased the attention for the extraskeletal effects of vitamin D. As a result, vitamin D deficiency has now not only been linked to bone health, but also for example cancer, cardiovascular diseases and autoimmune diseases.[9]

3 VITAMIN D AND AUTOIMMUNE DISEASES

Since the discovery of the VDR on blood lymphocytes [11, 12], the effects of vitamin D on the immune system and immune-related diseases became the subject of a large number of studies. In this context, it was discovered that supplementation with $1,25(OH)_2D_3$ could prevent both the initiation and progression of experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis (CIA), experimental models of MS and RA, respectively.[13-15] In addition, VDR deficiency aggravated arthritis severity in human TNFα transgenic mice.[16] Similarly, vitamin D deficiency increased enterocolitis severity in IL-10 knock-out (KO) mice, which are used as a model system for inflammatory bowel diseases

(IBD). Treatment with $1,25(OH)_2D_3$ decreased disease symptoms in both the IL-10 KO mice and in the dextran sulfate sodium (DSS)-induced colitis model.[17, 18] Finally, treatment with $1,25(OH)_2D_3$ reduced the incidence of diabetes in non-obese diabetic (NOD) mice [19, 20] and the severity of systemic lupus erythematosus in MRL/1 mice.[21]

These studies in experimental autoimmune models underscore the need to examine whether there is a protective role for vitamin D in human autoimmune diseases. In the last decades numerous studies have investigated the link between vitamin D and the incidence and severity of autoimmune diseases. One of the first indications was the correlation between increasing MS prevalence and increasing latitude, and consequently with decreasing sunlight exposure. Exceptions to this gradient can at least partially be explained by genetic variants (like the HLA-DRB1 allele) or lifestyle differences, such as high fish consumption.[22] The relation between latitude and disease prevalence was also found for other autoimmune diseases such as type I diabetes mellitus (T1D) and IBD.[23, 24] Further strengthening the link between sun exposure and autoimmunity is the finding that the risk of developing MS is correlated with the month of birth, with for the northern hemisphere a higher risk in April and a lower risk in October and November.[25, 26] Importantly, this correlation can only be found in areas where the UV exposure changes during the year.[25]

Next to UV exposure, vitamin D can also be obtained from dietary sources and supplements. A meta-analysis by Song *et al.* found that the incidence of RA is inversely correlated with vitamin D intake, both when considering dietary intake and supplements or supplements alone.[27] In addition, vitamin D supplementation in early childhood might reduce the risk of developing T1D up to 30% depending on the supplementation frequency.[28, 29] Also the effect of maternal vitamin D intake on the risk of T1D in the offspring has been investigated, but due to the limited amount of studies there is currently not sufficient evidence to prove a correlation.[29]

Investigating the correlation between vitamin D intake and prevalence of autoimmunity is challenging because the measurements of dietary intake and UV exposure are often based on estimations. Therefore, it might be more useful to analyze the correlation between the serum $25(OH)D_3$ level and autoimmunity. Indeed, in many autoimmune diseases patients have a lower serum $25(OH)D_3$ than healthy controls.[30-36] In addition, patients with a lower $25(OH)D_3$ level are implicated to have higher disease activity.[32, 35, 37] Although it is not clear whether the lower $25(OH)D_3$ level also increases the risk of autoimmunity, the study by

Hiraki *et al.* suggests there is a strong correlation between the risk of developing RA and the $25(OH)D_3$ level between 3 months and 4 years before diagnosis.[38] It should be noted that all these studies merely demonstrate correlations, so it is still under debate whether the low $25(OH)D_3$ level is the cause or the result of the autoimmune disease.

Another line of evidence that indicates a role for vitamin D in human autoimmunity is the correlation with polymorphisms in the VDR. There are four well-known VDR polymorphisms that have been extensively studied for their potential role in autoimmunity: ApaI, BsmI, TaqI and FokI. All of these polymorphisms have been associated with the risk of developing an autoimmune disease, although it differs between diseases and polymorphisms whether it is protective or a risk factor. Also, ethnicity plays a role in the correlation between the polymorphisms and autoimmune diseases.[39-47]

In summary, autoimmune diseases are correlated with $25(OH)D_3$ serum levels, vitamin D intake, UV exposure and VDR polymorphisms. Furthermore, $1,25(OH)_2D_3$ suppresses disease in experimental autoimmune models. Although these data do not prove a causal relationship between vitamin D and autoimmune diseases, they warrant further investigation into whether at-risk individuals and patients could benefit from vitamin D supplementation.

4 VITAMIN D AS A THERAPEUTIC AGENT IN HUMAN AUTOIMMUNE DISEASES

Despite the beneficial effects of $1,25(OH)_2D_3$ supplementation in experimental autoimmune models, the application of vitamin D derivatives in clinical practice is currently limited to topical use for the treatment of psoriasis.[48] The systemic use of vitamin D in the treatment of other autoimmune diseases is still under investigation. Table 1 gives an overview of the placebo-controlled clinical trials investigating the effect of vitamin D supplementation in autoimmune diseases other than psoriasis. Here we discuss these trials and what this means for the therapeutic potential of vitamin D in each of these autoimmune diseases.

4.1 Multiple Sclerosis (MS)

In the field of MS, several trials have been performed in which cholecalciferol was given to the patients, but the results are contradictory. Beneficial effects of cholecalciferol supplementation that have been reported include decrease in expanded disability status scale (EDSS), decrease in MRI lesions, increased functionality and reduced relapse

rates.[49, 50] Importantly, cholecalciferol has an added effect when used as a supplement to interferon β (IFNβ) treatment.[50] On the other hand, two other trials reported no difference in any of these parameters.[51, 52] Vitamin D supplementation might also be important in the pre-MS stage, since cholecalciferol supplementation decreased the conversion rate of optic neuritis to chronic MS.[53]

Due to the small sample size (no more than 35 patients per group) of these trials, it is difficult to draw conclusions from these data. Although the effect of cholecalciferol on conversion to chronic effect appears promising, this was only one study with 13 treated patients and 11 placebo controls. Therefore, more research is necessary to determine whether therapy with cholecalciferol is beneficial for MS patients.

4.2 Rheumatoid Arthritis (RA)

Despite the beneficial effect of $1,25(OH)_2D_3$ supplementation on experimental arthritis.[15] there are to date only three randomized trials investigating the effect of supplementation on disease activity in rheumatoid arthritis. Although the studies performed by Salesi *et al.* and Dehghan *et al.* suggest a beneficial effect on disease activity and relapse rate respectively, neither results reach statistical significance.[54, 55] However, Dehghan *et al.* point out that for every ten patients treated with cholecalciferol, relapse would be prevented in one patient. Considering the costs and safety profile of cholecalciferol supplementation, this might be worth following up. Ergocalciferol, the less potent fungal equivalent of human cholecalciferol, had no effect on disease activity and was associated with worse patient-related health assessments.[56] Similarly to studies in MS, the major limitation in the three RA studies is the group size, which limits the power of the analyses. Therefore no definitive conclusion can be drawn yet whether vitamin D can be used as a therapeutic agent in RA.

4.3 Crohn's Disease (CD)

Crohn's disease (CD) is a subtype of the inflammatory bowel diseases and investigated intensively for the effect of vitamin D supplementation. However, the difficulty with this disease is that the intestinal inflammation may lead to decreased absorption of the supplemented vitamin D. Nevertheless, for adult patients cholecalciferol supplementation might reduce the risk of relapses, although the difference does not reach statistical significance ($p = 0.06$).[57] Correspondingly, cholecalciferol prevented further increase of intestinal permeability, which may be an early marker of relapse.[58] This is even more pronounced when the patients are stratified based on their serum $25(OH)D_3$ level.

Additionally, patients with a serum level above 75 nmol/L have significantly lower serum levels of C-reactive protein (CRP, a marker of inflammation) and a non-significant decrease in disease activity as measured with Crohn's Disease Activity Index.[58] These studies used 1,200-2,000 IU cholecalciferol daily in adults, but in children there is no difference in disease activity between supplementing 400 and 2,000 IU daily despite a serum $25(OH)D_3$ level that is 25 nmol/L higher in the latter group.[59]

When compared to RA and MS, the results for adult CD are more consistently showing a beneficial effect of cholecalciferol treatment. Since group sizes are again small, more research is required to confirm these data.

4.4 Type I Diabetes Mellitus (T1D)

In contrast to the other autoimmune diseases where cholecalciferol supplementation is investigated, in T1D almost all trials use $1,25(OH)_2D_3$ or an analogue. Both forms appear to delay, but not prevent, the progression of β cell destruction in three studies.[60-62] On the other hand, no effect of $1,25(OH)_2D_3$ on T1D was observed in studies performed by Bizzarri *et al.* and Walter *et al.*.[63, 64] This lack of effect could be due to the low level of remaining β cell function at the start of the study, suggesting that the therapeutic window for vitamin D supplementation is in the earliest phases of the disease. The study by Li *et al.* found that the protective effect is only visible when the disease duration was less than one year, supporting this hypothesis.[62] In T1D the beneficial effects of $1,25(OH)_2D_3$ may lie more in the prevention of disease onset[28, 29] than in treatment of disease, since the destruction of β cells cannot be reversed.

4.5 Systemic Lupus Erythematosus (SLE)

Vitamin D supplementation in SLE might even be more relevant than in the other autoimmune diseases, since 80% of the patients is sensitive for sunlight and therefore protect themselves against UV exposure.[65] Two studies supplementing either 2,000 IU daily or 50,000 IU weekly demonstrate decreasing disease activity score, auto-antibody levels and fatigue.[66, 67] Conversely, the type I interferon (IFN) signature was unchanged after 12 weeks of 2,000 or 4,000 IU cholecalciferol in another study.[68] Since this study was performed in patients with inactive disease, had a short supplementation period and the signature was based on the expression of only three genes, it remains to be determined whether cholecalciferol supplementation truly does not affect the complete IFN signature in patients with active disease.

Table 1. Overview of randomized controlled trials with vitamin D supplementation in autoimmune diseases. ASA 5-aminosalicylzuur (sulfasalazine); CDAI Crohn's disease activity index; CQ Chloroquine; CRP C-reactive protein; ECLAM European consensus lupus activity measurement; EDSS Expanded disability status scale; ESR Erythrocyte sedimentation rate; FCP Fasting c-peptide; Gd Gadolinium; HAQ Health assessment questionnaire; HCQ Hydroxychloroquine; IU International Units; LADA Latent autoimmune diabetes in adults; MTX Methotrexate; PCP C-peptide after 75g glucose; QoL Quality of life; RCT Randomized controlled trial; RRMS Relapsing-remitting multiple sclerosis; SLEDAI Systemic lupus erythematosus disease activity index; DAS28 Disease activity score for 28 joints; VAS Visual analogue scale.

Trial	Disease	Trial design	Inclusion criteria	Groups	Supplementation dosage	Supplemental calcium	Other medication	Baseline 25(OH)D_3 in treated group (nmol/L)	Endpoint 25(OH)D_3 in treated group (nmol/L)	Main clinical findings
Burton 2010 (49)	MS	Open-label RCT, 52 weeks.	MS without a relapse within 60 days. EDSS 0-6.5. Serum 25(OH)D_3 < 150 nmol/L.	N=25 cholecalciferol, N=24 placebo	Dose escalation: up to 280.000 IU per week in 23 weeks, stay 6 weeks, then reduce to 0 in 20 weeks, then 3 weeks without	1200 mg daily	Continuation of MS medication, placebo-treated patients could take up to 4000IU cholecalciferol and supplemental calcium if desired. In case of relapse patients received steroids as judged by the treating physician	80	Up to 400 nmol/L after the peak of dosage, 200 nmol/L at the end of the trial	Lower proportion of patients with an increase in EDSS at the end of the trial. Trend towards reduced relapse rate.
Mosayebi 2011 (52)	MS	Double-blind RCT, 6 months (October-March).	MS with a relapse in the last year. More than 3 lesions on MRI. EDSS 0-3.5.	N=28 cholecalciferol, N=34 placebo	300.000 IU monthly (intramuscular)	No	IFNB-1a	25	150	No effect on EDSS. No effect on Gd-enhancing lesions.

Trial	Disease	Trial design	Inclusion criteria	Groups	Supplementation dosage	Supplemental calcium	Other medication	Baseline $25(OH)D_3$ in treated group (nmol/L)	Endpoint $25(OH)D_3$ in treated group (nmol/L)	Main clinical findings
Soilu-Hänninen 2012 (50)	MS	Double-blind RCT, 12 months.	RRMS with at least 1 month IFNB-1b treatment. Serum $25(OH)D_3$ < 85nmol/L.	N=34 cholecalciferol, N=32 placebo	20.000 IU weekly	No	IFNB-1b	54	110	Reduced number of Gd-enhancing lesions, but no effect on other MRI parameters. Trend towards reduced EDSS.
Kampman 2012 (51)	MS	Double-blind RCT, 96 weeks.	MS with an EDSS<4.5.	N=35 cholecalciferol, N=33 placebo	20.000 IU weekly	500 mg daily	46% of patients in both groups were treated with IFNβ, 3% with glatiramer acetate and 3% in the placebo group with natalizumab	55	123	No effects on EDSS, relapse rate, function or fatigue.
Derakhshandi 2013 (53)	MS	Double-blind pilot RCT, 12 months.	Optic neuritis patients without MS.	N=13 cholecalciferol, N=11 placebo	50.000 IU weekly, when reaching serum $25(OH)D_3$ of 250 nmol/L switch to a maintenance dose	No	3x 1g methylprednisolone per day i.v., then oral prednisolon	38	Unknown	Decreased incidence-rate ratio of demyelinating plaques. Reduced risk of progression to MS.

Trial	Disease	Trial design	Inclusion criteria	Groups	Supplementation dosage	Supplemental calcium	Other medication	Baseline $25(OH)D_3$ in treated group (nmol/L)	Endpoint $25(OH)D_3$ in treated group (nmol/L)	Main clinical findings
Salesi 2012 (54)	RA	Double-blind RCT, 12 weeks.	RA with DAS28>3.2. At least 24 weeks MTX treatment.	N=50 $25(OH)D_3$, N=48 placebo	50.000 IU weekly	No	MTX Prednison, HCQ and CQ were allowed	107	125	Modest, non-significant, improvement in tender joint count, swollen joint count, ESR and VAS.
Dehghan 2014 (55)	RA	Double-blind RCT, 6 months.	RA in remission for at least 2 months. Serum $25(OH)D_3$ <75 nmol/L	N=40 cholecalciferol, N=40 placebo	50.000 IU weekly	No	Prednison, MTX and HCQ allowed	<75	Unknown	Non-significant decrease in relapse rate.
Hansen 2014 (56)	RA	Double-blind RCT 12 months.	RA. Serum $25(OH)D_3$ between 15,25 and 62,25 nmol/L	N=11 cholecalciferol, N=11 placebo	4 weeks: 50.000 IU 3x weekly; 11 months: 50.000 IU 2x monthly; when serum was below 62,5 nmol/L: 50.000IU weekly for 8 weeks	500 mg 3x daily	SPF65	63	75 (after two months)	No effects on DAS28, HAQ or physician global assessment of RA. Non-significant increase in pain. Increased patient assessment of global health and patient global assessment of RA.

Trial	Disease	Trial design	Inclusion criteria	Groups	Supplementation dosage	Supplemental calcium	Other medication	Baseline $25(OH)D_3$ in treated group (nmol/L)	Endpoint $25(OH)D_3$ in treated group (nmol/L)	Main clinical findings
Jørgensen 2010 (57)	CD	Double-blind RCT, 1 year.	Crohn's disease in remission (CDAI<150) for at least 4 weeks.	N=46 cholecalciferol, N=48 placebo	1200 IU daily	1200 mg daily	Azothioprine (39-44% of participants)	70	95	Trend towards reduced relapse (hazard ratio of 0.44)
Wingate 2014 (59)	CD	Double-blind RCT, 6 months.	Children with quiescent Crohn's disease	N=35 2000 IU cholecalciferol, N=34 400 IU cholecalciferol	400 IU or 2000 IU daily depending on randomization	No	Multivitamins (without vitamin D). Normal IBD medication (36% 5-ASA, 57% immunomodulator, 30% biologics)	63	70 (400IU) or 86 (2000IU)	No difference between the groups in CDAI, ESR or CRP.
Raftery 2015 (58)	CD	Double-blind RCT, 3 months.	Adults with CD in remision (CDAI<150) and stable therapy for 3 months.	N=13 cholecalciferol, N=14 placebo	2000 IU daily	Only when already on it for bone health	Normal IBD medication (51% 5-ASA, 67% immunomodulator, 7% anti-TNFα).	70	90	Intestinal permeability was stable in the treated group, but increased in the placebo group. Reduced CRP, increased QoL and trend towards decreased CDAI in patients with serum $25(OH)D_3$ > 75 nmol/L.

Trial	Disease	Trial design	Inclusion criteria	Groups	Supplementation dosage	Supplemental calcium	Other medication	Baseline $25(OH)D_3$ in treated group (nmol/L)	Endpoint $25(OH)D_3$ in treated group (nmol/L)	Main clinical findings
Li 2009 (62)	T1D	Prospective RCT, 12 months.	LADA patients with diagnosis < 5 years	N=17 alfacalcidol, N=18 unsupplemented	0,25 µg twice daily	No	Insulin therapy in both groups	63	Unknown	Stable FCP while decline in control group, same trend for PCP. Especially pronounced when disease duration < 1 year.
Bizzarri 2010 (64)	T1D	Double-blind RCT, 24 months.	Recent-onset T1D	N=15 calcitriol, N=12 placebo	0,25 µg daily	No	Insulin therapy in both groups	<50	+ 3.9%	After 12 months the decline is FCP is slower in treated group, but not anymore after 24 months.
Walter 2010 (63)	T1D	Double-blind RCT, 18 months.	Adults with recent-onset T1D	N=20 calcitriol, N=18 placebo	0,25 µg daily	No	Insulin therapy in both groups	25 pg/ml (1,25OHD3)	30 pg/ml (1,25OHD3)	No changes in C-peptide or insulin dose.
Gabbay 2012 (61)	T1D	Double-blind RCT, 18 months.	Patients with recent onset T1D (age > 7). PCP > 0,06 ng/mL.	N=17 cholecalciferol, N=19 placebo	2000 IU daily	No	Insulin therapy in both groups	65	150	Decreased progression to undetectable C-peptide. Enhanced stimulated C-peptide after 12 months. Decreased decay of stimulated C-peptide after 18 months.

Trial	Disease	Trial design	Inclusion criteria	Groups	Supplementation dosage	Supplemental calcium	Other medication	Baseline $25(OH)D_3$ in treated group (nmol/L)	Endpoint $25(OH)D_3$ in treated group (nmol/L)	Main clinical findings
Ataie-Jafari 2013 (60)	T1D	Single-blind RCT, 6 months.	Patients with recent onset T1D	N=29 alfacalcidol, N=25 placebo	0,25 μg once daily, or twice if blood calcium levels allowed it	No	Insulin therapy in both groups	32.5	Unknown	Better preservation of C-peptide and lower insulin dose. Stronger effect in males than in females.
Abou-Raya 2013 (66)	SLE	Double-blind RCT, 12 months.	SLE with SLEDAI>1. Serum $25(OH)D_3$ <75 nmol/L.	N=158 cholecalciferol, N=89 placebo	2000 IU daily	Yes, unknown dose	6% corticosteroids, 80% antimalarials, 26% AZA, 27% ACE inhibitors/ ARB	50	98	Decrease in SLEDAI and ESR.
Lima 2014 (67)	SLE	Double-blind RCT, 24 weeks.	Juvenile onset SLE SLEDAI<12	N=20 cholecalciferol, N=20 placebo	50.000 IU weekly	No	Unknown, but stable during trial	50	78	Decrease in SLEDAI, trend to decrease in ECLAM and decrease of fatigue related to social life.
Aranow 2015 (68)	SLE	Double-blind RCT, 12 weeks.	Adult SLE with IFNa signature. Stable inactive disease. Anti-dsDNA positive. Serum $25(OH)D_3$ <50 nmol/L.	N=18 4000 IU cholecalciferol, N=17 2000 IU cholecalciferol N=19 placebo	2000 IU or 4000 IU daily	No	Unknown	28	75	No difference in IFN signature (based on 3 genes) or disease activity.

SLE is the only autoimmune disease is which a larger study was done, with 158 cholecalciferol-treated patients and 89 placebo controls.[66] The promising results in this clinical trial await further confirmation before vitamin D can be used therapeutically in these patients.

5 IMMUNE MODULATION BY VITAMIN D

In addition to exploring the potential of therapeutic vitamin D supplementation, there has been a great deal of research towards the working mechanisms of $1,25(OH)_2D_3$ in cells of the immune system. Since autoimmune diseases are characterized by an over-active immune response, it seems logical that the beneficial effects of vitamin D on autoimmunity are due to effects on the immune system. Furthermore, virtually all immune cells express the VDR, making them susceptible to $1,25(OH)_2D_3$-mediated modulation.[11, 12, 69, 70] Various immune cells, including monocytes, dendritic cells, macrophages, B cells and T cells, also have the capability to convert $25(OH)D_3$ into $1,25(OH)_2D_3$.[71-78] This allows for local regulation of the concentration of $1,25(OH)_2D_3$ at the site of inflammation and illustrates an important role for the cells of the immune system in the systemic effects of vitamin D.

Therefore, insight into how $1,25(OH)_2D_3$ modulates the immune system could uncover new therapeutic targets in autoimmune diseases. Here we discuss the effects of vitamin D on various cell types involved in the immune response, the current knowledge about the underlying mechanisms and what this means for the therapeutic potential of vitamin D in autoimmunity (figure 2).

5.1 Dendritic cells

Dendritic cells (DCs) are antigen-presenting cells (APCs), which means that their main function is to take up foreign antigens and present them as peptides to T cells on the human leukocyte antigen (HLA) molecules. DCs are predominantly found in an immature state in peripheral tissues such as the skin, gut and lungs, where they probe the surroundings for potential pathogens. Upon encountering a foreign antigen, they mature and migrate to the lymphoid tissues to stimulate antigen-specific T cells. Depending on the cytokines secreted by the DC, the T cell will differentiate into an effector cell with appropriate pro- or anti-inflammatory properties. Through these actions APCs are crucial in initiating effective adaptive immune responses against pathogens, but also for maintaining self-tolerance and immune homeostasis.

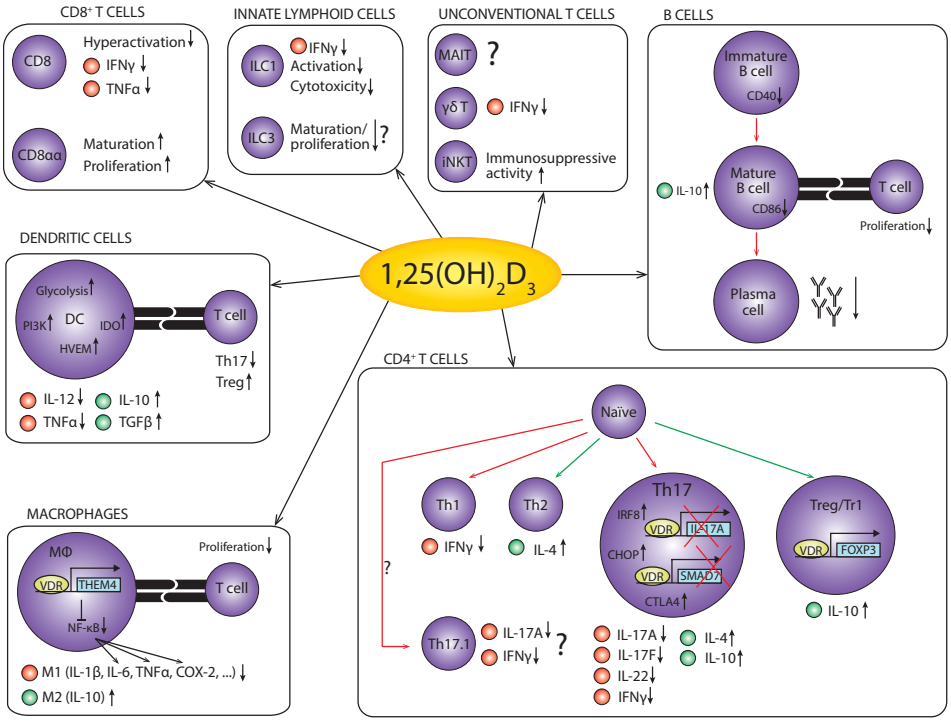


Figure 2. The anti-inflammatory effects of $1,25(OH)_2D_3$ on cells of the immune system. An overview of the anti-inflammatory effects of $1,25(OH)_2D_3$ on the cells of the immune system in autoimmunity. Red dots represent pro-inflammatory cytokines, while green dots represent anti-inflammatory cytokines. Red arrows indicate decreased differentiation, green arrows indicate increased differentiation. References: $CD8^+$ T cells [201, 202, 204]; ILC [203, 211-213, 220]; Unconventional T cells [144, 161, 208]; B cells [75, 133-136, 138, 139, 143]; DC [85-87, 91, 93-95]; Macrophages [115, 125-128]; $CD4^+$ T cells [141, 150, 155, 159, 163-167, 177-182, 184, 194].

The important role of DCs in autoimmune pathogenesis is illustrated in experimental autoimmune models, where deletion of specific DC subtypes ameliorates, or even prevents, disease onset.[79-82] In addition, APCs, including DCs but also macrophages and B cells, are associated with human autoimmunity through the correlation between specific HLA alleles and the risk of developing an autoimmune disease. For example, HLA-DRB1*15:01 is associated with an increased risk for MS.[83] while HLA-DRB1*04:01 confers a greater susceptibility to RA.[84]

DCs differentiated *in vitro* from monocytes or bone marrow cells in the presence of $1,25(OH)_2D_3$ will remain in an immature-like tolerogenic state. This is characterized by decreased production of pro-inflammatory factors like IL-12 and TNFα and increased

anti-inflammatory IL-10 production. These tolerogenic DCs are less capable of promoting proliferation and cytokine production of pro-inflammatory T cells, while they induce the differentiation of T regulatory (Treg) cells.[85-87] Furthermore, they specifically induce apoptosis in autoreactive T cells, while not affecting proliferation of other T cells.[88] Of note, $1,25(OH)_2D_3$ can only induce this tolerogenic phenotype in DCs when it is added before their maturation. Once a maturation stimulus like lipopolysaccharide (LPS) is present or when the cells have already matured, the effects of $1,25(OH)_2D_3$ on DCs are minimal.[89] Aside from *in vitro* differentiated DCs, $1,25(OH)_2D_3$ also induces a tolerogenic phenotype in dermal DCs, Langerhans cells and plasmacytoid DCs, even though there are subtle differences between the effects on these subsets.[90-92]

While the tolerizing effects of $1,25(OH)_2D_3$ on DCs are well described, the underlying mechanisms are less clear. Recently, Ferreira *et al.* suggested that a metabolic switch towards glycolysis and activation of the PI3K-Akt-mTOR pathway are the first steps for the generation of tolerogenic DCs by $1,25(OH)_2D_3$.[93] Also the induction of indoleamine 2,3-dioxygenase (IDO) on DCs has been reported to be essential for the induction of a tolerogenic DC (tDC) phenotype and thereby for the beneficial effect of $1,25(OH)_2D_3$ on EAE.[94] Although all tDCs promote regulatory T cells (Tregs), the mechanism by which they do this depends on the type of DC. While tDC derived *in vitro* from bone marrow cells promote Tregs via induction of herpesvirus entry mediator (HVEM), tolerized Langerhans cells use TGFβ for this.[91, 95] Dermal DCs induce the differentiation of T regulatory 1 (Tr1) cells, another type of regulatory T cell, via IL-10.[91] So in recent years advances have been made to fully understand how $1,25(OH)_2D_3$ modulates DCs, but the picture is not yet complete.

Despite the incomplete understanding of the molecular mechanism behind the effects of $1,25(OH)_2D_3$ on DCs, tDCs generated with $1,25(OH)_2D_3$ alone or in combination with dexamethasone are considered for therapy in autoimmune diseases.[96] Their persistent tolerogenic state and the possibility to pulse them with tissue-specific antigens has made them valuable candidates to treat various diseases, including autoimmune diseases.[87, 88, 97] This is illustrated in experimental disease models for T1D, MS and RA, where administered antigen-specific tDCs migrate to inflammatory sites and reduce disease activity upon administration.[94, 98-100] Importantly, DCs with an increased activation status from patients with autoimmune diseases can become equally tolerogenic in response to $1,25(OH)_2D_3$ as healthy DCs.[101-105] Because they can also be pulsed with auto-antigens and they can be generated under current Good Manufacturing Practice (cGMP) conditions, this

opens up the way for the use of autologous tDCs in the treatment of human autoimmune diseases.[101, 106] Currently the use of tDCs generated with $1,25(OH)_2D_3$ has not been clinically tested. However, tDCs generated using antisense oligonucleotides or Bay11-7082 were found to be safe upon administration in patients with T1D or RA, respectively.[107, 108]

It remains to be determined whether these tDCs also have effects on disease activity and whether tDCs generated using $1,25(OH)_2D_3$ could also be used in this context. Increased understanding on how $1,25(OH)_2D_3$, with or without dexamethasone, modulates the DCs can provide insights in how to further optimize the tolerogenic potential of the DCs.

5.2 Macrophages

Macrophages are known for their supreme phagocytic capacities, but they are also important APCs. In a normal immune response, an infection activates tissue-resident macrophages after which they produce inflammatory mediators and recruit other immune cells to eradicate the pathogen. Macrophages can roughly be divided in two categories: the M1 and M2 macrophages. M1 macrophages produce pro-inflammatory mediators like nitric oxide, TNFα, IL-23, IL-12 and IL-1β, whereby they kill pathogens and promote the polarization of T helper cells to Th1 and Th17 cells to assist in the immune response. On the other hand, M2 macrophages produce the anti-inflammatory cytokine IL-10 and are important in wound repair and restoring tissue homeostasis.[109]

The role of macrophages in the pathogenesis in autoimmune diseases is illustrated by an increase in macrophages at inflammatory sites.[110-113] In addition, macrophages are hyper-activated and produce more pro-inflammatory cytokines, suggesting a dysregulated balance between M1 and M2 cells.[111, 114, 115] As a result of their hyper-inflammatory state, they are essential for the development and activation of β-cell specific cytotoxic T cells which leads to insulitis in NOD mice.[116] Interestingly, the suppression of EAE by $1,25(OH)_2D_3$ is preceded by a rapid reduction of macrophages in the CNS. This suggests that macrophages are another important target for vitamin D in the suppression of autoimmunity.[117]

Notably, $1,25(OH)_2D_3$ has dual roles in macrophage differentiation and activation. In the early stages of infection, $1,25(OH)_2D_3$ stimulates differentiation of monocytes into macrophages.[118] Furthermore, toll-like receptor (TLR) triggering or IFNγ-induced activation activates Cyp27B1 and thereby potentiates the conversion of $25(OH)D_3$ into $1,25(OH)_2D_3$.[119, 120] $1,25(OH)_2D_3$ obtained via this pathway is then required for producing

cathelicidin and for the antimicrobial activity of human monocytes and macrophages.[121, 122] In addition, $1,25(OH)_2D_3$ induces IL-1β, either directly or via upregulation of C/EBPβ or Erk1/2.[123, 124] So initially, $1,25(OH)_2D_3$ is essential for effective pathogen clearance.

The hyper-responsiveness of $VDR^{-/-}$ mice to LPS stimulation indicates that in the later stages of infection, $1,25(OH)_2D_3$ plays a role in the contraction of the immune response.[125] The anti-inflammatory effect of $1,25(OH)_2D_3$ on macrophages is characterized by decreased production of pro-inflammatory factors like IL-1β, IL-6, TNFα, RANKL, COX-2 and nitric oxide and increased anti-inflammatory IL-10.[115, 125-128] These changes suggest that $1,25(OH)_2D_3$ promotes the M2 phenotype while inhibiting the M1 phenotype, thereby restoring the balance between these subsets. Finally, $1,25(OH)_2D_3$-treated macrophages have reduced T cell stimulatory capacity.[128]

In recent years some advances were made with unraveling the mechanism behind this anti-inflammatory effect of $1,25(OH)_2D_3$ on macrophages. An important target of $1,25(OH)_2D_3$ is thioesterase superfamily member 4 (THEM4), an inhibitor of the NFκB signaling pathway. THEM4 inhibits the direct binding of NFκB to the COX-2 locus and thereby prevents COX-2 transcription.[126] Furthermore, THEM4 inhibits IL-6 and TNFα expression by preventing the signaling cascade in which NFκB induces miR-155 to suppress SOCS .[125] Whether this THEM4-dependent pathway also inhibits the other pro-inflammatory mediators is not yet clear.[115]

The balancing effect of $1,25(OH)_2D_3$ between the pro- and anti-inflammatory status of macrophages is of particular interest in the treatment of autoimmune diseases. Currently, many inflammatory mediators secreted by M1 macrophages, like IL-1β, COX-2, IL-6 and especially TNFα, are already successful therapeutic targets in various autoimmune diseases. However, since current therapies result in systemic reduction of these mediators, patients may become prone to infections. Therefore it is of interest to understand the mechanism by which $1,25(OH)_2D_3$ balances between pro- and anti-inflammatory actions. This may provide insights in how to suppress the pro-inflammatory cytokines only in case of hyper-activation, without affecting the normal immune response.

5.3 B cells

B cells are mostly known for their crucial role in the immune response via the differentiation towards plasma cells and the production of antibodies. However, they also modulate the immune response via antigen presentation and cytokine secretion. In

the context of autoimmunity, B cells play a crucial role by the production of autoreactive antibodies. These auto-antibodies, like anti-nuclear antibodies (ANA) in SLE and anti-citrullinated peptide antibodies (ACPA) in RA, can be found in >95% and 70% of patients, respectively.[129, 130]

Interestingly, the VDR binds to the promoter region of genes involved in the immune system in lymphoblastoid B cell lines, suggesting a role for B cells in the effect of vitamin D on autoimmune diseases.[131] Here we discuss what is known about the direct effects of $1,25(OH)_2D_3$ on B cell differentiation and the three B cell functions of antibody production, cytokine secretion and antigen presentation.

Before B cells become plasma cells that secrete high-affinity antibodies, they have to go through various stages of differentiation, class-switch recombination and somatic hypermutation.[132] Various reports indicate that $1,25(OH)_2D_3$ reduces the proliferation of B cells, induces their apoptosis and inhibits immunoglobulin class switching.[133-135] This inhibition of differentiation may involve preventing nuclear translocation of NF-κB p65 and thereby inhibiting the signaling pathway downstream of CD40 costimulation.[136] On the other hand, $1,25(OH)_2D_3$ stimulates plasma cell development when added to terminally differentiating B cells. Furthermore, it induces the chemokine receptor CCR10 on these plasma cells, promoting their migration towards mucosal sites of inflammation.[137] Therefore, it appears that the effect of $1,25(OH)_2D_3$ depends on the activation and differentiation status of the B cells.

Independent of the effect of $1,25(OH)_2D_3$ on B cell differentiation, there is ample evidence that it decreases the antibody production.[133-135, 138, 139] Interestingly, the presence of ANA is correlated with a lower serum $25(OH)D_3$ level even in healthy people without SLE,[140] while cholecalciferol supplementation decreases auto-antibody titers.[66, 141]

Next to antibody production, B cells also secrete cytokines to influence the inflammatory milieu. Interestingly, VDR binds directly to the promoter region of IL-10 in B cells, thereby inducing the expression of IL-10.[75] However, in a cohort of healthy controls and relapsing-remitting MS patients there was no correlation between IL-10 producing B cells and serum $25(OH)D_3$ levels.[142]

There has been limited research towards the effect of $1,25(OH)_2D_3$ on the APC function of B cells. However one study suggests that B cells primed with $1,25(OH)_2D_3$ have decreased

CD86 surface expression. Thereby, these B cells are less potent stimulators of naïve T cell proliferation and cytokine production.[143]

Altogether, the effect of $1,25(OH)_2D_3$ on B cells is still not completely clear. Currently it is hypothesized that $1,25(OH)_2D_3$ inhibits the pathogenic function of B cells in autoimmunity by preventing plasma cell differentiation and thereby auto-antibody production, by inducing IL-10 production and by inhibiting the antigen presentation capabilities. However, the limited amount of studies warrants further research to support this hypothesis and what role these effects play in the suppression of autoimmunity by $1,25(OH)_2D_3$.

5.4 T cells

Historically, it was thought DCs were the main target of vitamin D and that effects observed on T cells were mediated via DCs. However, it has now become clear that upon activation various T cell populations express the VDR, including $CD4^+$ T helper (Th) cells, $CD8^+$ cytotoxic T cells and TCRγδ cells.[12, 144, 145] This makes the T cell another direct immunological target for $1,25(OH)_2D_3$. The effects of $1,25(OH)_2D_3$ on T cells include modulation of cytokine secretion and differentiation, but VDR is also required for the activation of T cell by propagating TCR signaling.[77] Since T cells are proposed to play an important role in the pathogenesis of autoimmunity, we will discuss the effects of $1,25(OH)_2D_3$ on the various T cell populations.

5.4.1 $CD4^+$ T cells

$CD4^+$ T cells are a heterogeneous group of cells, including T-helper 1 (Th1), Th2, Th17 and Treg cells. In the normal immune response, Th1 cells are important for fighting intracellular pathogens, Th2 cells for helminth infections and Th17 cells for extracellular pathogens and fungi. On the other hand, Tregs mediate immunological tolerance against self-antigens and harmless foreign antigens such as food and intestinal microbiota. Furthermore, they control the immune response via various mechanisms, including the secretion of anti-inflammatory mediators such as IL-10 and TGF-β.[146] However, in autoimmune diseases T cells mediate an immune response against the body itself, suggesting either hyper-activation of the pro-inflammatory T cells or insufficient control by Treg cells, or both.

The importance of the T cells as a target of $1,25(OH)_2D_3$ in experimental autoimmune diseases is illustrated by Mayne *et al.*, who showed that $1,25(OH)_2D_3$ is not able to suppress EAE when the VDR is absent in T cells.[147] For these studies they used the CD4-Cre

system, resulting in VDR deficiency in both $CD4^+$ and $CD8^+$ T cells. However, in this disease model $CD4^+$ are likely the prime $1,25(OH)_2D_3$ target cells, since other studies show that in this model $CD8^+$ T cells are dispensable for the effects of $1,25(OH)_2D_3$.[148] Further strengthening the hypothesis that the suppression of EAE by $1,25(OH)_2D_3$ is driven by modulation of $CD4^+$ T cells, is the finding that $1,25(OH)_2D_3$ prevents $CD4^+$ T helper cell migration into the CNS.[149] Finally, VDR binding is enriched near SNPs associated with autoimmune diseases in human $CD4^+$ T cells, suggesting that these cells are also important in the effects of $1,25(OH)_2D_3$ in human autoimmunity.

Because the effects of $1,25(OH)_2D_3$ differ between the various $CD4^+$ Th cell subsets,[150] we will give an overview of the current knowledge on how these individual subsets are modulated by $1,25(OH)_2D_3$ to suppress the autoimmune response.

5.4.1.1 Th1 and Th2 cells

Classically, $CD4^+$ T cells were subdivided into two classes: Th1 and Th2 cells. Th1 cells are characterized by the expression of IFNγ and T-bet, while Th2 cells produce IL-4, IL-5 and IL-13 and express the transcription factor GATA3. In the context of autoimmunity it was long thought that Th1 cells mediate the disease pathogenesis, since mice lacking the transcription factor T-bet are protected against EAE.[151] However, the discovery of Th17 cells, which will be discussed in the next section, and the finding that IFNγ is not required for induction of autoimmunity have led to a debate as to whether Th1 cells are important for autoimmune pathogenesis.[152, 153] However, since adoptive transfer of myelin-specific $IFN\gamma^+$ cells induces EAE,[154] Th1 cells may still play a role in the disease pathogenesis.

Within Th1 cells, some studies suggest that $1,25(OH)_2D_3$ inhibits IFNγ production when added at the first phases of differentiation.[155, 156] On the other hand, another study found no effects on IFNγ.[150] This contradiction could be explained by the addition of exogenous IL-2 in the first two studies. Since $1,25(OH)_2D_3$ directly downregulates IL-2, exogenous IL-2 might be required for the inhibition of IFNγ by $1,25(OH)_2D_3$.[157, 158] Although these studies indicate that $1,25(OH)_2D_3$ modulates Th1 cells under certain circumstances, given their relatively small role in autoimmune pathogenesis and the low expression of VDR compared to other $CD4^+$ T cell subsets, it is unlikely that they play an important role in the suppression of autoimmunity by $1,25(OH)_2D_3$.[150, 159]

In contrast to Th1 cells, Th2 cells might be protective in Th17-driven autoimmune diseases even though they are pathogenic in the development of asthma and allergies. Studies

in experimental arthritis demonstrate that T cell specific overexpression of GATA3 is protective in autoimmunity due to suppression of Th17 responses.[160] Interestingly, IL-4 is required for $1,25(OH)_2D_3$ to inhibit EAE, suggesting an important role for this cytokine in the effect of $1,25(OH)_2D_3$.[161] In the same model, $1,25(OH)_2D_3$ induces GATA3 and its regulator STAT6. The functional relevance of this upregulation is demonstrated in STAT6-KO mice, where $1,25(OH)_2D_3$ is unable to inhibit EAE development.[162] Altogether these studies suggest a role for Th2 induction in the immune suppression by $1,25(OH)_2D_3$.

However, the data on the effect of $1,25(OH)_2D_3$ on Th2 cytokines like IL-4 seems contradictory. When naïve $CD4^+$ T cells or the entire $CD4^+$ T cell population are cultured without polarizing cytokines, $1,25(OH)_2D_3$ induces IL-4 and GATA3.[163, 164] Also, in PBMC of treatment-naïve early RA patients, where IL-4 production is diminished, $1,25(OH)_2D_3$ restores the IL-4 levels to the levels of healthy controls.[165] However, when naïve $CD4^+$ T cells, effector $CD4^+$ T cells or total $CD4^+$ T cells are cultured in the presence of IL-4 to induce Th2 polarization, cellular IL-4 production is unaffected or even inhibited by $1,25(OH)_2D_3$.[155, 156] Also when patients are supplemented with cholecalciferol, there is no increased IL-4 production by their T cells.[141, 166, 167] Combining these data leads to the hypothesis that $1,25(OH)_2D_3$ promotes Th2 differentiation and IL-4 production to assist in suppression of autoimmunity, but only when no sufficient IL-4 is present. The mechanism behind the precise regulation of IL-4 is of interest, not only for treatment of autoimmunity, but also of allergies and asthma where Th2 cytokines play an important pathogenic role.

5.4.1.2 Th17 cells

In most autoimmune diseases, Th17 cells are considered to be important drivers of disease pathogenesis. Th17 cells are characterized by production of cytokines such as IL-17A, IL-17F, TNFα and GM-CSF and the transcription factor RORC2 (RORγt in mice). They can also be distinguished based on the expression of the chemokine receptor CCR6, which directs migration towards the chemokine CCL20. Their differentiation can be driven by TGFβ, IL-6 and IL-1β, but they require IL-23 to become pathogenic Th17 cells.[168] In 2003 two hallmark studies showed that IL-23, and not IL-12, is required for the induction of EAE and CIA [169, 170], suggesting an important role for the IL-23/IL-17 immune pathway in the pathogenesis of autoimmune diseases. Indeed, local IL-17A overexpression in mouse knee joints induces an arthritis-like phenotype with inflammation, bone erosions and damaged cartilage.[171] In EAE the pathogenic cells appear to be the ex-Th17 cells, which now express IFNγ and T-bet, indicating the importance of Th17 plasticity in autoimmune diseases.[172] In human autoimmunity, for example in RA and SLE, levels of Th17 cells are

elevated in the peripheral blood and synovial fluid of patients and correlate with disease activity.[173-175] Furthermore, specifically the $CCR6^+$ memory Th cells, which include Th17 cells, are potent activators of synovial fibroblasts.[173] We have previously shown that this interaction leads to a pro-inflammatory feedback loop with increased production of IL-17A, IL-6, IL-8 and tissue-destructive enzymes. Via this mechanism, Th17 cells may contribute to local joint inflammation in RA.[173] Combining the important role of Th17 cells in autoimmunity and the beneficial effect of $1,25(OH)_2D_3$ on autoimmune diseases, it is hypothesized that $1,25(OH)_2D_3$ suppresses autoimmunity at least partially via the inhibition of Th17 activity.

In support of this hypothesis, the effect of $1,25(OH)_2D_3$ on an experimental model for anti-retinal autoimmunity depends on inhibiting Th17 activity.[176] Also *in vitro* $1,25(OH)_2D_3$ decreases expression of pro-inflammatory cytokines like IL-17A, IL-17F and IL-22 in $CD4^+$ T cells, $CD4^+$ memory cells or $CD4^+CCR6^+$ memory cells.[165, 177-179] Functionally, this decrease in Th17 activity diminishes activation of synovial fibroblasts, thereby inhibiting the pro-inflammatory loop between these cell types.[179] Interestingly, $1,25(OH)_2D_3$ also inhibits the secretion of IL-17A and other Th17 cytokines in the presence of Th17 polarizing cytokines.[178, 180]

$1,25(OH)_2D_3$ not only inhibits the activity of Th17 cells, but also Th17 differentiation. When naïve $CD4^+$ T cells are differentiated towards the Th17 lineage *in vitro*, the presence of $1,25(OH)_2D_3$ inhibits Th17-related cytokines and transcription factors such as IL-17A, IL-17F, RORC and CCR6.[150, 159, 181] Functionally, MOG-specific Th17 cells differentiated in the presence of $1,25(OH)_2D_3$ are less capable of inducing EAE upon adoptive transfer.[178] Aside from the decreased pathogenicity of the cells, this effect may also be due to a decrease in CCR6, the chemokine receptor required for migration to the CNS.[182]

Although the inhibitory effect on Th17 activity is well described, the mechanisms behind it are less clear. First of all, Joshi *et al.* showed that the regulation of IL-17A can be mediated via direct binding of the VDR to the IL-17A promoter. VDR-RXR complexes compete with NFAT for the binding sites in the promoter, after which they recruit RUNX1 and HDAC (histone deacetylase) to inhibit IL-17A gene expression.[178] This competition for the NFAT binding site also occurs at the promoter of IL-2, a known primary $1,25(OH)_2D_3$ target gene, suggesting that this may be a general mechanism that also applies to other NFAT-regulated genes.[157] Recruitment of HDAC indicates that epigenetic regulation is also important in the inhibition of IL-17A by $1,25(OH)_2D_3$, especially given the relative

epigenetic instability of the IL-17A gene locus.[183] Aside from this direct regulation of IL-17A, other mechanisms have also been proposed. One study showed that CHOP is crucial for the inhibitory effect of $1,25(OH)_2D_3$, while a second study indicated IRF8 to be important.[159, 181] Yet another study indicated that VDR forms a complex with VDR, RXR, HDAC2 and Smad3 to inhibit Smad7 transcription, thereby preventing IL-17A production.[184] Of note, TGFβ is the cytokine that induces Smad3 and Erk, leading to this inhibition of IL-17A, but it is also the cytokine responsible for inducing the VDR.[180] How these mechanisms relate to each other remains to be investigated.

5.4.1.3 Th17.1 cells

Before the discovery of Th17 cells it was thought that Th1 cells, characterized by expression of IFNγ, T-bet and CXCR3, were the major drivers of the autoimmune response. The finding that IL-23, and not IL-12, was required for experimental autoimmunity, at first completely shifted the viewpoint towards Th17 cells as the pathogenic drivers of autoimmunity. However, lately more and more studies indicate that the subdivision into Th17 and Th1 is not as linear as previously assumed. Upon stimulation by IL-12 or TNFα Th17 cells can become double producers of IL-17A and IFNγ or even shift towards high IFNγ production with little or no IL-17A. Since these latter cells still express CCR6 and RORC, together with T-bet and CXCR3, they are called non-classic Th1 or Th17.1 cells.[185] Currently, it is hypothesized that the Th17.1 cells are more pathogenic than Th17 cells in autoimmune diseases, because they are enriched at the sites of inflammation in several diseases.[186, 187]

Interestingly, we have shown that in $CCR6^+$ cells, which includes Th17 and Th17.1 cells, $1,25(OH)_2D_3$ reduces the frequency of $IFN\gamma^+$, $IL\text{-}17A^+$ and $IFN\gamma^+IL\text{-}17A^+$ cells.[179] This suggests that $1,25(OH)_2D_3$ can inhibit T helper cell pathogenicity in autoimmunity via the inhibition of Th17 and Th17.1 cells. A similar effect was found in the $CD4^+$ T cells of SLE patients supplemented with 10400 IU cholecalciferol for 6 months.[188] Other supplementation studies have not addressed the combined or single expression of IFNγ and IL-17A, but the results on total $IL\text{-}17A^+$ or total $IFN\gamma^+$ cells are ambiguous.[141, 166, 167]

5.4.1.4 Regulatory T cells

In contrast to the pro-inflammatory T helper subsets mentioned above, regulatory T cells, or Tregs, suppress the immune response. Tregs express FoxP3, the anti-inflammatory cytokines IL-10 and TGFβ, the inhibitory co-receptor CTLA4 and a high level of CD25. They exert immunomodulatory effects on other immune cells such as macrophages,

dendritic cells, $CD8^+$ T cells but also other $CD4^+$ T cells, thereby maintaining immune homeostasis. Their essential role in preventing autoimmunity is demonstrated in patients with a mutation in FoxP3. These patients are suffering from the IPEX syndrome, which is characterized by massive autoimmunity.[189] In the autoimmune diseases discussed here it is hypothesized that an imbalance between pro-inflammatory T cells, such as Th17 or Th17.1, and regulatory T cells underlies the immune pathogenesis. $1,25(OH)_2D_3$ may act by restoring this balance and thereby restoring immune homeostasis.

Indeed, $1,25(OH)_2D_3$ induces $FoxP3^+$ Tregs in the spleen, lymph nodes and spinal cord of EAE mice.[178, 184] Additionally, without IL-10 or IL-10-mediated signaling, $1,25(OH)_2D_3$ cannot inhibit EAE.[190] In *in vitro* cultures of Tregs, either obtained via *in vitro* polarization or sorted from peripheral blood, $1,25(OH)_2D_3$ induces the production of IL-10, but not FoxP3.[164, 191, 192] Polarized Tregs express a higher level of Treg-associated markers such as CTLA4, PD1 and CD25 and their suppressive capacity is enhanced by $1,25(OH)_2D_3$.[192] Also, the suppressive capacity of Tregs is positively correlated with the serum $25(OH)D_3$ level in MS patients.[193] However, when sorted Tregs are used, $1,25(OH)_2D_3$ does not further enhance their suppressive capacity.[164, 191] This suggests that $1,25(OH)_2D_3$ optimizes Treg function in order to suppress autoimmunity.

Interestingly, $1,25(OH)_2D_3$ also induces IL-10 production when $CD4^+$ cells are cultured under neutral conditions, and even further in the presence of Th17 polarizing cytokines. Furthermore, in these cultures $1,25(OH)_2D_3$ also induces FoxP3 and CTLA4, while enhancing the suppressive capacity of the cells.[163, 177, 178, 180, 181, 184, 194] Because $1,25(OH)_2D_3$ inhibits Th17 polarization while inducing IL-10 in these cultures, it was postulated that $1,25(OH)_2D_3$ may inhibit Th17 activity via IL-10 induction. However, IL-10 is dispensable for the inhibition of IL-17A, suggesting that Th17 inhibition and Treg induction are two independent mechanisms of $1,25(OH)_2D_3$.[150]

On a molecular level three mechanisms have been proposed by which $1,25(OH)_2D_3$ can stimulate a Treg-like phenotype even under Th17 polarizing conditions. Firstly, the VDR can bind to three VDREs in the conserved non-coding sequence of the FoxP3 promoter, thereby directly controlling FoxP3 transcription.[178, 194] The second mechanism is by reversing the inhibitory effect of Th17 polarizing cytokines on CTLA4, leading to upregulation of CTLA4.[180] Finally, $1,25(OH)_2D_3$ induces the expression of IDO, which increases the number of Tregs.[76] The latter finding is interesting, since IDO was also

reported to be important for the induction of tDCs (see section 5.1).[94] suggesting it might be a general target of $1,25(OH)_2D_3$ in the immune system.

Although the *in vitro* data demonstrate that $1,25(OH)_2D_3$ induces Treg cells, not all cholecalciferol supplementation studies find an effect on Tregs. Several studies suggest an increase in the proportion or number of Treg cells based on surface marker expression [141, 166, 195] or based on IL-10 production.[52, 167] However, another study did not find this induction in Treg cells.[61] and Treg suppressive function is unaffected by cholecalciferol supplementation.[167]

Overall, in $CD4^+$ T cells $1,25(OH)_2D_3$ inhibits the pro-inflammatory Th cell functions while stimulating Treg activity. These effects are observed under both healthy and pathogenic conditions, such as in patients with autoimmune diseases.[191] Therefore, restoring the disturbed balance between effector T cells and Treg cells may underlie the beneficial effects of $1,25(OH)_2D_3$ on autoimmunity.

5.4.2 $CD8^+$ cytotoxic T cells

In addition to $CD4^+$ T cells, cytotoxic $CD8^+$ T cells comprise the second important class within the T cells. These cells contribute to the immune response by inducing apoptosis in abnormal cells, for example in case of infection or uncontrolled growth in cancer. In addition they modulate other immune cells by secreting cytokines.[196] Although the role of $CD8^+$ T cells in autoimmune diseases is not as well characterized as the role of $CD4^+$ T cells, various studies indicate that they play a role in disease pathogenesis. For example, myelin-specific $CD8^+$ T cells induce EAE in mice, with characteristics of human MS that are not conferred by myelin-specific $CD4^+$ T cells.[197, 198] Similarly, hsp60-specific $CD8^+$ T cells induce autoimmune intestinal inflammation.[199] More recently it was shown that IL-$17A^+CD8^+$ T cells are enriched in the synovial fluid of psoriatic arthritis patients. These cells do not express cytolytic markers, but their levels are positively correlated with markers of disease activity.[200] Since $CD8^+$ T cells have a higher expression of VDR than $CD4^+$ T cells,[145] $CD8^+$ T cells may also be a target for $1,25(OH)_2D_3$ in the suppression of autoimmunity.

Indeed, adoptive transfer of $VDR^{-/-}$ $CD8^+$ T cells in Rag-deficient mice induces intestinal inflammation. When $VDR^{-/-}IL\text{-}10^{-/-}$ $CD8^+$ T cells are transferred the intestinal inflammation is even worse and leads to wasting disease.[201] The increased proliferation of $VDR^{-/-}$ $CD8^+$ T cells, even in the naive state, suggests that VDR-induced signaling is required for

maintaining quiescence of these cells. Thereby $1,25(OH)_2D_3$ prevented hyper-activation of $CD8^+$ T cells and subsequent autoimmune pathology in diseases such as Crohn's disease.[201] In addition to maintaining quiescence, $1,25(OH)_2D_3$ also inhibits the secretion of IFNγ and TNFα by activated $CD8^+$ T cells.[202] Finally, topical treatment with calcipotriol decreases the frequency of $IL\text{-}17A^+CD8^+$ cells in psoriatic lesions, which is interesting in light of the correlations between these cells and disease activity in psoriatic arthritis.[200, 203]

Aside from modulating the activity of the classical $CD8^+$ T cells to reduce autoimmunity, $1,25(OH)_2D_3$ is also important in the development of $CD8\alpha\alpha^+$ T cells. $CD8\alpha\alpha^+$ T cells are self-reactive cells that have a regulatory function by maintaining homeostasis in the gut. In $VDR^{-/-}$ mice the number of these cells is reduced, which may explain the susceptibility of these animals to intestinal inflammation.[204]

It is important to note that the effect of $1,25(OH)_2D_3$ is not mediated via the $CD8^+$ T cells in every autoimmune disease, since they were dispensable for the attenuation of EAE by $1,25(OH)_2D_3$.[148] However, it seems that in IBD and psoriatic arthritis the $CD8^+$ T cells are target for $1,25(OH)_2D_3$. It will be of great interest to determine what the role of the $CD8^+$ T cells is in the effect of $1,25(OH)_2D_3$ on other autoimmune diseases. This will not only provide insight into the mechanisms behind the effect of vitamin D, but also about the differences in pathogenesis in the various autoimmune diseases.

5.4.3 Unconventional T cells

Next to the traditional $CD4^+$ and $CD8^+$ T cells, there are also cells expressing the TCR but lacking both CD4 and CD8. These so-called unconventional T cells have a less diverse TCR repertoire and they are not restricted to MHC class I or II. The unconventional T cells include mucosal associated invariant T (MAIT) cells, TCRγδ T cells and natural killer T (NKT) cells.

Although MAIT cells have been implicated to be suppressive in autoimmunity, as reviewed by Godfrey *et al.,*[205] there is currently no data available on the effect of $1,25(OH)_2D_3$ on these cells.

TCRγδ T cells are rapid responders in the event of an infection with intracellular pathogens, due to their recognition of phospho-antigens. Interestingly, they are pathogenic in autoimmune models like EAE and CIA and they produce a wide range of pro-inflammatory cytokines like IL-17A, IL-17F, GM-CSF, TNFα and IFNγ.[206] There is only

one study that investigated the effect of $1,25(OH)_2D_3$ on the pro-inflammatory activity of these cells. They demonstrated that TCRγδ T cells express the VDR upon activation. In response to $1,25(OH)_2D_3$ the production of IFNγ and the proliferation of these cells was inhibited.[144] Currently it is thought that the main pathogenic action of the TCRγδ T cells in autoimmunity is the secretion of IL-17A.[206] Unfortunately, there is no data available yet that describes the effect of $1,25(OH)_2D_3$ on this cytokine, or any of the other cytokines secreted by the TCRγδ T cells.

The last subset of unconventional T cells that will be discussed here are the NKT cells. They recognize glycolipid antigens and are thereby involved in the protection against a wide range of pathogens. Upon TCR stimulation, NKT cells can rapidly secrete various pro-inflammatory cytokines, including IL-4, IFNγ and IL-17A. NKT cells can be divided into type I and type II NKT cells. Type I NKT cells are also called invariant NKT (iNKT) cells due to their invariant TCR. Type II NKT cells have a variable TCR and are therefore called the variant NKT cells. The exact role of NKT cells in the pathogenesis of autoimmune disease is not yet completely clear. They are pathogenic in CIA, but they are protective in EAE, T1D and SLE.[161, 207]

Interestingly, VDR is required in the thymus for the development of functionally mature iNKT cells. Furthermore, the iNKT cells in $VDR^{-/-}$ mice are hyporesponsive to TCR stimulation.[208] In addition, the protective effect of $1,25(OH)_2D_3$ in EAE is partially dependent on iNKT cells, possibly via inducing IL-4 in these cells.[161] These data suggest that $1,25(OH)_2D_3$ promotes a suppressive function of iNKT cells. However, given the two-sided effect of iNKT cells in the different autoimmune diseases, further research is needed to fully examine the effect of $1,25(OH)_2D_3$ on iNKT cell activity and what this means for each individual disease.

5.5 Innate lymphoid cells

Recently a new group of cells became the center of attention in the field of immunology; the innate lymphoid cells (ILC). ILCs play an important role in tissue repair, tissue homeostasis and the immune response against bacteria, viruses and fungi. ILCs can be grouped into three classes; (i) the group 1 ILCs (ILC1) that secrete IFNγ and depend on T-bet expression, (ii) the group 2 ILCs (ILC2) that secrete type 2 cytokines such as IL-5 and IL-13 and depend on GATA3 and (iii) the group 3 ILCs (ILC3) that secrete IL-17A and/or IL-22 and depend on RORC.[209]

The ILC1s include natural killer cells, which have been known for a longer time and play a role in the clearance of viruses. Since viral triggers are thought to play a role in the initiation of some autoimmune diseases, the NK cells have been investigated for their role in this context. However, under some circumstances NK cells are protective, while in others they can be pathogenic as recently reviewed by Poggi and Zocchi.[210] Also the data on the effect of $1,25(OH)_2D_3$ on NK cells are somewhat contradictory. In an NK cell line, $1,25(OH)_2D_3$ induces the cytolytic killing capacity of NK cells,[211] but this effect has not been found in healthy control peripheral blood.[212, 213] However, when $1,25(OH)_2D_3$ is added during the *in vitro* differentiation of NK cells from hematopoietic stem cells, the development of NK cells is impaired and their cytotoxicity and IFNγ production are reduced.[212] Interestingly, $1,25(OH)_2D_3$ specifically inhibits activation, cytotoxic capacity and pro-inflammatory cytokine production in over-activated NK cells in women with recurrent pregnancy losses.[213] This supports a hypothesis in which $1,25(OH)_2D_3$ is not a general inhibitor of the immune response, but rather a regulator of immune homeostasis. Therefore it is of interest whether this abnormal NK activation is also seen in autoimmune diseases and can be modulated by $1,25(OH)_2D_3$.

Based on their cytokine signature, it can be hypothesized that in the context of autoimmunity ILC3 cells play a role in disease pathogenesis. Indeed, an increase in ILC3 cells has been demonstrated in the lesional skin of psoriasis patient [214, 215], in the inflamed intestine of Crohn's disease patients,[216] in the peripheral blood of MS patients [217] and in the gut, peripheral blood, bone marrow and synovial fluid of patients with ankylosing spondylitis.[218] Furthermore, ILC3 were shown to be responsible for experimental innate-induced colitis.[219] Interestingly, in VDR-KO mice, which are susceptible for colitis, the levels of ILC1 and ILC3 are increased.[220] On the other hand, calcipotriol treatment did not affect the frequencies of ILC subsets in psoriatic skin lesions after two weeks.[203]

Since the research into ILC has only started to expand in recent years, the effects of $1,25(OH)_2D_3$ on these cells have not been investigated extensively. Current data suggests that $1,25(OH)_2D_3$ may also have anti-inflammatory effects on these cells, but more studies are required to distinguish the effects on the different subsets and its role in the protective effect of vitamin D in autoimmunity.

5.6 Indirect immunomodulatory effects

In the previous sections we discussed the direct modulatory effects of $1,25(OH)_2D_3$ on various cells of the immune system. However, $1,25(OH)_2D_3$ and the VDR also affect tissue

resident cells, such as hepatic and pancreatic stellate cells, and the inflammatory mediators that they secrete.[221, 222] This indirect mechanism of immune modulation by $1,25(OH)_2D_3$ is also relevant in autoimmune diseases. For example, in RA the interaction between T cells and synovial fibroblasts contributes to disease pathogenesis.[173] Therefore it is also of interest to study the effect of $1,25(OH)_2D_3$ on the tissue-resident cells in the context of autoimmunity.

Similar to the tissue-resident tissue cells in liver and pancreas, $1,25(OH)_2D_3$ also directly affects RA synovial fibroblasts. Not only is the IL-1β-induced production of tissue-degrading matrix metalloprotease 1 (MMP1) inhibited, also the infiltration capacity of RA fibroblasts is reduced upon treatment with $1,25(OH)_2D_3$.[223] But this effect on tissue-resident cells is not only found in the synovial cells. It was also shown that the VDR is required for intestinal homeostasis by limiting the production of IL-6 by epithelial cells through inhibition of the NFκB pathway.[224] Finally, $1,25(OH)_2D_3$ also affects brain pericytes, which may be relevant for MS. The pericytes line the epithelial cells of blood vessels and in the brain they are important for maintaining the blood-brain-barrier and neuron functioning. Brain pericytes cells produce less pro-inflammatory genes when exposed to $1,25(OH)_2D_3$ while upregulating anti-inflammatory genes. Interestingly, brain pericytes express Cyp27B1 upon stimulation with TNFα and IFNγ. This indicates that an inflammatory environment promotes the conversion of $25(OH)D_3$ into $1,25(OH)_2D_3$, which then can dampen the inflammation by modulating the pericytes.[225]

Overall, the indirect effects of vitamin D and the VDR on immune cells via tissue-resident cells have been underexposed in the past years. However, if we truly want to understand the molecular mechanisms by which $1,25(OH)_2D_3$ acts in autoimmune diseases, these effects are very important for future studies.

6 FUTURE DIRECTIONS

In this review we have discussed the advancements that have been made regarding the clinical effects of vitamin D and the molecular mechanisms that underlie these effects. However, there is still a lot that is unclear at the moment which will be subject of investigation in the coming years.

6.1 Vitamin D supplementation

Based on the current data on the effect of vitamin D supplementation it is still not possible to draw conclusions about the added value for the treatment of autoimmunity. This is due to the low number of trials, small patient numbers and heterogeneity in trial setup. In order to determine the therapeutic value of vitamin D supplementation, there are two big open questions that need to be addressed.

Firstly it is important to assess what serum $25(OH)D_3$ level is required for a beneficial effect of vitamin D in autoimmune diseases. Based on the requirements for calcium homeostasis, current guidelines indicate that a level below 50 nmol/L corresponds with deficiency, between 50 and 74 nmol/L as insufficiency and above 75 nmol/L as a sufficient $25(OH)D_3$ level.[226, 227] However, in the context of autoimmunity it is not known whether it is enough to correct deficiency or whether we should strive for an even higher serum $25(OH)D_3$ level. Using 75 nmol/L as a cut-off point, Raftery *et al.* showed that CD patients with sufficient serum $25(OH)D_3$ have significantly higher quality of life and less severe disease as measured by intestinal permeability, LL-37 expression and CDAI.[58] Furthermore, in healthy individuals the serum $25(OH)D_3$ level is correlated with number of VDR binding sites in $CD4^+$ T cells. When they have a level above 75 nmol/L, the VDR binding is enriched near genes associated with autoimmune diseases and regulatory T cells. However, clinical trials, either with or without placebo controls, do not consistently find immune modulation regardless of the baseline and endpoint serum $25(OH)D_3$ level (table 2). It should be noted that these measurements have been done in the peripheral blood or in cells from the peripheral blood, which is not the site of inflammation and therefore may not be the most relevant place to look for immunological effects.

The second question that is still matter of debate is in what form and dosage vitamin D should be supplemented. In the experimental autoimmune models animals are mostly supplemented with a high dose of $1,25(OH)_2D_3$, but in humans this strategy may lead to hypercalcemia. Therefore most clinical trials use cholecalciferol as the form of choice, although some use $1,25(OH)_2D_3$ or less calcemic analogues like alfacalcidiol. Of note, a study comparing the effects of alfacalcidiol (analogue for $1,25(OH)_2D_3$) with colecalciferol (analogue for cholecalciferol) indicates that in the short term alfacalcidiol might be more effective, but this effect disappears after 12 months.[228] Analogues like calcipotriol that are used in the topical treatment of psoriasis have not been tested in the other autoimmune diseases that were discussed here. Other analogues have been developed, which show equal or better immunomodulatory potential and have been

successfully used in experimental autoimmune diseases.[191, 229-233] The only analogue that was used in clinical trials was alfacalcidiol, mainly in type 1 diabetes patients (table 1). However, the effects of alfacalcidiol do not seem better than calcitriol, and at the same dosage there were no severe side effects from either alfacalcidiol or calcitriol.[60, 63, 64] More research into the actual effects of vitamin D analogues on human autoimmune disease is required for establishing whether these analogues can be used safely and effectively. Furthermore, in the clinical trials performed so far there were no serious adverse events after cholecalciferol supplementation. Therefore it is important to establish the added value of the vitamin D analogues compared to cholecalciferol supplementation. Currently, cholecalciferol is the most used supplementation form in clinical practice. Vitamin D supplementation guidelines indicate a maximum safe dose of 4,000 IU cholecalciferol per day for healthy adults.[226] However, no adverse effects were found with dosages of up to 50,000 IU cholecalciferol weekly for 12 weeks, or 100,000 IU weekly for 1 month followed by 100,000 IU monthly for 5 months.[54, 141, 167] Interestingly, the dose-escalation regime used by Burton *et al.* and 20,000 IU weekly by Smolders *et al.* did not elicit hypercalcemia despite reaching a serum $25(OH)D_3$ level of 400 and 380 nmol/L, respectively.[49, 167]

In considering the best strategy for cholecalciferol supplementation it should also not be forgotten that $1,25(OH)_2D_3$ may have a synergistic effect with other treatments. For example, *in vitro* studies have shown that $1,25(OH)_2D_3$ synergizes with retinoic acid (an active vitamin A metabolite) or dexamethason in the inhibition of Th17 pathogenicity.[165, 234] Also in monocytes the combination of dexamethasone and $1,25(OH)_2D_3$ has added effects over the compounds separately, partially because $1,25(OH)_2D_3$ enhances the effects of the glucocorticoid receptor.[235, 236] Furthermore, we have previously shown that $1,25(OH)_2D_3$ has an added effect on TNFα blockade in inhibiting the pro-inflammatory loop between Th17 cells and RASF in RA, suggesting that vitamin D combined with anti-TNFα could yield a better treatment response in the treatment of RA patients.[179] Finally, combining $1,25(OH)_2D_3$ with Lovastatin has an added therapeutic effect on EAE. This is due to the inhibition of RhoA-ROCK signaling in autoreactive T cells, leading to decreased expression of Cyp24A1 and thereby less inactivation of $1,25(OH)_2D_3$.[237] Altogether, these data indicate that it may be worthwhile to investigate the addition of cholecalciferol to current treatments like anti-TNFα, or to combine cholecalciferol with for example retinoic acid or statins. Due to the synergy between $1,25(OH)_2D_3$ and these already approved drugs, a lower dose of cholecalciferol may be sufficient for achieving beneficial clinical effects.

Currently several clinical trials are ongoing and recruiting patients in MS (clinicaltrials.gov identifier NCT01490502), RA (NCT02243800) and IBD (NCT02704624, NCT01046773, NCT02208310) for which the results are expected in the coming 3 to 5 years. Hopefully they can provide more insight into the answers on these remaining questions. However, to firmly establish the added value of cholecalciferol supplementation, large multi-center trials are required. Ideally, in these trials the patients should be randomized into different treat-to-target arms, in which every arm has a target $25(OH)D_3$ serum level, such as 75, 100 and 150 nmol/L. Since the effect of cholecalciferol alone is probably not sufficient to control disease activity, patients should receive standard care following pre-defined, harmonized treatment protocols in addition to the cholecalciferol supplementation.

6.2 Molecular mechanisms underlying immunomodulation

In addition to the studies where cholecalciferol has been supplemented, attention has also focused on understanding the immunomodulatory effects of $1,25(OH)_2D_3$ on a cellular level. Based on the current knowledge, $1,25(OH)_2D_3$ reduced the pathogenicity of dendritic cells, macrophages, $CD4^+$ T cells, $CD8^+$ T cells and B cells. Similar effects have been observed in γδ T cells, iNKT cells and ILCs, but more research is necessary to confirm these data (see section 5). It should be noted that $1,25(OH)_2D_3$ does not merely work as an anti-inflammatory agent. Instead, $1,25(OH)_2D_3$ assists in maintaining the balance between a pro- and anti-inflammatory state and is thereby able to restore the disturbed balance that is associated with autoimmunity.

This balancing effect of $1,25(OH)_2D_3$ is best illustrated in monocytes and macrophages, where it has pro-inflammatory effects in the early stages of activation but later shifts to an anti-inflammatory state.[238] Therefore it is interesting to study the effects of $1,25(OH)_2D_3$ in more detail in the various stages of differentiation and activation from monocyte to macrophage. The Carlberg lab has performed ChIP-seq experiments in the monocytic THP-1 cell line at early time points. Detailed studies have revealed several primary target genes such as ASAP2 and THBD,[239-241] but also identified Bcl6 as a primary target that mediates important secondary responses.[242] Next to the primary target genes, combining the ChIP-seq dataset with publically available ChIA-PET and FAIRE-seq datasets has improved the knowledge on VDR binding kinetics.[243, 244]

This is just an example of how next generation sequencing techniques can be combined to yield more understanding of the molecular mechanisms behind the effects of $1,25(OH)_2D_3$. Since it has already been shown that $1,25(OH)_2D_3$ has different effects on every cell type,

even closely related cell types such as Th1 and Th17,[150] it will be interesting to study VDR DNA binding and identify primary target genes in separate cell types. This will give insight into the similarities and differences between the effects of $1,25(OH)_2D_3$ on each cell, and what will be important to balance the immune response in patients with autoimmune diseases.

7 CONCLUSION

Although various studies have shown a beneficial effect of cholecalciferol supplementation in autoimmune diseases, there are also studies that do not find any effect on disease parameters. This might be due to the supplementation strategy or the subjects included in the study, which are issues that should be addressed in properly designed multi-center clinical trials.

However, it is also possible that systemic cholecalciferol supplementation is not sufficient to establish effects in every patient. Therefore, another way to use the immunomodulatory effects of vitamin D to the advantage of patients with autoimmune diseases, is to mimic the effects by targeting important pathways within immune cells. In order to do this, it is crucial to understand the working mechanisms of $1,25(OH)_2D_3$. In the coming years attention should be paid towards unraveling these molecular mechanisms to optimize the therapeutic potential of vitamin D.

Table 2. Overview of clinical trials looking at immunological parameters after vitamin D supplementation. aTreg Activated memory regulatory T cells; BAFF B-cell activating factor; CM Central memory; CS Class-switched memory; DN Double negative; EM Effector memory; iTreg Induced regulatory T cells; IU International Units; moDC Monocyte-derived dendritic cell; MZ Marginal zone; rTreg Resting regulatory T cells; TE Terminal effector; tTreg Thymic regulatory T cells; # number; [ ] concentration.

Trial	Disease	Supplementation strategy	Mean baseline $25(OH)D_3$	Mean endpoint $25(OH)D_3$	PBMC	T cells		B cells	Innate immune cells (DC, NK)	Cytokines and antibodies in serum or plasma
						$CD4^+$	$CD8^+$			
Bock 2011 (195)	Healthy	3 months 140.000 IU cholecalciferol monthly or placebo	64±29 nmol/l	~138 nmol/l		Increased % of Tregs				
Smolders 2010,[167] Knippenberg 2011,[142] Peelen 2013 (158)	MS	12 weeks 20.000 IU cholecalciferol daily (no placebo group)	50 (31-175) nmol/l	308 (151-535) nmol/l		No difference in % or function of Tregs, either naive or memory. Increased production of IL-10 and decreased IL-17A/IL-4 ratio in T cells from PBMC cultures.	No relation between % $IL\text{-}10^+$ or $IL\text{-}17^+$ $CD8^+$ and serum $25(OH)D_3$. No change in % $IL\text{-}10^+$ or $IL\text{-}17^+$ $CD8^+$.	No difference in %, # or differentiation status of circulating B cells.		No difference in BAFF. No change in immunoglobulins.
Kimball 2011 (245)	MS	Dose escalation: up to 280.000 IU per week in 23 weeks, stay 6 weeks, then reduce to 0 in 20 weeks, then 3 weeks without (trial: Burton *et al*, 2010)	78±27 nmol/l	179±76 nmol/l	Decreased PBMC proliferation in response to certain MS-associated antigens					

Trial	Disease	Supplementation strategy	Mean baseline 25(OH)D_3	Mean endpoint 25(OH)D_3	PBMC	T cells		B cells	Innate immune cells (DC, NK)	Cytokines and antibodies in serum or plasma
						$CD4^+$	$CD8^+$			
Mosayebi 2011 (52)	MS	6 months 300.000 IU cholecalciferol or placebo i.m. monthly	~25 nmol/l	~140nmol/l	Decreased PBMC proliferation upon PHA stimulation. No difference in IFNγ, but increase in IL-10 and TGFβ production in these cultures.					
Sotirchos 2016 (188)	MS	6 months 10400 or 800 IU cholecalciferol daily	10400: 68±22 nmol/l 800: 70±21 nmol/l	10400: + 87 (63-112) nmol/l compared to baseline 800: +17 (3-34) nmol/l compared to baseline		High dose, but not low dose, decreases % $IL\text{-}17^+$, but not % $IFN\gamma^+$ or % $IFN\gamma^+IL\text{-}17^+$. High dose, but not low dose, decreases % of EM and $CD161^+$, while decreasing % of CM and naïve. % $IL17^+$ is correlated with % EM For every 12.5 nmol/l increase in serum 25(OH)D_3, the % $IL\text{-}17^+$ $CD4^+$ decreases by 1% (when serum 25(OH)D_3 increases more than 45 nmol/l)	High dose, but not low dose, decreases $CD85j^+$			

Trial	Disease	Supplementation strategy	Mean baseline $25(OH)D_3$	Mean endpoint $25(OH)D_3$	PBMC	T cells		B cells	Innate immune cells (DC, NK)	Cytokines and antibodies in serum or plasma
						$CD4^+$	$CD8^+$			
Bendix-Struve 2010,[246] Bartels 2014 (103)	CD	1 year placebo vs 1200 IU cholecalciferol daily (trial Jorgensen *et al.* 2010)	33 (16-66) nmol/l	118 (62-154) nmol/l		Over time decrease of IL-6 production is prevented upon supplementation. Increased $CD4^+$ proliferation which is inversely correlated with the IL-10 production.			MoDCs have decreased IL-10, IL-6, IL-8 and IL-1β, CD80 and HLA-DR. The allogeneic stimulatory capacities of moDCs are unaffected.	
Yang 2013 (247)	CD	24 weeks, start with 1000IU cholecalciferol daily, increase to 5000IU daily or until serum $25(OH)D_3$ is 100 nmol/L (no placebo group)	40±25 nmol/L	113±48 nmol/L						No change in IL-17, TNFα or IL-10

Trial	Disease	Supplementation strategy	Mean baseline 25(OH)D_3	Mean endpoint 25(OH)D_3	PBMC	T cells		B cells	Innate immune cells (DC, NK)	Cytokines and antibodies in serum or plasma
						$CD4^+$	$CD8^+$			
Gabbay 2012 (61)	T1D	18 months 2000 IU cholecalciferol daily or placebo	66±16 nmol/l	152±54 nmol/l		No change in % Tregs				No difference in IL-12, TNFα, CXCL10 or IL-10, but close-to-significant increase of CCL2 after 12 months (not after 18 months)
Terrier 2012 (141)	SLE	4 weeks 100.000 IU cholecalciferol weekly, then 6 months 100.000 IU monthly (no placebo group)	47±17 nmol/L	129±35 nmol/L		No change in total % or #. Increase in # naive at 6 months, but not %. No change in other activation stages. Increase in % and # of Tregs, aTregs and rTregs. Increase of % $CTLA4^+$ and $GITR^+$, but not LAP^+ Tregs. Decrease in % of Th1 and Th17 at 2 months, but only of Th1 at 6 months. No change in Th2.	No change in total % or #. Decrease in % effector memory at 2 and 6 months, but not #. No change in other activation stages. Decrease in $IFN\gamma^+$ at 2 months.	Decrease in % and # after 2 months, but after 6 months only in %. Increase in MZ % and # after 6 months. Decrease in % and # DN after 6 months. No change in naive or CS B cells	No change in % or # of NK cells	Anti-dsDNA decreased

Trial	Disease	Supplementation strategy	Mean baseline 25(OH)D_3	Mean endpoint 25(OH)D_3	PBMC	T cells		B cells	Innate immune cells (DC, NK)	Cytokines and antibodies in serum or plasma
						$CD4^+$	$CD8^+$			
Abou-Raya 2013 (66)	SLE	12 months placebo vs 2000 IU cholecalciferol daily	50±41nmol/L	95±41 nmol/L						Decrease in IL-1β, IL-6, IL-18 and TNFα Decrease in anti-dsDNA, anti-Sm and C4, but not anticardiolipin IgG or IgM
Piantoni 2015,[166] Andreoli 2015 (248)	SLE	12 months 25.000 IU cholecalciferol monthly (standard regime, SR) or 300.000 IU at baseline followed by 50.000 IU monthly (intensive regime, IR), compared with healthy control immune parameters	SR: 79 (20-211) nmol/L IR: 80 (47-188) nmol/L	SR: 68 nmol/l IR 96 nmol/l		Upon SR increase in % and [ ] of iTreg but not tTreg. In IR increased % iTreg and %tTreg, but not [ ]. In SR and IR increase in [ ] highly experienced Tmem, but only in % in SR. Increase in total CD4 % in SR and IR, but only in [ ] in IR. No change in % of IL-17^+, IFNγ^+ or IL-4^+ $CD4^+$ T cells after SR and IR.	Increase in % but not [ ] of $CD8^+$ in SR and IR. No change in % of IL-17^+, IFNγ^+ or IL-4^+ $CD8^+$ cells after both SR and IR, but in IR a decreased IFNγ/IL-4 ratio			No difference in anti-dsDNA between SR and IR

REFERENCES

1. Lerner A, Jeremias P, Matthias T. The World Incidence and Prevalence of Autoimmune Diseases is Increasing. *International Journal of Celiac Disease* (2015) **3**(4):151-5. PubMed PMID: doi:10.12691/ijcd-3-4-8.
2. Chang C. Unmet needs in the treatment of autoimmunity: from aspirin to stem cells. *Autoimmun Rev* (2014) **13**(4-5):331-46. Epub 2014/01/28. doi: S1568-9972(14)00064-0 [pii] 10.1016/j.autrev.2014.01.052. PubMed PMID: 24462645.
3. Christakos S, Ajibade DV, Dhawan P, Fechner AJ, Mady LJ. Vitamin D: metabolism. *Endocrinol Metab Clin North Am* (2010) **39**(2):243-53, table of contents. Epub 2010/06/01. doi: S0889-8529(10)00004-6 [pii] 10.1016/j.ecl.2010.02.002. PubMed PMID: 20511049; PubMed Central PMCID: PMC2879391.
4. Pike JW, Meyer MB. The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D(3). *Endocrinol Metab Clin North Am* (2010) **39**(2):255-69, table of contents. Epub 2010/06/01. doi: S0889-8529(10)00009-5 [pii] 10.1016/j.ecl.2010.02.007. PubMed PMID: 20511050; PubMed Central PMCID: PMC2879406.
5. Heikkinen S, Vaisanen S, Pehkonen P, Seuter S, Benes V, Carlberg C. Nuclear hormone 1alpha,25-dihydroxyvitamin D3 elicits a genome-wide shift in the locations of VDR chromatin occupancy. *Nucleic Acids Res* (2011) **39**(21):9181-93. Epub 2011/08/19. doi: gkr654 [pii] 10.1093/nar/gkr654. PubMed PMID: 21846776; PubMed Central PMCID: PMC3241659.
6. Evans RM, Mangelsdorf DJ. Nuclear Receptors, RXR, and the Big Bang. *Cell* (2014) **157**(1):255-66. Epub 2014/04/01. doi: S0092-8674(14)00346-8 [pii] 10.1016/j.cell.2014.03.012. PubMed PMID: 24679540; PubMed Central PMCID: PMC4029515.
7. Meyer MB, Goetsch PD, Pike JW. VDR/RXR and TCF4/beta-catenin cistromes in colonic cells of colorectal tumor origin: impact on c-FOS and c-MYC gene expression. *Mol Endocrinol* (2012) **26**(1):37-51. Epub 2011/11/24. doi: me.2011-1109 [pii] 10.1210/me.2011-1109. PubMed PMID: 22108803; PubMed Central PMCID: PMC3248320.
8. Handel AE, Sandve GK, Disanto G, Berlanga-Taylor AJ, Gallone G, Hanwell H, et al. Vitamin D receptor ChIP-seq in primary CD4+ cells: relationship to serum 25-hydroxyvitamin D levels and autoimmune disease. *BMC Med* (2013) **11**:163. Epub 2013/07/16. doi: 1741-7015-11-163 [pii] 10.1186/1741-7015-11-163. PubMed PMID: 23849224; PubMed Central PMCID: PMC3710212.
9. Carmeliet G, Dermauw V, Bouillon R. Vitamin D signaling in calcium and bone homeostasis: a delicate balance. *Best Pract Res Clin Endocrinol Metab* (2015) **29**(4):621-31. Epub 2015/08/26. doi: S1521-690X(15)00062-7 [pii] 10.1016/j.beem.2015.06.001. PubMed PMID: 26303088.

10. Ellman P, Anderson KH. Calciferol in tuberculous peritonitis with disseminated tuberculosis. *Br Med J* (1948) **1**(4547):394. Epub 1948/02/28. PubMed PMID: 18905261; PubMed Central PMCID: PMC2089577.
11. Bhalla AK, Amento EP, Clemens TL, Holick MF, Krane SM. Specific high-affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. *J Clin Endocrinol Metab* (1983) **57**(6):1308-10. Epub 1983/12/01. doi: 10.1210/jcem-57-6-1308. PubMed PMID: 6313738.
12. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. 1,25-dihydroxyvitamin D3 receptors in human leukocytes. *Science* (1983) **221**(4616):1181-3. Epub 1983/09/16. PubMed PMID: 6310748.
13. Lemire JM, Archer DC. 1,25-dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. *J Clin Invest* (1991) **87**(3):1103-7. Epub 1991/03/01. doi: 10.1172/JCI115072. PubMed PMID: 1705564; PubMed Central PMCID: PMC329907.
14. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc Natl Acad Sci U S A* (1996) **93**(15):7861-4. Epub 1996/07/23. PubMed PMID: 8755567; PubMed Central PMCID: PMC38839.
15. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxycholecalciferol inhibits the progression of arthritis in murine models of human arthritis. *J Nutr* (1998) **128**(1):68-72. Epub 1998/01/31. PubMed PMID: 9430604.
16. Zwerina K, Baum W, Axmann R, Heiland GR, Distler JH, Smolen J, et al. Vitamin D receptor regulates TNF-mediated arthritis. *Ann Rheum Dis* (2011) **70**(6):1122-9. Epub 2011/03/19. doi: ard.2010.142331 [pii] 10.1136/ard.2010.142331. PubMed PMID: 21415051.
17. Zhang H, Wu H, Liu L, Li H, Shih DQ, Zhang X. 1,25-dihydroxyvitamin D3 regulates the development of chronic colitis by modulating both T helper (Th)1 and Th17 activation. *APMIS* (2015) **123**(6):490-501. Epub 2015/04/25. doi: 10.1111/apm.12378. PubMed PMID: 25907285.
18. 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**(11):2648-52. Epub 2000/10/29. PubMed PMID: 11053501.
19. Mathieu C, Laureys J, Sobis H, Vandeputte M, Waer M, Bouillon R. 1,25-Dihydroxyvitamin D3 prevents insulitis in NOD mice. *Diabetes* (1992) **41**(11):1491-5. Epub 1992/11/01. PubMed PMID: 1397723.
20. Mathieu C, Waer M, Laureys J, Rutgeerts O, Bouillon R. Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3. *Diabetologia* (1994) **37**(6):552-8. Epub 1994/06/01. PubMed PMID: 7926338.

21. Lemire JM, Ince A, Takashima M. 1,25-Dihydroxyvitamin D3 attenuates the expression of experimental murine lupus of MRL/l mice. *Autoimmunity* (1992) **12**(2):143-8. Epub 1992/01/01. doi: 10.3109/08916939209150321. PubMed PMID: 1617111.
22. Simpson S, Jr., Blizzard L, Otahal P, Van der Mei I, Taylor B. Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. *J Neurol Neurosurg Psychiatry* (2011) **82**(10):1132-41. Epub 2011/04/12. doi: jnnp.2011.240432 [pii] 10.1136/jnnp.2011.240432. PubMed PMID: 21478203.
23. Mohr SB, Garland CF, Gorham ED, Garland FC. The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide. *Diabetologia* (2008) **51**(8):1391-8. Epub 2008/06/13. doi: 10.1007/s00125-008-1061-5. PubMed PMID: 18548227.
24. Szilagyi A, Leighton H, Burstein B, Xue X. Latitude, sunshine, and human lactase phenotype distributions may contribute to geographic patterns of modern disease: the inflammatory bowel disease model. *Clin Epidemiol* (2014) **6**:183-98. Epub 2014/06/28. doi: 10.2147/CLEP.S59838 clep-6-183 [pii]. PubMed PMID: 24971037; PubMed Central PMCID: PMC4070862.
25. Dobson R, Giovannoni G, Ramagopalan S. The month of birth effect in multiple sclerosis: systematic review, meta-analysis and effect of latitude. *J Neurol Neurosurg Psychiatry* (2013) **84**(4):427-32. Epub 2012/11/16. doi: jnnp-2012-303934 [pii] 10.1136/jnnp-2012-303934. PubMed PMID: 23152637.
26. Torkildsen O, Grytten N, Aarseth J, Myhr KM, Kampman MT. Month of birth as a risk factor for multiple sclerosis: an update. *Acta Neurol Scand Suppl* (2012) (195):58-62. Epub 2013/01/04. doi: 10.1111/ane.12040. PubMed PMID: 23278658.
27. Song GG, Bae SC, Lee YH. Association between vitamin D intake and the risk of rheumatoid arthritis: a meta-analysis. *Clin Rheumatol* (2012) **31**(12):1733-9. Epub 2012/09/04. doi: 10.1007/s10067-012-2080-7. PubMed PMID: 22941259.
28. Zipitis CS, Akobeng AK. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. *Arch Dis Child* (2008) **93**(6):512-7. Epub 2008/03/15. doi: adc.2007.128579 [pii] 10\.1136/adc.2007.128579. PubMed PMID: 18339654.
29. Dong JY, Zhang WG, Chen JJ, Zhang ZL, Han SF, Qin LQ. Vitamin D intake and risk of type 1 diabetes: a meta-analysis of observational studies. *Nutrients* (2013) **5**(9):3551-62. Epub 2013/09/17. doi: nu5093551 [pii] 10\.3390/nu5093551. PubMed PMID: 24036529; PubMed Central PMCID: PMC3798920.
30. Shen L, Zhuang QS, Ji HF. Assessment of vitamin D levels in type 1 and type 2 diabetes patients: Results from meta-analysis. *Mol Nutr Food Res* (2016). Epub 2016/02/24. doi: 10.1002/mnfr.201500937. PubMed PMID: 26898922.

31. Duan S, Lv Z, Fan X, Wang L, Han F, Wang H, et al. Vitamin D status and the risk of multiple sclerosis: a systematic review and meta-analysis. *Neurosci Lett* (2014) **570**:108-13. Epub 2014/04/29. doi: S0304-3940(14)00324-3 [pii] 10\.1016/j.neulet.2014.04.021. PubMed PMID: 24769422.
32. Lin J, Liu J, Davies ML, Chen W. Serum Vitamin D Level and Rheumatoid Arthritis Disease Activity: Review and Meta-Analysis. *PLoS One* (2016) **11**(1):e0146351. Epub 2016/01/12. doi: 10.1371/journal.pone.0146351 PONE-D-15-32843 [pii]. PubMed PMID: 26751969; PubMed Central PMCID: PMC4709104.
33. Del Pinto R, Pietropaoli D, Chandar AK, Ferri C, Cominelli F. Association Between Inflammatory Bowel Disease and Vitamin D Deficiency: A Systematic Review and Meta-analysis. *Inflamm Bowel Dis* (2015) **21**(11):2708-17. Epub 2015/09/09. doi: 10.1097/MIB.0000000000000546. PubMed PMID: 26348447; PubMed Central PMCID: PMC4615394.
34. Lu C, Yang J, Yu W, Li D, Xiang Z, Lin Y, et al. Association between 25(OH)D Level, Ultraviolet Exposure, Geographical Location, and Inflammatory Bowel Disease Activity: A Systematic Review and Meta-Analysis. *PLoS One* (2015) **10**(7):e0132036. Epub 2015/07/15. doi: 10.1371/journal.pone.0132036 PONE-D-15-11802 [pii]. PubMed PMID: 26172950; PubMed Central PMCID: PMC4501705.
35. Sadeghian M, Saneei P, Siassi F, Esmaillzadeh A. Vitamin D status in relation to Crohn's disease: Meta-analysis of observational studies. *Nutrition* (2016) **32**(5):505-14. Epub 2016/02/04. doi: S0899-9007(15)00469-4 [pii] 10\.1016/j.nut.2015.11.008. PubMed PMID: 26837598.
36. Feng R, Li Y, Li G, Li Z, Zhang Y, Li Q, et al. Lower serum 25 (OH) D concentrations in type 1 diabetes: A meta-analysis. *Diabetes Res Clin Pract* (2015) **108**(3):e71-5. Epub 2015/04/04. doi: S0168-8227(15)00008-X [pii] 10\.1016/j.diabres.2014.12.008. PubMed PMID: 25836943.
37. Sahebari M, Nabavi N, Salehi M. Correlation between serum 25(OH)D values and lupus disease activity: an original article and a systematic review with meta-analysis focusing on serum VitD confounders. *Lupus* (2014) **23**(11):1164-77. Epub 2014/06/26. doi: 0961203314540966 [pii] 10\.1177/0961203314540966. PubMed PMID: 24961748.
38. Hiraki LT, Arkema EV, Cui J, Malspeis S, Costenbader KH, Karlson EW. Circulating 25-hydroxyvitamin D level and risk of developing rheumatoid arthritis. *Rheumatology (Oxford)* (2014) **53**(12):2243-8. Epub 2014/07/30. doi: keu276 [pii] 10\.1093/rheumatology/keu276. PubMed PMID: 25065001; PubMed Central PMCID: PMC4241892.
39. Tizaoui K, Kaabachi W, Hamzaoui A, Hamzaoui K. Association between vitamin D receptor polymorphisms and multiple sclerosis: systematic review and meta-analysis of case-control studies. *Cell Mol Immunol* (2015) **12**(2):243-52. Epub 2014/07/08. doi: cmi201447 [pii] 10\.1038/cmi.2014.47. PubMed PMID: 24998351; PubMed Central PMCID: PMC4654294.

40. Tizaoui K, Hamzaoui K. Association between VDR polymorphisms and rheumatoid arthritis disease: Systematic review and updated meta-analysis of case-control studies. *Immunobiology* (2015) **220**(6):807-16. Epub 2015/01/13. doi: S0171-2985(14)00282-4 [pii] 10\.1016/j.imbio.2014.12.013. PubMed PMID: 25577294.
41. Lee YH, Bae SC, Choi SJ, Ji JD, Song GG. Associations between vitamin D receptor polymorphisms and susceptibility to rheumatoid arthritis and systemic lupus erythematosus: a meta-analysis. *Mol Biol Rep* (2011) **38**(6):3643-51. Epub 2010/11/27. doi: 10.1007/s11033-010-0477-4. PubMed PMID: 21110115.
42. Xue LN, Xu KQ, Zhang W, Wang Q, Wu J, Wang XY. Associations between vitamin D receptor polymorphisms and susceptibility to ulcerative colitis and Crohn's disease: a meta-analysis. *Inflamm Bowel Dis* (2013) **19**(1):54-60. Epub 2012/04/03. doi: 10.1002/ibd.22966. PubMed PMID: 22467262.
43. Wang L, Wang ZT, Hu JJ, Fan R, Zhou J, Zhong J. Polymorphisms of the vitamin D receptor gene and the risk of inflammatory bowel disease: a meta-analysis. *Genet Mol Res* (2014) **13**(2):2598-610. Epub 2014/05/02. doi: gmr2615 [pii] 10\.4238/2014.April.8.2. PubMed PMID: 24782048.
44. Tizaoui K, Kaabachi W, Hamzaoui A, Hamzaoui K. Contribution of VDR polymorphisms to type 1 diabetes susceptibility: Systematic review of case-control studies and meta-analysis. *J Steroid Biochem Mol Biol* (2014) **143**:240-9. Epub 2014/04/20. doi: S0960-0760(14)00082-X [pii] 10\.1016/j.jsbmb.2014.03.011. PubMed PMID: 24742873.
45. Wang G, Zhang Q, Xu N, Xu K, Wang J, He W, et al. Associations between two polymorphisms (FokI and BsmI) of vitamin D receptor gene and type 1 diabetes mellitus in Asian population: a meta-analysis. *PLoS One* (2014) **9**(3):e89325. Epub 2014/03/08. doi: 10.1371/journal.pone.0089325 PONE-D-13-40571 [pii]. PubMed PMID: 24603699; PubMed Central PMCID: PMC3945782.
46. Zhang J, Li W, Liu J, Wu W, Ouyang H, Zhang Q, et al. Polymorphisms in the vitamin D receptor gene and type 1 diabetes mellitus risk: an update by meta-analysis. *Mol Cell Endocrinol* (2012) **355**(1):135-42. Epub 2012/03/01. doi: S0303-7207(12)00080-9 [pii] 10\.1016/j.mce.2012.02.003. PubMed PMID: 22361322.
47. Zhou TB, Jiang ZP, Lin ZJ, Su N. Association of vitamin D receptor gene polymorphism with the risk of systemic lupus erythematosus. *J Recept Signal Transduct Res* (2015) **35**(1):8-14. Epub 2014/05/24. doi: 10.3109/10799893.2014.922577. PubMed PMID: 24853028.
48. Mason AR, Mason J, Cork M, Dooley G, Hancock H. Topical treatments for chronic plaque psoriasis. *Cochrane Db Syst Rev* (2013) (3). doi: ARTN CD005028 10\.1002/14651858.CD005028.pub3. PubMed PMID: WOS:000316887200017.

49. Burton JM, Kimball S, Vieth R, Bar-Or A, Dosch HM, Cheung R, et al. A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis. *Neurology* (2010) **74**(23):1852-9. Epub 2010/04/30. doi: WNL.0b013e3181e1cec2 [pii] 10\.1212/WNL.0b013e3181e1cec2. PubMed PMID: 20427749; PubMed Central PMCID: PMC2882221.
50. Soilu-Hanninen M, Aivo J, Lindstrom BM, Elovaara I, Sumelahti ML, Farkkila M, et al. A randomised, double blind, placebo controlled trial with vitamin D3 as an add on treatment to interferon beta-1b in patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry* (2012) **83**(5):565-71. Epub 2012/03/01. doi: jnnp-2011-301876 [pii] 10\.1136/jnnp-2011-301876. PubMed PMID: 22362918.
51. Kampman MT, Steffensen LH, Mellgren SI, Jorgensen L. Effect of vitamin D3 supplementation on relapses, disease progression, and measures of function in persons with multiple sclerosis: exploratory outcomes from a double-blind randomised controlled trial. *Mult Scler* (2012) **18**(8):1144-51. Epub 2012/02/23. doi: 1352458511434607 [pii] 10\.1177/1352458511434607. PubMed PMID: 22354743.
52. Mosayebi G, Ghazavi A, Ghasami K, Jand Y, Kokhaei P. Therapeutic effect of vitamin D3 in multiple sclerosis patients. *Immunol Invest* (2011) **40**(6):627-39. Epub 2011/05/06. doi: 10.3109/08820139.2011.573041. PubMed PMID: 21542721.
53. Derakhshandi H, Etemadifar M, Feizi A, Abtahi SH, Minagar A, Abtahi MA, et al. Preventive effect of vitamin D3 supplementation on conversion of optic neuritis to clinically definite multiple sclerosis: a double blind, randomized, placebo-controlled pilot clinical trial. *Acta Neurol Belg* (2013) **113**(3):257-63. Epub 2012/12/20. doi: 10.1007/s13760-012-0166-2. PubMed PMID: 23250818.
54. Salesi M, Farajzadegan Z. Efficacy of vitamin D in patients with active rheumatoid arthritis receiving methotrexate therapy. *Rheumatol Int* (2012) **32**(7):2129-33. Epub 2011/04/28. doi: 10.1007/s00296-011-1944-5. PubMed PMID: 21523344.
55. Dehghan A, Rahimpour S, Soleymani-Salehabadi H, Owlia MB. Role of vitamin D in flare ups of rheumatoid arthritis. *Z Rheumatol* (2014) **73**(5):461-4. Epub 2013/12/20. doi: 10.1007/s00393-013-1297-4. PubMed PMID: 24352479.
56. Hansen KE, Bartels CM, Gangnon RE, Jones AN, Gogineni J. An evaluation of high-dose vitamin D for rheumatoid arthritis. *J Clin Rheumatol* (2014) **20**(2):112-4. Epub 2014/02/25. doi: 10.1097/RHU.0000000000000072 00124743-201403000-00013 [pii]. PubMed PMID: 24561419; PubMed Central PMCID: PMC4089514.
57. Jorgensen SP, Agnholt J, Glerup H, Lyhne S, Villadsen GE, Hvas CL, et al. Clinical trial: vitamin D3 treatment in Crohn's disease - a randomized double-blind placebo-controlled study. *Aliment Pharmacol Ther* (2010) **32**(3):377-83. Epub 2010/05/25. doi: APT4355 [pii] 10\.1111/j.1365-2036.2010.04355.x. PubMed PMID: 20491740.

58. Raftery T, Martineau AR, Greiller CL, Ghosh S, McNamara D, Bennett K, et al. Effects of vitamin D supplementation on intestinal permeability, cathelicidin and disease markers in Crohn's disease: Results from a randomised double-blind placebo-controlled study. *United European Gastroenterol J* (2015) **3**(3):294-302. Epub 2015/07/03. doi: 10.1177/2050640615572176 10\.1177_2050640615572176 [pii]. PubMed PMID: 26137304; PubMed Central PMCID: PMC4480538.

59. Wingate KE, Jacobson K, Issenman R, Carroll M, Barker C, Israel D, et al. 25-Hydroxyvitamin D concentrations in children with Crohn's disease supplemented with either 2000 or 400 IU daily for 6 months: a randomized controlled study. *J Pediatr* (2014) **164**(4):860-5. Epub 2014/01/16. doi: S0022-3476(13)01506-0 [pii] 10\.1016/j.jpeds.2013.11.071. PubMed PMID: 24423431.

60. Ataie-Jafari A, Loke SC, Rahmat AB, Larijani B, Abbasi F, Leow MK, et al. A randomized placebo-controlled trial of alphacalcidol on the preservation of beta cell function in children with recent onset type 1 diabetes. *Clin Nutr* (2013) **32**(6):911-7. Epub 2013/02/12. doi: S0261-5614(13)00038-1 [pii] 10\.1016/j.clnu.2013.01.012. PubMed PMID: 23395257.

61. Gabbay MA, Sato MN, Finazzo C, Duarte AJ, Dib SA. Effect of cholecalciferol as adjunctive therapy with insulin on protective immunologic profile and decline of residual beta-cell function in new-onset type 1 diabetes mellitus. *Arch Pediatr Adolesc Med* (2012) **166**(7):601-7. Epub 2012/07/04. doi: 1212223 [pii] 10\.1001/archpediatrics.2012.164. PubMed PMID: 22751874.

62. Li X, Liao L, Yan X, Huang G, Lin J, Lei M, et al. Protective effects of 1-alpha-hydroxyvitamin D3 on residual beta-cell function in patients with adult-onset latent autoimmune diabetes (LADA). *Diabetes Metab Res Rev* (2009) **25**(5):411-6. Epub 2009/06/03. doi: 10.1002/dmrr.977. PubMed PMID: 19488999.

63. Walter M, Kaupper T, Adler K, Foersch J, Bonifacio E, Ziegler AG. No effect of the 1alpha,25-dihydroxyvitamin D3 on beta-cell residual function and insulin requirement in adults with new-onset type 1 diabetes. *Diabetes Care* (2010) **33**(7):1443-8. Epub 2010/04/02. doi: dc09-2297 [pii] 10\.2337/dc09-2297. PubMed PMID: 20357369; PubMed Central PMCID: PMC2890336.

64. Bizzarri C, Pitocco D, Napoli N, Di Stasio E, Maggi D, Manfrini S, et al. No protective effect of calcitriol on beta-cell function in recent-onset type 1 diabetes: the IMDIAB XIII trial. *Diabetes Care* (2010) **33**(9):1962-3. Epub 2010/09/02. doi: dc10-0814 [pii] 10\.2337/dc10-0814. PubMed PMID: 20805274; PubMed Central PMCID: PMC2928344.

65. Foering K, Chang AY, Piette EW, Cucchiara A, Okawa J, Werth VP. Characterization of clinical photosensitivity in cutaneous lupus erythematosus. *J Am Acad Dermatol* (2013) **69**(2):205-13. Epub 2013/05/08. doi: S0190-9622(13)00289-2 [pii] 10\.1016/j.jaad.2013.03.015. PubMed PMID: 23648190; PubMed Central PMCID: PMC3928014.
66. Abou-Raya A, Abou-Raya S, Helmii M. The effect of vitamin D supplementation on inflammatory and hemostatic markers and disease activity in patients with systemic lupus erythematosus: a randomized placebo-controlled trial. *J Rheumatol* (2013) **40**(3):265-72. Epub 2012/12/04. doi: jrheum.111594 [pii] 10\.3899/jrheum.111594. PubMed PMID: 23204220.
67. Lima GL, Paupitz J, Aikawa NE, Takayama L, Bonfa E, Pereira RM. Vitamin D Supplementation in Adolescents and Young Adults With Juvenile Systemic Lupus Erythematosus for Improvement in Disease Activity and Fatigue Scores: A Randomized, Double-Blind, Placebo-Controlled Trial. *Arthritis Care Res (Hoboken)* (2016) **68**(1):91-8. Epub 2015/05/20. doi: 10.1002/acr.22621. PubMed PMID: 25988278.
68. Aranow C, Kamen DL, Dall'Era M, Massarotti EM, Mackay MC, Koumpouras F, et al. Randomized, Double-Blind, Placebo-Controlled Trial of the Effect of Vitamin D3 on the Interferon Signature in Patients With Systemic Lupus Erythematosus. *Arthritis Rheumatol* (2015) **67**(7):1848-57. Epub 2015/03/18. doi: 10.1002/art.39108. PubMed PMID: 25777546; PubMed Central PMCID: PMC4732716.
69. Brennan A, Katz DR, Nunn JD, Barker S, Hewison M, Fraher LJ, et al. Dendritic cells from human tissues express receptors for the immunoregulatory vitamin D3 metabolite, dihydroxycholecalciferol. *Immunology* (1987) **61**(4):457-61. Epub 1987/08/01. PubMed PMID: 2832307; PubMed Central PMCID: PMC1453440.
70. Morgan JW, Kouttab N, Ford D, Maizel AL. Vitamin D-mediated gene regulation in phenotypically defined human B cell subpopulations. *Endocrinology* (2000) **141**(9):3225-34. Epub 2000/08/31. doi: 10.1210/endo.141.9.7666. PubMed PMID: 10965893.
71. Kongsbak M, von Essen MR, Levring TB, Schjerling P, Woetmann A, Odum N, et al. Vitamin D-binding protein controls T cell responses to vitamin D. *BMC Immunol* (2014) **15**(1):35. Epub 2014/09/19. doi: s12865-014-0035-2 [pii] 10\.1186/s12865-014-0035-2. PubMed PMID: 25230725; PubMed Central PMCID: PMC4177161.
72. Morgan JW, Reddy GS, Uskokovic MR, May BK, Omdahl JL, Maizel AL, et al. Functional block for 1 alpha,25-dihydroxyvitamin D3-mediated gene regulation in human B lymphocytes. *J Biol Chem* (1994) **269**(18):13437-43. Epub 1994/05/06. PubMed PMID: 8175775.
73. Sigmundsdottir H, Pan J, Debes GF, Alt C, Habtezion A, Soler D, et al. DCs metabolize sunlight-induced vitamin D3 to 'program' T cell attraction to the epidermal chemokine CCL27. *Nat Immunol* (2007) **8**(3):285-93. Epub 2007/01/30. doi: ni1433 [pii] 10\.1038/ni1433. PubMed PMID: 17259988.

74. Jeffery LE, Wood AM, Qureshi OS, Hou TZ, Gardner D, Briggs Z, et al. Availability of 25-hydroxyvitamin D(3) to APCs controls the balance between regulatory and inflammatory T cell responses. *J Immunol* (2012) **189**(11):5155-64. Epub 2012/10/23. doi: jimmunol.1200786 [pii] 10\.4049/jimmunol.1200786. PubMed PMID: 23087405; PubMed Central PMCID: PMC3504609.

75. Heine G, Niesner U, Chang HD, Steinmeyer A, Zugel U, Zuberbier T, et al. 1,25-dihydroxyvitamin D(3) promotes IL-10 production in human B cells. *Eur J Immunol* (2008) **38**(8):2210-8. Epub 2008/07/25. doi: 10.1002/eji.200838216. PubMed PMID: 18651709.

76. Correale J, Ysrraelit MC, Gaitan MI. Immunomodulatory effects of Vitamin D in multiple sclerosis. *Brain* (2009) **132**(Pt 5):1146-60. Epub 2009/03/27. doi: awp033 [pii] 10\.1093/brain/awp033. PubMed PMID: 19321461.

77. von Essen MR, Kongsbak M, Schjerling P, Olgaard K, Odum N, Geisler C. Vitamin D controls T cell antigen receptor signaling and activation of human T cells. *Nat Immunol* (2010) **11**(4):344-9. Epub 2010/03/09. doi: ni.1851 [pii] 10\.1038/ni.1851. PubMed PMID: 20208539.

78. Bakdash G, van Capel TM, Mason LM, Kapsenberg ML, de Jong EC. Vitamin D3 metabolite calcidiol primes human dendritic cells to promote the development of immunomodulatory IL-10-producing T cells. *Vaccine* (2014) **32**(47):6294-302. Epub 2014/09/23. doi: S0264-410X(14)01239-0 [pii] 10\.1016/j.vaccine.2014.08.075. PubMed PMID: 25236584.

79. Teichmann LL, Ols ML, Kashgarian M, Reizis B, Kaplan DH, Shlomchik MJ. Dendritic cells in lupus are not required for activation of T and B cells but promote their expansion, resulting in tissue damage. *Immunity* (2010) **33**(6):967-78. Epub 2010/12/21. doi: S1074-7613(10)00455-3 [pii] 10\.1016/j.immuni.2010.11.025. PubMed PMID: 21167752; PubMed Central PMCID: PMC3010763.

80. Wohn C, Ober-Blobaum JL, Haak S, Pantelyushin S, Cheong C, Zahner SP, et al. Langerin(neg) conventional dendritic cells produce IL-23 to drive psoriatic plaque formation in mice. *Proc Natl Acad Sci U S A* (2013) **110**(26):10723-8. Epub 2013/06/12. doi: 1307569110 [pii] 10\.1073/pnas.1307569110. PubMed PMID: 23754427; PubMed Central PMCID: PMC3696803.

81. Sisirak V, Ganguly D, Lewis KL, Couillault C, Tanaka L, Bolland S, et al. Genetic evidence for the role of plasmacytoid dendritic cells in systemic lupus erythematosus. *J Exp Med* (2014) **211**(10):1969-76. Epub 2014/09/03. doi: jem.20132522 [pii] 10\.1084/jem.20132522. PubMed PMID: 25180061; PubMed Central PMCID: PMC4172218.

82. Ferris ST, Carrero JA, Mohan JF, Calderon B, Murphy KM, Unanue ER. A minor subset of Batf3-dependent antigen-presenting cells in islets of Langerhans is essential for the development of autoimmune diabetes. *Immunity* (2014) **41**(4):657-69. Epub 2014/11/05. doi: S1074-7613(14)00346-X [pii] 10\.1016/j.immuni.2014.09.012. PubMed PMID: 25367577; PubMed Central PMCID: PMC4220295.

83. Schmidt H, Williamson D, Ashley-Koch A. HLA-DR15 haplotype and multiple sclerosis: a HuGE review. *Am J Epidemiol* (2007) **165**(10):1097-109. Epub 2007/03/03. doi: kwk118 [pii] 10\.1093/aje/kwk118. PubMed PMID: 17329717.

84. Mackie SL, Taylor JC, Martin SG, Consortium Y, Consortium U, Wordsworth P, et al. A spectrum of susceptibility to rheumatoid arthritis within HLA-DRB1: stratification by autoantibody status in a large UK population. *Genes Immun* (2012) **13**(2):120-8. Epub 2011/09/02. doi: gene201160 [pii] 10\.1038/gene.2011.60. PubMed PMID: 21881596.

85. Penna G, Adorini L. 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol* (2000) **164**(5):2405-11. Epub 2000/02/29. doi: ji_v164n5p2405 [pii]. PubMed PMID: 10679076.

86. Piemonti L, Monti P, Sironi M, Fraticelli P, Leone BE, Dal Cin E, et al. Vitamin D3 affects differentiation, maturation, and function of human monocyte-derived dendritic cells. *J Immunol* (2000) **164**(9):4443-51. Epub 2000/04/26. doi: ji_v164n9p4443 [pii]. PubMed PMID: 10779743.

87. Unger WW, Laban S, Kleijwegt FS, van der Slik AR, Roep BO. Induction of Treg by monocyte-derived DC modulated by vitamin D3 or dexamethasone: differential role for PD-L1. *Eur J Immunol* (2009) **39**(11):3147-59. Epub 2009/08/19. doi: 10.1002/eji.200839103. PubMed PMID: 19688742.

88. van Halteren AG, Tysma OM, van Etten E, Mathieu C, Roep BO. 1alpha,25-dihydroxyvitamin D3 or analogue treated dendritic cells modulate human autoreactive T cells via the selective induction of apoptosis. *J Autoimmun* (2004) **23**(3):233-9. Epub 2004/10/27. doi: S0896-8411(04)00073-3 [pii] 10\.1016/j.jaut.2004.06.004. PubMed PMID: 15501394.

89. Gambhir V, Kim J, Siddiqui S, Taylor M, Byford V, Petrof EO, et al. Influence of 1,25-dihydroxy vitamin D3 on TLR4-induced activation of antigen presenting cells is dependent on the order of receptor engagement. *Immunobiology* (2011) **216**(9):988-96. Epub 2011/05/03. doi: S0171-2985(11)00069-6 [pii] 10\.1016/j.imbio.2011.03.011. PubMed PMID: 21529994.

90. Penna G, Amuchastegui S, Giarratana N, Daniel KC, Vulcano M, Sozzani S, et al. 1,25-Dihydroxyvitamin D3 selectively modulates tolerogenic properties in myeloid but not plasmacytoid dendritic cells. *J Immunol* (2007) **178**(1):145-53. Epub 2006/12/22. doi: 178/1/145 [pii]. PubMed PMID: 17182549.

91. van der Aar AM, Sibiryak DS, Bakdash G, van Capel TM, van der Kleij HP, Opstelten DJ, et al. Vitamin D3 targets epidermal and dermal dendritic cells for induction of distinct regulatory T cells. *J Allergy Clin Immunol* (2011) **127**(6):1532-40 e7. Epub 2011/04/19. doi: S0091-6749(11)00363-0 [pii] 10\.1016/j.jaci.2011.01.068. PubMed PMID: 21497886.

92. Karthaus N, van Spriel AB, Looman MW, Chen S, Spilgies LM, Lieben L, et al. Vitamin D controls murine and human plasmacytoid dendritic cell function. *J Invest Dermatol* (2014) **134**(5):1255-64. Epub 2013/12/20. doi: S0022-202X(15)36791-9 [pii] 10\.1038/jid.2013.501. PubMed PMID: 24352045.
93. Ferreira GB, Vanherwegen AS, Eelen G, Gutierrez AC, Van Lommel L, Marchal K, et al. Vitamin D3 Induces Tolerance in Human Dendritic Cells by Activation of Intracellular Metabolic Pathways. *Cell Rep* (2015). Epub 2015/02/11. doi: S2211-1247(15)00026-1 [pii] 10\.1016/j.celrep.2015.01.013. PubMed PMID: 25660022.
94. Farias AS, Spagnol GS, Bordeaux-Rego P, Oliveira CO, Fontana AG, de Paula RF, et al. Vitamin D3 induces IDO+ tolerogenic DCs and enhances Treg, reducing the severity of EAE. *CNS Neurosci Ther* (2013) **19**(4):269-77. Epub 2013/03/26. doi: 10.1111/cns.12071. PubMed PMID: 23521914.
95. Huang Y, Zhao Y, Ran X, Wang C. Increased expression of herpesvirus entry mediator in 1,25-dihydroxyvitamin D3-treated mouse bone marrow-derived dendritic cells promotes the generation of CD4(+)CD25(+)Foxp3(+) regulatory T cells. *Mol Med Rep* (2014) **9**(3):813-8. Epub 2013/12/25. doi: 10.3892/mmr.2013.1874. PubMed PMID: 24366217.
96. Hu J, Wan Y. Tolerogenic dendritic cells and their potential applications. *Immunology* (2011) **132**(3):307-14. Epub 2011/01/07. doi: 10.1111/j.1365-2567.2010.03396.x. PubMed PMID: 21208205; PubMed Central PMCID: PMC3044897.
97. Griffin MD, Lutz W, Phan VA, Bachman LA, McKean DJ, Kumar R. Dendritic cell modulation by 1alpha,25 dihydroxyvitamin D3 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 U S A* (2001) **98**(12):6800-5. Epub 2001/05/24. doi: 10.1073/pnas.121172198 121172198 [pii]. PubMed PMID: 11371626; PubMed Central PMCID: PMC34433.
98. Stoop JN, Harry RA, von Delwig A, Isaacs JD, Robinson JH, Hilkens CM. Therapeutic effect of tolerogenic dendritic cells in established collagen-induced arthritis is associated with a reduction in Th17 responses. *Arthritis Rheum* (2010) **62**(12):3656-65. Epub 2010/09/24. doi: 10.1002/art.27756. PubMed PMID: 20862679.
99. Ferreira GB, Gysemans CA, Demengeot J, da Cunha JP, Vanherwegen AS, Overbergh L, et al. 1,25-Dihydroxyvitamin D3 promotes tolerogenic dendritic cells with functional migratory properties in NOD mice. *J Immunol* (2014) **192**(9):4210-20. Epub 2014/03/26. doi: jimmunol.1302350 [pii] 10\.4049/jimmunol.1302350. PubMed PMID: 24663679.
100. Mansilla MJ, Selles-Moreno C, Fabregas-Puig S, Amoedo J, Navarro-Barriuso J, Teniente-Serra A, et al. Beneficial effect of tolerogenic dendritic cells pulsed with MOG autoantigen in experimental autoimmune encephalomyelitis. *CNS Neurosci Ther* (2015) **21**(3):222-30. Epub 2014/11/19. doi: 10.1111/cns.12342. PubMed PMID: 25403984.

101. Harry RA, Anderson AE, Isaacs JD, Hilkens CM. Generation and characterisation of therapeutic tolerogenic dendritic cells for rheumatoid arthritis. *Ann Rheum Dis* (2010) **69**(11):2042-50. Epub 2010/06/17. doi: ard.2009.126383 [pii] 10\.1136/ard.2009.126383. PubMed PMID: 20551157; PubMed Central PMCID: PMC3002758.

102. Bartosik-Psujek H, Tabarkiewicz J, Pocinska K, Stelmasiak Z, Rolinski J. Immunomodulatory effects of vitamin D on monocyte-derived dendritic cells in multiple sclerosis. *Mult Scler* (2010) **16**(12):1513-6. Epub 2010/08/27. doi: 1352458510379611 [pii] 10\.1177/1352458510379611. PubMed PMID: 20739336.

103. Bartels LE, Jorgensen SP, Bendix M, Hvas CL, Agnholt J, Agger R, et al. 25-Hydroxy vitamin D3 modulates dendritic cell phenotype and function in Crohn's disease. *Inflammopharmacology* (2013) **21**(2):177-86. Epub 2013/01/24. doi: 10.1007/s10787-012-0168-y. PubMed PMID: 23341164.

104. Volchenkov R, Brun JG, Jonsson R, Appel S. In vitro suppression of immune responses using monocyte-derived tolerogenic dendritic cells from patients with primary Sjogren's syndrome. *Arthritis Res Ther* (2013) **15**(5):R114. Epub 2013/09/13. doi: ar4294 [pii] 10\.1186/ar4294. PubMed PMID: 24025795; PubMed Central PMCID: PMC3978468.

105. Wahono CS, Rusmini H, Soelistyoningsih D, Hakim R, Handono K, Endharti AT, et al. Effects of 1,25(OH)2D3 in immune response regulation of systemic lupus erithematosus (SLE) patient with hypovitamin D. *Int J Clin Exp Med* (2014) **7**(1):22-31. Epub 2014/02/01. PubMed PMID: 24482685; PubMed Central PMCID: PMC3902237.

106. Raiotach-Regue D, Grau-Lopez L, Naranjo-Gomez M, Ramo-Tello C, Pujol-Borrell R, Martinez-Caceres E, et al. Stable antigen-specific T-cell hyporesponsiveness induced by tolerogenic dendritic cells from multiple sclerosis patients. *Eur J Immunol* (2012) **42**(3):771-82. Epub 2012/04/11. doi: 10.1002/eji.201141835. PubMed PMID: 22488365.

107. Giannoukakis N, Phillips B, Finegold D, Harnaha J, Trucco M. Phase I (safety) study of autologous tolerogenic dendritic cells in type 1 diabetic patients. *Diabetes Care* (2011) **34**(9):2026-32. Epub 2011/06/18. doi: dc11-0472 [pii] 10\.2337/dc11-0472. PubMed PMID: 21680720; PubMed Central PMCID: PMC3161299.

108. Benham H, Nel HJ, Law SC, Mehdi AM, Street S, Ramnoruth N, et al. Citrullinated peptide dendritic cell immunotherapy in HLA risk genotype-positive rheumatoid arthritis patients. *Sci Transl Med* (2015) **7**(290):290ra87. Epub 2015/06/05. doi: 7/290/290ra87 [pii] 10\.1126/scitranslmed.aaa9301. PubMed PMID: 26041704.

109. Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nat Rev Immunol* (2011) **11**(11):723-37. Epub 2011/10/15. doi: nri3073 [pii] 10\.1038/nri3073. PubMed PMID: 21997792; PubMed Central PMCID: PMC3422549.

110. Tak PP, Smeets TJ, Daha MR, Kluin PM, Meijers KA, Brand R, et al. Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. *Arthritis Rheum* (1997) **40**(2):217-25. Epub 1997/02/01. PubMed PMID: 9041933.

111. Kamada N, Hisamatsu T, Okamoto S, Chinen H, Kobayashi T, Sato T, et al. Unique CD14 intestinal macrophages contribute to the pathogenesis of Crohn disease via IL-23/IFN-gamma axis. *J Clin Invest* (2008) **118**(6):2269-80. Epub 2008/05/24. doi: 10.1172/JCI34610. PubMed PMID: 18497880; PubMed Central PMCID: PMC2391067.

112. van Horssen J, Singh S, van der Pol S, Kipp M, Lim JL, Peferoen L, et al. Clusters of activated microglia in normal-appearing white matter show signs of innate immune activation. *J Neuroinflammation* (2012) **9**:156. Epub 2012/07/04. doi: 1742-2094-9-156 [pii] 10\.1186/1742-2094-9-156. PubMed PMID: 22747960; PubMed Central PMCID: PMC3411485.

113. Singh S, Metz I, Amor S, van der Valk P, Stadelmann C, Bruck W. Microglial nodules in early multiple sclerosis white matter are associated with degenerating axons. *Acta Neuropathol* (2013) **125**(4):595-608. Epub 2013/01/29. doi: 10.1007/s00401-013-1082-0. PubMed PMID: 23354834; PubMed Central PMCID: PMC3611040.

114. Orme J, Mohan C. Macrophage subpopulations in systemic lupus erythematosus. *Discov Med* (2012) **13**(69):151-8. Epub 2012/03/01. PubMed PMID: 22369974.

115. Neve A, Corrado A, Cantatore FP. Immunomodulatory effects of vitamin D in peripheral blood monocyte-derived macrophages from patients with rheumatoid arthritis. *Clin Exp Med* (2014) **14**(3):275-83. Epub 2013/07/05. doi: 10.1007/s10238-013-0249-2. PubMed PMID: 23824148.

116. Jun HS, Yoon CS, Zbytnuik L, van Rooijen N, Yoon JW. The role of macrophages in T cell-mediated autoimmune diabetes in nonobese diabetic mice. *J Exp Med* (1999) **189**(2):347-58. Epub 1999/01/20. PubMed PMID: 9892617; PubMed Central PMCID: PMC2192977.

117. Nashold FE, Miller DJ, Hayes CE. 1,25-dihydroxyvitamin D3 treatment decreases macrophage accumulation in the CNS of mice with experimental autoimmune encephalomyelitis. *J Neuroimmunol* (2000) **103**(2):171-9. Epub 2000/03/04. doi: S0165572899002477 [pii]. PubMed PMID: 10696912.

118. Xu H, Soruri A, Gieseler RK, Peters JH. 1,25-Dihydroxyvitamin D3 exerts opposing effects to IL-4 on MHC class-II antigen expression, accessory activity, and phagocytosis of human monocytes. *Scand J Immunol* (1993) **38**(6):535-40. Epub 1993/12/01. PubMed PMID: 8256111.

119. Krutzik SR, Hewison M, Liu PT, Robles JA, Stenger S, Adams JS, et al. IL-15 links TLR2/1-induced macrophage differentiation to the vitamin D-dependent antimicrobial pathway. *J Immunol* (2008) **181**(10):7115-20. Epub 2008/11/05. doi: 181/10/7115 [pii]. PubMed PMID: 18981132; PubMed Central PMCID: PMC2678236.

120. Fabri M, Stenger S, Shin DM, Yuk JM, Liu PT, Realegeno S, et al. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. *Sci Transl Med* (2011) **3**(104):104ra2. Epub 2011/10/15. doi: 3/104/104ra102 [pii] 10\.1126/scitranslmed.3003045. PubMed PMID: 21998409; PubMed Central PMCID: PMC3269210.

121. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* (2006) **311**(5768):1770-3. Epub 2006/02/25. doi: 1123933 [pii] 10\.1126/science.1123933. PubMed PMID: 16497887.

122. Liu PT, Stenger S, Tang DH, Modlin RL. Cutting edge: vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. *J Immunol* (2007) **179**(4):2060-3. Epub 2007/08/07. doi: 179/4/2060 [pii]. PubMed PMID: 17675463.

123. Lee BN, Kim TH, Jun JB, Yoo DH, Woo JH, Choi SJ, et al. Upregulation of interleukin-1beta production by 1,25-dihydroxyvitamin D(3) in activated human macrophages. *Mol Biol Rep* (2011) **38**(3):2193-201. Epub 2010/09/18. doi: 10.1007/s11033-010-0348-z. PubMed PMID: 20848209.

124. Verway M, Bouttier M, Wang TT, Carrier M, Calderon M, An BS, et al. Vitamin D induces interleukin-1beta expression: paracrine macrophage epithelial signaling controls M. tuberculosis infection. *PLoS Pathog* (2013) **9**(6):e1003407. Epub 2013/06/14. doi: 10.1371/journal.ppat.1003407 PPATHOGENS-D-12-02616 [pii]. PubMed PMID: 23762029; PubMed Central PMCID: PMC3675149.

125. Chen Y, Liu W, Sun T, Huang Y, Wang Y, Deb DK, et al. 1,25-Dihydroxyvitamin D promotes negative feedback regulation of TLR signaling via targeting microRNA-155-SOCS1 in macrophages. *J Immunol* (2013) **190**(7):3687-95. Epub 2013/02/26. doi: jimmunol.1203273 [pii] 10\.4049/jimmunol.1203273. PubMed PMID: 23436936; PubMed Central PMCID: PMC3608760.

126. Wang Q, He Y, Shen Y, Zhang Q, Chen D, Zuo C, et al. Vitamin D inhibits COX-2 expression and inflammatory response by targeting thioesterase superfamily member 4. *J Biol Chem* (2014) **289**(17):11681-94. Epub 2014/03/13. doi: M113.517581 [pii] 10\.1074/jbc.M113.517581. PubMed PMID: 24619416; PubMed Central PMCID: PMC4002078.

127. Zhang X, Zhou M, Guo Y, Song Z, Liu B. 1,25-Dihydroxyvitamin D(3) Promotes High Glucose-Induced M1 Macrophage Switching to M2 via the VDR-PPARgamma Signaling Pathway. *Biomed Res Int* (2015) **2015**:157834. Epub 2015/05/12. doi: 10.1155/2015/157834. PubMed PMID: 25961000; PubMed Central PMCID: PMC4417570.

128. Korf H, Wenes M, Stijlemans B, Takiishi T, Robert S, Miani M, et al. 1,25-Dihydroxyvitamin D3 curtails the inflammatory and T cell stimulatory capacity of macrophages through an IL-10-dependent mechanism. *Immunobiology* (2012) **217**(12):1292-300. Epub 2012/09/05. doi: S0171-2985(12)00182-9 [pii] 10\.1016/j.imbio.2012.07.018. PubMed PMID: 22944250.

129. Kumar V, Abbas AK, Fausto N, Mitchell R. Robbins Basic Pathology. 8 ed: Elsevier Health Sciences (2007).

130. van Venrooij WJ, van Beers JJ, Pruijn GJ. Anti-CCP antibodies: the past, the present and the future. *Nat Rev Rheumatol* (2011) **7**(7):391-8. Epub 2011/06/08. doi: nrrheum.2011.76 [pii] 10\.1038/nrrheum.2011.76. PubMed PMID: 21647203.

131. Ramagopalan SV, Heger A, Berlanga AJ, Maugeri NJ, Lincoln MR, Burrell A, et al. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. *Genome Res* (2010) **20**(10):1352-60. Epub 2010/08/26. doi: gr.107920.110 [pii] 10\.1101/gr.107920.110. PubMed PMID: 20736230; PubMed Central PMCID: PMC2945184.

132. Pieper K, Grimbacher B, Eibel H. B-cell biology and development. *J Allergy Clin Immunol* (2013) **131**(4):959-71. Epub 2013/03/08. doi: S0091-6749(13)00256-X [pii] 10\.1016/j.jaci.2013.01.046. PubMed PMID: 23465663.

133. Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. *J Immunol* (2007) **179**(3):1634-47. Epub 2007/07/21. doi: 179/3/1634 [pii]. PubMed PMID: 17641030.

134. Lemire JM, Adams JS, Sakai R, Jordan SC. 1 alpha,25-dihydroxyvitamin D3 suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells. *J Clin Invest* (1984) **74**(2):657-61. Epub 1984/08/01. doi: 10.1172/JCI111465. PubMed PMID: 6611355; PubMed Central PMCID: PMC370520.

135. Iho S, Takahashi T, Kura F, Sugiyama H, Hoshino T. The effect of 1,25-dihydroxyvitamin D3 on in vitro immunoglobulin production in human B cells. *J Immunol* (1986) **136**(12):4427-31. Epub 1986/06/15. PubMed PMID: 3519769.

136. Geldmeyer-Hilt K, Heine G, Hartmann B, Baumgrass R, Radbruch A, Worm M. 1,25-dihydroxyvitamin D3 impairs NF-kappaB activation in human naive B cells. *Biochem Biophys Res Commun* (2011) **407**(4):699-702. Epub 2011/03/23. doi: S0006-291X(11)00475-X [pii] 10\.1016/j.bbrc.2011.03.078. PubMed PMID: 21420936.

137. Shirakawa AK, Nagakubo D, Hieshima K, Nakayama T, Jin Z, Yoshie O. 1,25-dihydroxyvitamin D3 induces CCR10 expression in terminally differentiating human B cells. *J Immunol* (2008) **180**(5):2786-95. Epub 2008/02/23. doi: 180/5/2786 [pii]. PubMed PMID: 18292499.

138. Chen WC, Vayuvegula B, Gupta S. 1,25-Dihydroxyvitamin D3-mediated inhibition of human B cell differentiation. *Clin Exp Immunol* (1987) **69**(3):639-46. Epub 1987/09/01. PubMed PMID: 3117462; PubMed Central PMCID: PMC1542374.

139. Heine G, Anton K, Henz BM, Worm M. 1alpha,25-dihydroxyvitamin D3 inhibits anti-CD40 plus IL-4-mediated IgE production in vitro. *Eur J Immunol* (2002) **32**(12):3395-404. Epub 2002/11/15. doi: 10.1002/1521-4141(200212)32:12<3395::AID-IMMU3395>3.0.CO;2-#. PubMed PMID: 12432570.

140. Ritterhouse LL, Crowe SR, Niewold TB, Kamen DL, Macwana SR, Roberts VC, et al. Vitamin D deficiency is associated with an increased autoimmune response in healthy individuals and in patients with systemic lupus erythematosus. *Ann Rheum Dis* (2011) **70**(9):1569-74. Epub 2011/05/19. doi: ard.2010.148494 [pii] 10\.1136/ard.2010.148494. PubMed PMID: 21586442; PubMed Central PMCID: PMC3149865.

141. Terrier B, Derian N, Schoindre Y, Chaara W, Geri G, Zahr N, et al. Restoration of regulatory and effector T cell balance and B cell homeostasis in systemic lupus erythematosus patients through vitamin D supplementation. *Arthritis Res Ther* (2012) **14**(5):R221. Epub 2012/10/19. doi: ar4060 [pii] 10\.1186/ar4060. PubMed PMID: 23075451; PubMed Central PMCID: PMC3580532.

142. Knippenberg S, Peelen E, Smolders J, Thewissen M, Menheere P, Cohen Tervaert JW, et al. Reduction in IL-10 producing B cells (Breg) in multiple sclerosis is accompanied by a reduced naive/memory Breg ratio during a relapse but not in remission. *J Neuroimmunol* (2011) **239**(1-2):80-6. Epub 2011/09/24. doi: S0165-5728(11)00236-0 [pii] 10\.1016/j.jneuroim.2011.08.019. PubMed PMID: 21940055.

143. Drozdenko G, Scheel T, Heine G, Baumgrass R, Worm M. Impaired T cell activation and cytokine production by calcitriol-primed human B cells. *Clin Exp Immunol* (2014) **178**(2):364-72. Epub 2014/06/27. doi: 10.1111/cei.12406. PubMed PMID: 24965738; PubMed Central PMCID: PMC4233385.

144. Chen L, Cencioni MT, Angelini DF, Borsellino G, Battistini L, Brosnan CF. Transcriptional profiling of gamma delta T cells identifies a role for vitamin D in the immunoregulation of the V gamma 9V delta 2 response to phosphate-containing ligands. *J Immunol* (2005) **174**(10):6144-52. Epub 2005/05/10. doi: 174/10/6144 [pii]. PubMed PMID: 15879110.

145. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. *Arch Biochem Biophys* (2000) **374**(2):334-8. Epub 2000/02/10. doi: 10.1006/abbi.1999.1605 S0003-9861(99)91605-3 [pii]. PubMed PMID: 10666315.

146. Hirahara K, Nakayama T. CD4+ T-cell subsets in inflammatory diseases: beyond the Th1/Th2 paradigm. *Int Immunol* (2016). Epub 2016/02/14. doi: dxw006 [pii] 10\.1093/intimm/dxw006. PubMed PMID: 26874355.

147. Mayne CG, Spanier JA, Relland LM, Williams CB, Hayes CE. 1,25-Dihydroxyvitamin D3 acts directly on the T lymphocyte vitamin D receptor to inhibit experimental autoimmune encephalomyelitis. *Eur J Immunol* (2011) **41**(3):822-32. Epub 2011/02/03. doi: 10.1002/eji.201040632. PubMed PMID: 21287548.

148. Meehan TF, DeLuca HF. CD8(+) T cells are not necessary for 1 alpha,25-dihydroxyvitamin D(3) to suppress experimental autoimmune encephalomyelitis in mice. *Proc Natl Acad Sci U S A* (2002) **99**(8):5557-60. Epub 2002/04/04. doi: 10.1073/pnas.082100699 082100699 [pii]. PubMed PMID: 11929984; PubMed Central PMCID: PMC122808.

149. Grishkan IV, Fairchild AN, Calabresi PA, Gocke AR. 1,25-Dihydroxyvitamin D3 selectively and reversibly impairs T helper-cell CNS localization. *Proc Natl Acad Sci U S A* (2013) **110**(52):21101-6. Epub 2013/12/11. doi: 1306072110 [pii] 10\.1073/pnas.1306072110. PubMed PMID: 24324134; PubMed Central PMCID: PMC3876241.

150. Palmer MT, Lee YK, Maynard CL, Oliver JR, Bikle DD, Jetten AM, et al. Lineage-specific effects of 1,25-dihydroxyvitamin D(3) on the development of effector CD4 T cells. *J Biol Chem* (2011) **286**(2):997-1004. Epub 2010/11/05. doi: M110.163790 [pii] 10\.1074/jbc.M110.163790. PubMed PMID: 21047796; PubMed Central PMCID: PMC3020784.

151. Nath N, Prasad R, Giri S, Singh AK, Singh I. T-bet is essential for the progression of experimental autoimmune encephalomyelitis. *Immunology* (2006) **118**(3):384-91. Epub 2006/07/11. doi: IMM2385 [pii] 10\.1111/j.1365-2567.2006.02385.x. PubMed PMID: 16827899; PubMed Central PMCID: PMC1782298.

152. Ferber IA, Brocke S, Taylor-Edwards C, Ridgway W, Dinisco C, Steinman L, et al. Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). *J Immunol* (1996) **156**(1):5-7. Epub 1996/01/01. PubMed PMID: 8598493.

153. Damsker JM, Hansen AM, Caspi RR. Th1 and Th17 cells: adversaries and collaborators. *Ann N Y Acad Sci* (2010) **1183**:211-21. Epub 2010/02/12. doi: NYAS5133 [pii] 10\.1111/j.1749-6632.2009.05133.x. PubMed PMID: 20146717; PubMed Central PMCID: PMC2914500.

154. Waldburger KE, Hastings RC, Schaub RG, Goldman SJ, Leonard JP. Adoptive transfer of experimental allergic encephalomyelitis after in vitro treatment with recombinant murine interleukin-12. Preferential expansion of interferon-gamma-producing cells and increased expression of macrophage-associated inducible nitric oxide synthase as immunomodulatory mechanisms. *Am J Pathol* (1996) **148**(2):375-82. Epub 1996/02/01. PubMed PMID: 8579100; PubMed Central PMCID: PMC1861690.

155. Pichler J, Gerstmayr M, Szepfalusi Z, Urbanek R, Peterlik M, Willheim M. 1 alpha,25(OH)2D3 inhibits not only Th1 but also Th2 differentiation in human cord blood T cells. *Pediatr Res* (2002) **52**(1):12-8. Epub 2002/06/27. doi: 10.1203/00006450-200207000-00005. PubMed PMID: 12084841.

156. Staeva-Vieira TP, Freedman LP. 1,25-dihydroxyvitamin D3 inhibits IFN-gamma and IL-4 levels during in vitro polarization of primary murine CD4+ T cells. *J Immunol* (2002) **168**(3):1181-9. Epub 2002/01/22. PubMed PMID: 11801653.

157. Takeuchi A, Reddy GS, Kobayashi T, Okano T, Park J, Sharma S. Nuclear factor of activated T cells (NFAT) as a molecular target for 1alpha,25-dihydroxyvitamin D3-mediated effects. *J Immunol* (1998) **160**(1):209-18. Epub 1998/04/29. PubMed PMID: 9551973.

158. Peelen E, Thewissen M, Knippenberg S, Smolders J, Muris AH, Menheere P, et al. Fraction of IL-10+ and IL-17+ CD8 T cells is increased in MS patients in remission and during a relapse, but is not influenced by immune modulators. *J Neuroimmunol* (2013) **258**(1-2):77-84. Epub 2013/03/23. doi: S0165-5728(13)00052-0 [pii] 10\.1016/j.jneuroim.2013.02.014. PubMed PMID: 23517930.

159. Chang SH, Chung Y, Dong C. Vitamin D suppresses Th17 cytokine production by inducing C/EBP homologous protein (CHOP) expression. *J Biol Chem* (2010) **285**(50):38751-5. Epub 2010/10/27. doi: C110.185777 [pii] 10\.1074/jbc.C110.185777. PubMed PMID: 20974859; PubMed Central PMCID: PMC2998156.

160. van Hamburg JP, Mus AM, de Bruijn MJ, de Vogel L, Boon L, Cornelissen F, et al. GATA-3 protects against severe joint inflammation and bone erosion and reduces differentiation of Th17 cells during experimental arthritis. *Arthritis Rheum* (2009) **60**(3):750-9. Epub 2009/02/28. doi: 10.1002/art.24329. PubMed PMID: 19248112.

161. Waddell A, Zhao J, Cantorna MT. NKT cells can help mediate the protective effects of 1,25-dihydroxyvitamin D3 in experimental autoimmune encephalomyelitis in mice. *Int Immunol* (2015) **27**(5):237-44. Epub 2015/01/13. doi: dxu147 [pii] 10\.1093/intimm/dxu147. PubMed PMID: 25574039; PubMed Central PMCID: PMC4406266.

162. Sloka S, Silva C, Wang J, Yong VW. Predominance of Th2 polarization by vitamin D through a STAT6-dependent mechanism. *J Neuroinflammation* (2011) **8**:56. Epub 2011/05/25. doi: 1742-2094-8-56 [pii] 10\.1186/1742-2094-8-56. PubMed PMID: 21605467; PubMed Central PMCID: PMC3118349.

163. Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF, O'Garra A. 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. *J Immunol* (2001) **167**(9):4974-80. Epub 2001/10/24. PubMed PMID: 11673504.

164. Khoo AL, Joosten I, Michels M, Woestenenk R, Preijers F, He XH, et al. 1,25-Dihydroxyvitamin D3 inhibits proliferation but not the suppressive function of regulatory T cells in the absence of antigen-presenting cells. *Immunology* (2011) **134**(4):459-68. Epub 2011/11/03. doi: 10.1111/j.1365-2567.2011.03507.x. PubMed PMID: 22044285; PubMed Central PMCID: PMC3230799.

165. Colin EM, Asmawidjaja PS, van Hamburg JP, Mus AM, van Driel M, Hazes JM, et al. 1,25-dihydroxyvitamin D3 modulates Th17 polarization and interleukin-22 expression by memory T cells from patients with early rheumatoid arthritis. *Arthritis Rheum* (2010) **62**(1):132-42. Epub 2009/12/30. doi: 10.1002/art.25043. PubMed PMID: 20039421.

166. Piantoni S, Andreoli L, Scarsi M, Zanola A, Dall'Ara F, Pizzorni C, et al. Phenotype modifications of T-cells and their shift toward a Th2 response in patients with systemic lupus erythematosus supplemented with different monthly regimens of vitamin D. *Lupus* (2015) **24**(4-5):490-8. Epub 2015/03/25. doi: 24/4-5/490 [pii] 10\.1177/0961203314559090. PubMed PMID: 25801892.

167. Smolders J, Peelen E, Thewissen M, Cohen Tervaert JW, Menheere P, Hupperts R, et al. Safety and T cell modulating effects of high dose vitamin D3 supplementation in multiple sclerosis. *PLoS One* (2010) **5**(12):e15235. Epub 2010/12/24. doi: 10.1371/journal.pone.0015235. PubMed PMID: 21179201; PubMed Central PMCID: PMC3001453.

168. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, et al. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat Immunol* (2007) **8**(12):1390-7. Epub 2007/11/13. doi: ni1539 [pii] 10\.1038/ni1539. PubMed PMID: 17994024.

169. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* (2003) **421**(6924):744-8. Epub 2003/03/01. doi: 10.1038/nature01355 nature01355 [pii]. PubMed PMID: 12610626.

170. Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, et al. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. *J Exp Med* (2003) **198**(12):1951-7. Epub 2003/12/10. doi: 10.1084/jem.20030896 jem.20030896 [pii]. PubMed PMID: 14662908; PubMed Central PMCID: PMC2194162.

171. Lubberts E, Joosten LA, van de Loo FA, Schwarzenberger P, Kolls J, van den Berg WB. Overexpression of IL-17 in the knee joint of collagen type II immunized mice promotes collagen arthritis and aggravates joint destruction. *Inflamm Res* (2002) **51**(2):102-4. Epub 2002/04/05. PubMed PMID: 11930902.

172. Hirota K, Duarte JH, Veldhoen M, Hornsby E, Li Y, Cua DJ, et al. Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat Immunol* (2011) **12**(3):255-63. Epub 2011/02/01. doi: ni.1993 [pii] 10\.1038/ni.1993. PubMed PMID: 21278737; PubMed Central PMCID: PMC3040235.

173. van Hamburg JP, Asmawidjaja PS, Davelaar N, Mus AM, Colin EM, Hazes JM, et al. Th17 cells, but not Th1 cells, from patients with early rheumatoid arthritis are potent inducers of matrix metalloproteinases and proinflammatory cytokines upon synovial fibroblast interaction, including autocrine interleukin-17A production. *Arthritis Rheum* (2011) **63**(1):73-83. Epub 2010/10/19. doi: 10.1002/art.30093. PubMed PMID: 20954258.

174. Yang J, Chu Y, Yang X, Gao D, Zhu L, Yang X, et al. Th17 and natural Treg cell population dynamics in systemic lupus erythematosus. *Arthritis Rheum* (2009) **60**(5):1472-83. Epub 2009/05/01. doi: 10.1002/art.24499. PubMed PMID: 19404966.

175. Leipe J, Grunke M, Dechant C, Reindl C, Kerzendorf U, Schulze-Koops H, et al. Role of Th17 cells in human autoimmune arthritis. *Arthritis Rheum* (2010) **62**(10):2876-85. Epub 2010/06/29. doi: 10.1002/art.27622. PubMed PMID: 20583102.

176. Tang J, Zhou R, Luger D, Zhu W, Silver PB, Grajewski RS, et al. Calcitriol suppresses antiretinal autoimmunity through inhibitory effects on the Th17 effector response. *J Immunol* (2009) **182**(8):4624-32. Epub 2009/04/04. doi: 182/8/4624 [pii] 10\.4049/jimmunol.0801543. PubMed PMID: 19342637; PubMed Central PMCID: PMC2756755.

177. Jeffery LE, Burke F, Mura M, Zheng Y, Qureshi OS, Hewison M, et al. 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. *J Immunol* (2009) **183**(9):5458-67. Epub 2009/10/22. doi: 183/9/5458 [pii] 10\.4049/jimmunol.0803217. PubMed PMID: 19843932; PubMed Central PMCID: PMC2810518.

178. Joshi S, Pantalena LC, Liu XK, Gaffen SL, Liu H, Rohowsky-Kochan C, et al. 1,25-dihydroxyvitamin D(3) ameliorates Th17 autoimmunity via transcriptional modulation of interleukin-17A. *Mol Cell Biol* (2011) **31**(17):3653-69. Epub 2011/07/13. doi: MCB.05020-11 [pii] 10\.1128/MCB.05020-11. PubMed PMID: 21746882; PubMed Central PMCID: PMC3165548.

179. van Hamburg JP, Asmawidjaja PS, Davelaar N, Mus AM, Cornelissen F, van Leeuwen JP, et al. TNF blockade requires 1,25(OH)2D3 to control human Th17-mediated synovial inflammation. *Ann Rheum Dis* (2012) **71**(4):606-12. Epub 2012/01/06. doi: annrheumdis-2011-200424 [pii] 10\.1136/annrheumdis-2011-200424. PubMed PMID: 22219138.

180. Jeffery LE, Qureshi OS, Gardner D, Hou TZ, Briggs Z, Soskic B, et al. Vitamin D Antagonises the Suppressive Effect of Inflammatory Cytokines on CTLA-4 Expression and Regulatory Function. *PLoS One* (2015) **10**(7):e0131539. Epub 2015/07/03. doi: 10.1371/journal.pone.0131539 PONE-D-15-16310 [pii]. PubMed PMID: 26134669; PubMed Central PMCID: PMC4489761.

181. Tian Y, Wang C, Ye Z, Xiao X, Kijlstra A, Yang P. Effect of 1,25-dihydroxyvitamin D3 on Th17 and Th1 response in patients with Behcet's disease. *Invest Ophthalmol Vis Sci* (2012) **53**(10):6434-41. Epub 2012/08/25. doi: iovs.12-10398 [pii] 10\.1167/iovs.12-10398. PubMed PMID: 22918640.

182. Chang JH, Cha HR, Lee DS, Seo KY, Kweon MN. 1,25-Dihydroxyvitamin D3 inhibits the differentiation and migration of T(H)17 cells to protect against experimental autoimmune encephalomyelitis. *PLoS One* (2010) **5**(9):e12925. Epub 2010/10/05. doi: 10.1371/journal.pone.0012925. PubMed PMID: 20886077; PubMed Central PMCID: PMC2944871.

183. Mukasa R, Balasubramani A, Lee YK, Whitley SK, Weaver BT, Shibata Y, et al. Epigenetic instability of cytokine and transcription factor gene loci underlies plasticity of the T helper 17 cell lineage. *Immunity* (2010) **32**(5):616-27. Epub 2010/05/18. doi: S1074-7613(10)00165-2 [pii] 10\.1016/j.immuni.2010.04.016. PubMed PMID: 20471290; PubMed Central PMCID: PMC3129685.

184. Nanduri R, Mahajan S, Bhagyaraj E, Sethi K, Kalra R, Chandra V, et al. The Active Form of Vitamin D Transcriptionally Represses Smad7 Signaling and Activates Extracellular Signal-regulated Kinase (ERK) to Inhibit the Differentiation of a Inflammatory T Helper Cell Subset and Suppress Experimental Autoimmune Encephalomyelitis. *J Biol Chem* (2015) **290**(19):12222-36. Epub 2015/03/27. doi: M114.621839 [pii] 10\.1074/jbc.M114.621839. PubMed PMID: 25809484; PubMed Central PMCID: PMC4424354.

185. Maggi L, Santarlasci V, Capone M, Rossi MC, Querci V, Mazzoni A, et al. Distinctive features of classic and nonclassic (Th17 derived) human Th1 cells. *Eur J Immunol* (2012) **42**(12):3180-8. Epub 2012/09/12. doi: 10.1002/eji.201242648. PubMed PMID: 22965818.

186. Cosmi L, Liotta F, Maggi E, Romagnani S, Annunziato F. Th17 and non-classic Th1 cells in chronic inflammatory disorders: two sides of the same coin. *Int Arch Allergy Immunol* (2014) **164**(3):171-7. Epub 2014/07/19. doi: 000363502 [pii] 10\.1159/000363502. PubMed PMID: 25033972.

187. Paulissen SM, van Hamburg JP, Dankers W, Lubberts E. The role and modulation of CCR6+ Th17 cell populations in rheumatoid arthritis. *Cytokine* (2015) **74**(1):43-53. Epub 2015/04/02. doi: S1043-4666(15)00057-5 [pii] 10\.1016/j.cyto.2015.02.002. PubMed PMID: 25828206.

188. Sotirchos ES, Bhargava P, Eckstein C, Van Haren K, Baynes M, Ntranos A, et al. Safety and immunologic effects of high- vs low-dose cholecalciferol in multiple sclerosis. *Neurology* (2016) **86**(4):382-90. Epub 2016/01/01. doi: WNL.0000000000002316 [pii] 10\.1212/WNL.0000000000002316. PubMed PMID: 26718578; PubMed Central PMCID: PMC4776090.

189. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* (2001) **27**(1):20-1. Epub 2001/01/04. doi: 10.1038/83713. PubMed PMID: 11137993.

190. Spach KM, Nashold FE, Dittel BN, Hayes CE. IL-10 signaling is essential for 1,25-dihydroxyvitamin D3-mediated inhibition of experimental autoimmune encephalomyelitis. *J Immunol* (2006) **177**(9):6030-7. Epub 2006/10/24. doi: 177/9/6030 [pii]. PubMed PMID: 17056528.

191. Van Belle TL, Vanherwegen AS, Feyaerts D, De Clercq P, Verstuyf A, Korf H, et al. 1,25-Dihydroxyvitamin D3 and its analog TX527 promote a stable regulatory T cell phenotype in T cells from type 1 diabetes patients. *PLoS One* (2014) **9**(10):e109194. Epub 2014/10/04. doi: 10.1371/journal.pone.0109194 PONE-D-14-12459 [pii]. PubMed PMID: 25279717; PubMed Central PMCID: PMC4184870.

192. Urry Z, Chambers ES, Xystrakis E, Dimeloe S, Richards DF, Gabrysova L, et al. The role of 1alpha,25-dihydroxyvitamin D3 and cytokines in the promotion of distinct Foxp3+ and IL-10+ CD4+ T cells. *Eur J Immunol* (2012) **42**(10):2697-708. Epub 2012/08/21. doi: 10.1002/eji.201242370. PubMed PMID: 22903229; PubMed Central PMCID: PMC3471131.

193. Smolders J, Thewissen M, Peelen E, Menheere P, Tervaert JW, Damoiseaux J, et al. Vitamin D status is positively correlated with regulatory T cell function in patients with multiple sclerosis. *PLoS One* (2009) **4**(8):e6635. Epub 2009/08/14. doi: 10.1371/journal.pone.0006635. PubMed PMID: 19675671; PubMed Central PMCID: PMC2721656.

194. Kang SW, Kim SH, Lee N, Lee WW, Hwang KA, Shin MS, et al. 1,25-Dihyroxyvitamin D3 promotes FOXP3 expression via binding to vitamin D response elements in its conserved noncoding sequence region. *J Immunol* (2012) **188**(11):5276-82. Epub 2012/04/25. doi: jimmunol.1101211 [pii] 10\.4049/jimmunol.1101211. PubMed PMID: 22529297; PubMed Central PMCID: PMC3358577.

195. Bock G, Prietl B, Mader JK, Holler E, Wolf M, Pilz S, et al. The effect of vitamin D supplementation on peripheral regulatory T cells and beta cell function in healthy humans: a randomized controlled trial. *Diabetes Metab Res Rev* (2011) **27**(8):942-5. Epub 2011/11/10. doi: 10.1002/dmrr.1276. PubMed PMID: 22069289.

196. Zhang N, Bevan MJ. CD8(+) T cells: foot soldiers of the immune system. *Immunity* (2011) **35**(2):161-8. Epub 2011/08/27. doi: S1074-7613(11)00303-7 [pii] 10\.1016/j.immuni.2011.07.010. PubMed PMID: 21867926; PubMed Central PMCID: PMC3303224.

197. Huseby ES, Liggitt D, Brabb T, Schnabel B, Ohlen C, Goverman J. A pathogenic role for myelin-specific CD8(+) T cells in a model for multiple sclerosis. *J Exp Med* (2001) **194**(5):669-76. Epub 2001/09/06. PubMed PMID: 11535634; PubMed Central PMCID: PMC2195947.

198. Sun D, Whitaker JN, Huang Z, Liu D, Coleclough C, Wekerle H, et al. Myelin antigen-specific CD8+ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. *J Immunol* (2001) **166**(12):7579-87. Epub 2001/06/08. PubMed PMID: 11390514.

199. Steinhoff U, Brinkmann V, Klemm U, Aichele P, Seiler P, Brandt U, et al. Autoimmune intestinal pathology induced by hsp60-specific CD8 T cells. *Immunity* (1999) **11**(3):349-58. Epub 1999/10/08. doi: S1074-7613(00)80110-7 [pii]. PubMed PMID: 10514013.

200. Menon B, Gullick NJ, Walter GJ, Rajasekhar M, Garrood T, Evans HG, et al. Interleukin-17+CD8+ T cells are enriched in the joints of patients with psoriatic arthritis and correlate with disease activity and joint damage progression. *Arthritis Rheumatol* (2014) **66**(5):1272-81. Epub 2014/01/29. doi: 10.1002/art.38376. PubMed PMID: 24470327; PubMed Central PMCID: PMC4158887.

201. Chen J, Bruce D, Cantorna MT. Vitamin D receptor expression controls proliferation of naive CD8+ T cells and development of CD8 mediated gastrointestinal inflammation. *BMC Immunol* (2014) **15**:6. Epub 2014/02/08. doi: 1471-2172-15-6 [pii] 10\.1186/1471-2172-15-6. PubMed PMID: 24502291; PubMed Central PMCID: PMC3923390.

202. Lysandropoulos AP, Jaquiery E, Jilek S, Pantaleo G, Schluep M, Du Pasquier RA. Vitamin D has a direct immunomodulatory effect on CD8+ T cells of patients with early multiple sclerosis and healthy control subjects. *J Neuroimmunol* (2011) **233**(1-2):240-4. Epub 2010/12/28. doi: S0165-5728(10)00519-9 [pii] 10\.1016/j.jneuroim.2010.11.008. PubMed PMID: 21186064.

203. Dyring-Andersen B, Bonefeld CM, Bzorek M, Lovendorf MB, Lauritsen JP, Skov L, et al. The Vitamin D Analogue Calcipotriol Reduces the Frequency of CD8+ IL-17+ T Cells in Psoriasis Lesions. *Scand J Immunol* (2015) **82**(1):84-91. Epub 2015/04/24. doi: 10.1111/sji.12304. PubMed PMID: 25904071.

204. Bruce D, Cantorna MT. Intrinsic requirement for the vitamin D receptor in the development of CD8alphaalpha-expressing T cells. *J Immunol* (2011) **186**(5):2819-25. Epub 2011/01/29. doi: jimmunol.1003444 [pii] 10\.4049/jimmunol.1003444. PubMed PMID: 21270396; PubMed Central PMCID: PMC3127166.

205. Godfrey DI, Uldrich AP, McCluskey J, Rossjohn J, Moody DB. The burgeoning family of unconventional T cells. *Nat Immunol* (2015) **16**(11):1114-23. Epub 2015/10/21. doi: ni.3298 [pii] 10\.1038/ni.3298. PubMed PMID: 26482978.

206. Edwards SC, McGinley AM, McGuinness NC, Mills KH. gammadelta T Cells and NK Cells - Distinct Pathogenic Roles as Innate-Like Immune Cells in CNS Autoimmunity. *Front Immunol* (2015) **6**:455. Epub 2015/10/07. doi: 10.3389/fimmu.2015.00455. PubMed PMID: 26441960; PubMed Central PMCID: PMC4561808.

207. Wu L, Van Kaer L. Natural killer T cells in health and disease. *Front Biosci (Schol Ed)* (2011) **3**:236-51. Epub 2011/01/05. doi: 148 [pii]. PubMed PMID: 21196373; PubMed Central PMCID: PMC3626278.

208. Yu S, Cantorna MT. The vitamin D receptor is required for iNKT cell development. *Proc Natl Acad Sci U S A* (2008) **105**(13):5207-12. Epub 2008/03/28. doi: 0711558105 [pii] 10\.1073/pnas.0711558105. PubMed PMID: 18364394; PubMed Central PMCID: PMC2278204.

209. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, et al. Innate lymphoid cells--a proposal for uniform nomenclature. *Nat Rev Immunol* (2013) **13**(2):145-9. Epub 2013/01/26. doi: nri3365 [pii] 10\.1038/nri3365. PubMed PMID: 23348417.

210. Poggi A, Zocchi MR. NK cell autoreactivity and autoimmune diseases. *Front Immunol* (2014) **5**:27. Epub 2014/02/20. doi: 10.3389/fimmu.2014.00027. PubMed PMID: 24550913; PubMed Central PMCID: PMC3912987.

211. Balogh G, de Boland AR, Boland R, Barja P. Effect of 1,25(OH)(2)-vitamin D(3) on the activation of natural killer cells: role of protein kinase C and extracellular calcium. *Exp Mol Pathol* (1999) **67**(2):63-74. Epub 1999/10/21. doi: 10.1006/exmp.1999.2264 S0014-4800(99)92264-5 [pii]. PubMed PMID: 10527758.

212. Weeres MA, Robien K, Ahn YO, Neulen ML, Bergerson R, Miller JS, et al. The effects of 1,25-dihydroxyvitamin D3 on in vitro human NK cell development from hematopoietic stem cells. *J Immunol* (2014) **193**(7):3456-62. Epub 2014/08/26. doi: jimmunol.1400698 [pii] 10\.4049/jimmunol.1400698. PubMed PMID: 25149465; PubMed Central PMCID: PMC4363084.

213. Ota K, Dambaeva S, Kim MW, Han AR, Fukui A, Gilman-Sachs A, et al. 1,25-Dihydroxyvitamin D3 regulates NK-cell cytotoxicity, cytokine secretion, and degranulation in women with recurrent pregnancy losses. *Eur J Immunol* (2015) **45**(11):3188-99. Epub 2015/08/11. doi: 10.1002/eji.201545541. PubMed PMID: 26257123.

214. Villanova F, Flutter B, Tosi I, Grys K, Sreeneebus H, Perera GK, et al. Characterization of innate lymphoid cells in human skin and blood demonstrates increase of NKp44+ ILC3 in psoriasis. *J Invest Dermatol* (2014) **134**(4):984-91. Epub 2013/12/20. doi: S0022-202X(15)36698-7 [pii] 10\.1038/jid.2013.477. PubMed PMID: 24352038; PubMed Central PMCID: PMC3961476.

215. Teunissen MB, Munneke JM, Bernink JH, Spuls PI, Res PC, Te Velde A, et al. Composition of innate lymphoid cell subsets in the human skin: enrichment of NCR(+) ILC3 in lesional skin and blood of psoriasis patients. *J Invest Dermatol* (2014) **134**(9):2351-60. Epub 2014/03/25. doi: S0022-202X(15)36976-1 [pii] 10\.1038/jid.2014.146. PubMed PMID: 24658504.

216. Geremia A, Arancibia-Carcamo CV, Fleming MP, Rust N, Singh B, Mortensen NJ, et al. IL-23-responsive innate lymphoid cells are increased in inflammatory bowel disease. *J Exp Med* (2011) **208**(6):1127-33. Epub 2011/05/18. doi: jem.20101712 [pii] 10\.1084/jem.20101712. PubMed PMID: 21576383; PubMed Central PMCID: PMC3173242.

217. Perry JS, Han S, Xu Q, Herman ML, Kennedy LB, Csako G, et al. Inhibition of LTi cell development by CD25 blockade is associated with decreased intrathecal inflammation in multiple sclerosis. *Sci Transl Med* (2012) **4**(145):145ra06. Epub 2012/08/03. doi: 4/145/145ra106 [pii] 10\.1126/scitranslmed.3004140. PubMed PMID: 22855463; PubMed Central PMCID: PMC3846177.

218. Ciccia F, Guggino G, Giardina A, Ferrante A, Carrubbi F, Giacomelli R, et al. The role of innate and lymphoid IL-22-producing cells in the immunopathology of primary Sjogren's syndrome. *Expert Rev Clin Immunol* (2014) **10**(4):533-41. Epub 2014/02/05. doi: 10.1586/1744666X.2014.884461. PubMed PMID: 24490899.

219. Buonocore S, Ahern PP, Uhlig HH, Ivanov, II, Littman DR, Maloy KJ, et al. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature* (2010) **464**(7293):1371-5. Epub 2010/04/16. doi: nature08949 [pii] 10\.1038/nature08949. PubMed PMID: 20393462; PubMed Central PMCID: PMC3796764.

220. Chen J, Waddell A, Lin YD, Cantorna MT. Dysbiosis caused by vitamin D receptor deficiency confers colonization resistance to Citrobacter rodentium through modulation of innate lymphoid cells. *Mucosal Immunol* (2015) **8**(3):618-26. Epub 2014/10/16. doi: mi201494 [pii] 10\.1038/mi.2014.94. PubMed PMID: 25315967; PubMed Central PMCID: PMC4398576.

221. Ding N, Yu RT, Subramaniam N, Sherman MH, Wilson C, Rao R, et al. A vitamin D receptor/SMAD genomic circuit gates hepatic fibrotic response. *Cell* (2013) **153**(3):601-13. doi: 10.1016/j.cell.2013.03.028. PubMed PMID: 23622244; PubMed Central PMCID: PMC3673534.

222. Sherman MH, Yu RT, Engle DD, Ding N, Atkins AR, Tiriac H, et al. Vitamin D receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy. *Cell* (2014) **159**(1):80-93. doi: 10.1016/j.cell.2014.08.007. PubMed PMID: 25259922; PubMed Central PMCID: PMC4177038.

223. Laragione T, Shah A, Gulko PS. The vitamin D receptor regulates rheumatoid arthritis synovial fibroblast invasion and morphology. *Mol Med* (2012) **18**:194-200. Epub 2011/11/09. doi: molmed.2011.00410 [pii] 10\.2119/molmed.2011.00410. PubMed PMID: 22064970; PubMed Central PMCID: PMC3320133.

224. Wu S, Liao AP, Xia Y, Li YC, Li JD, Sartor RB, et al. Vitamin D receptor negatively regulates bacterial-stimulated NF-kappaB activity in intestine. *Am J Pathol* (2010) **177**(2):686-97. Epub 2010/06/23. doi: S0002-9440(10)60126-5 [pii] 10\.2353/ajpath.2010.090998. PubMed PMID: 20566739; PubMed Central PMCID: PMC2913341.

225. Nissou MF, Guttin A, Zenga C, Berger F, Issartel JP, Wion D. Additional clues for a protective role of vitamin D in neurodegenerative diseases: 1,25-dihydroxyvitamin D3 triggers an anti-inflammatory response in brain pericytes. *J Alzheimers Dis* (2014) **42**(3):789-99. Epub 2014/06/18. doi: N22Q7774R7N85287 [pii] 10\.3233/JAD-140411. PubMed PMID: 24934545.

226. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* (2011) **96**(7):1911-30. Epub 2011/06/08. doi: jc.2011-0385 [pii] 10\.1210/jc.2011-0385. PubMed PMID: 21646368.

227. Aspray TJ, Bowring C, Fraser W, Gittoes N, Javaid MK, Macdonald H, et al. National Osteoporosis Society vitamin D guideline summary. *Age Ageing* (2014) **43**(5):592-5. Epub 2014/07/31. doi: afu093 [pii] 10\.1093/ageing/afu093. PubMed PMID: 25074538.

228. Miheller P, Muzes G, Hritz I, Lakatos G, Pregun I, Lakatos PL, et al. Comparison of the effects of 1,25 dihydroxyvitamin D and 25 hydroxyvitamin D on bone pathology and disease activity in Crohn's disease patients. *Inflamm Bowel Dis* (2009) **15**(11):1656-62. Epub 2009/05/02. doi: 10.1002/ibd.20947. PubMed PMID: 19408329.

229. Ferreira GB, Overbergh L, Verstuyf A, Mathieu C. 1alpha,25-Dihydroxyvitamin D3 and its analogs as modulators of human dendritic cells: a comparison dose-titration study. *J Steroid Biochem Mol Biol* (2013) **136**:160-5. Epub 2012/10/27. doi: S0960-0760(12)00209-9 [pii] 10\.1016/j.jsbmb.2012.10.009. PubMed PMID: 23098690.

230. Verlinden L, Leyssens C, Beullens I, Marcelis S, Mathieu C, De Clercq P, et al. The vitamin D analog TX527 ameliorates disease symptoms in a chemically induced model of inflammatory bowel disease. *J Steroid Biochem Mol Biol* (2013) **136**:107-11. Epub 2012/09/25. doi: S0960-0760(12)00173-2 [pii] 10\.1016/j.jsbmb.2012.09.017. PubMed PMID: 23000190.

231. Kiekhaefer CM, Weber B, Huggins M, Gorichanaz C, Nehring JA, DeLuca HF. 2alpha-Methyl-19-nor-(20S)-1,25-dihydroxyvitamin D(3) protects the insulin 2 knockout non-obese diabetic mouse from developing type 1 diabetes without hypercalcaemia. *Clin Exp Immunol* (2011) **166**(3):325-32. Epub 2011/11/09. doi: 10.1111/j.1365-2249.2011.04481.x. PubMed PMID: 22059989; PubMed Central PMCID: PMC3232379.

232. Sochorova K, Budinsky V, Rozkova D, Tobiasova Z, Dusilova-Sulkova S, Spisek R, et al. Paricalcitol (19-nor-1,25-dihydroxyvitamin D2) and calcitriol (1,25-dihydroxyvitamin D3) exert potent immunomodulatory effects on dendritic cells and inhibit induction of antigen-specific T cells. *Clin Immunol* (2009) **133**(1):69-77. Epub 2009/08/08. doi: S1521-6616(09)00728-1 [pii] 10\.1016/j.clim.2009.06.011. PubMed PMID: 19660988.

233. Larsson P, Mattsson L, Klareskog L, Johnsson C. A vitamin D analogue (MC 1288) has immunomodulatory properties and suppresses collagen-induced arthritis (CIA) without causing hypercalcaemia. *Clin Exp Immunol* (1998) **114**(2):277-83. Epub 1998/11/20. PubMed PMID: 9822288; PubMed Central PMCID: PMC1905103.

234. Ikeda U, Wakita D, Ohkuri T, Chamoto K, Kitamura H, Iwakura Y, et al. 1alpha,25-Dihydroxyvitamin D3 and all-trans retinoic acid synergistically inhibit the differentiation and expansion of Th17 cells. *Immunol Lett* (2010) **134**(1):7-16. Epub 2010/07/27. doi: S0165-2478(10)00189-6 [pii] 10\.1016/j.imlet.2010.07.002. PubMed PMID: 20655952.

235. Ferreira GB, Kleijwegt FS, Waelkens E, Lage K, Nikolic T, Hansen DA, et al. Differential protein pathways in 1,25-dihydroxyvitamin d(3) and dexamethasone modulated tolerogenic human dendritic cells. *J Proteome Res* (2012) **11**(2):941-71. Epub 2011/11/23. doi: 10.1021/pr200724e. PubMed PMID: 22103328.

236. Zhang Y, Leung DY, Goleva E. Vitamin D enhances glucocorticoid action in human monocytes: involvement of granulocyte-macrophage colony-stimulating factor and mediator complex subunit 14. *J Biol Chem* (2013) **288**(20):14544-53. Epub 2013/04/11. doi: M112.427054 [pii] 10\.1074/jbc.M112.427054. PubMed PMID: 23572530; PubMed Central PMCID: PMC3656308.

237. Paintlia AS, Paintlia MK, Hollis BW, Singh AK, Singh I. Interference with RhoA-ROCK signaling mechanism in autoreactive CD4+ T cells enhances the bioavailability of 1,25-dihydroxyvitamin D3 in experimental autoimmune encephalomyelitis. *Am J Pathol* (2012) **181**(3):993-1006. Epub 2012/07/17. doi: S0002-9440(12)00433-6 [pii] 10\.1016/j.ajpath.2012.05.028. PubMed PMID: 22796435; PubMed Central PMCID: PMC3432427.

238. Matilainen JM, Husso T, Toropainen S, Seuter S, Turunen MP, Gynther P, et al. Primary effect of 1alpha,25(OH)(2)D(3) on IL-10 expression in monocytes is short-term down-regulation. *Biochim Biophys Acta* (2010) **1803**(11):1276-86. Epub 2010/08/10. doi: S0167-4889(10)00203-X [pii] 10\.1016/j.bbamcr.2010.07.009. PubMed PMID: 20691220.

239. Seuter S, Heikkinen S, Carlberg C. Chromatin acetylation at transcription start sites and vitamin D receptor binding regions relates to effects of 1alpha,25-dihydroxyvitamin D3 and histone deacetylase inhibitors on gene expression. *Nucleic Acids Res* (2013) **41**(1):110-24. Epub 2012/10/25. doi: gks959 [pii] 10\.1093/nar/gks959. PubMed PMID: 23093607; PubMed Central PMCID: PMC3592476.

240. Seuter S, Ryynanen J, Carlberg C. The ASAP2 gene is a primary target of 1,25-dihydroxyvitamin D3 in human monocytes and macrophages. *J Steroid Biochem Mol Biol* (2014) **144 Pt A**:12-8. Epub 2013/09/04. doi: S0960-0760(13)00157-X [pii] 10\.1016/j.jsbmb.2013.08.014. PubMed PMID: 23999061.

241. Ryynanen J, Carlberg C. Primary 1,25-dihydroxyvitamin D3 response of the interleukin 8 gene cluster in human monocyte- and macrophage-like cells. *PLoS One* (2013) **8**(10):e78170. Epub 2013/11/20. doi: 10.1371/journal.pone.0078170 PONE-D-13-31741 [pii]. PubMed PMID: 24250750; PubMed Central PMCID: PMC3824026.

242. Nurminen V, Neme A, Ryynanen J, Heikkinen S, Seuter S, Carlberg C. The transcriptional regulator BCL6 participates in the secondary gene regulatory response to vitamin D. *Biochim Biophys Acta* (2015) **1849**(3):300-8. Epub 2014/12/09. doi: S1874-9399(14)00295-8 [pii] 10\.1016/j.bbagrm.2014.12.001. PubMed PMID: 25482012.

243. Seuter S, Pehkonen P, Heikkinen S, Carlberg C. Dynamics of 1alpha,25-dihydroxyvitamin D3-dependent chromatin accessibility of early vitamin D receptor target genes. *Biochim Biophys Acta* (2013) **1829**(12):1266-75. Epub 2013/11/05. doi: S1874-9399(13)00152-1 [pii] 10\.1016/j.bbagrm.2013.10.003. PubMed PMID: 24185200.

244. Seuter S, Neme A, Carlberg C. Characterization of genomic vitamin D receptor binding sites through chromatin looping and opening. *PLoS One* (2014) **9**(4):e96184. Epub 2014/04/26. doi: 10.1371/journal.pone.0096184 PONE-D-14-00646 [pii]. PubMed PMID: 24763502; PubMed Central PMCID: PMC3999108.

245. Kimball S, Vieth R, Dosch HM, Bar-Or A, Cheung R, Gagne D, et al. Cholecalciferol plus calcium suppresses abnormal PBMC reactivity in patients with multiple sclerosis. *J Clin Endocrinol Metab* (2011) **96**(9):2826-34. Epub 2011/06/24. doi: jc.2011-0325 [pii] 10\.1210/jc.2011-0325. PubMed PMID: 21697250; PubMed Central PMCID: PMC3417163.

246. Bendix-Struve M, Bartels LE, Agnholt J, Dige A, Jorgensen SP, Dahlerup JF. Vitamin D3 treatment of Crohn's disease patients increases stimulated T cell IL-6 production and proliferation. *Aliment Pharmacol Ther* (2010) **32**(11-12):1364-72. Epub 2010/11/06. doi: 10.1111/j.1365-2036.2010.04463.x. PubMed PMID: 21050239.

247. Yang L, Weaver V, Smith JP, Bingaman S, Hartman TJ, Cantorna MT. Therapeutic effect of vitamin d supplementation in a pilot study of Crohn's patients. *Clin Transl Gastroenterol* (2013) **4**:e33. Epub 2013/04/19. doi: ctg20131 [pii] 10\.1038/ctg.2013.1. PubMed PMID: 23594800; PubMed Central PMCID: PMC3636524.

248. Andreoli L, Dall'Ara F, Piantoni S, Zanola A, Piva N, Cutolo M, et al. A 24-month prospective study on the efficacy and safety of two different monthly regimens of vitamin D supplementation in pre-menopausal women with systemic lupus erythematosus. *Lupus* (2015) **24**(4-5):499-506. Epub 2015/03/25. doi: 24/4-5/499 [pii] 10\.1177/0961203314559089. PubMed PMID: 25801893.