Vitamin D in rheumatoid arthritis—towards clinical application

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Abstract | In addition to its well-documented involvement in mineral homeostasis, vitamin D seems to have broad effects on human health that go beyond the skeletal system. Prominent among these so-called nonclassical effects of vitamin D are its immunomodulatory properties. *In vitro* studies have shown anti-inflammatory effects of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the active form of vitamin D. In addition, epidemiological analysis of patients with established inflammatory disease identified associations between vitamin D deficiency (low serum concentrations of inactive 25-hydroxyvitamin D, abbreviated to 25(OH)D) and inflammatory conditions, including rheumatoid arthritis (RA). The association of vitamin D deficiency with RA severity supports the hypothesis of a role for vitamin D in the initiation or progression of the disease, or possibly both. However, whether 25(OH)D status is a cause or consequence of RA is still incompletely understood and requires further analysis in prospective vitamin D supplementation trials. The characterization of factors that promote the transition from preclinical to clinical phases of RA has become a major focus of research, with the aim to facilitate earlier diagnosis and treatment, and improve therapeutic outcomes. In this Review, we aim to describe the current knowledge of vitamin D and the immune system specifically in RA, and discuss the potential benefits that vitamin D might have on slowing RA progression.

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Introduction

Rheumatoid arthritis (RA) is a progressive inflammatory disease characterized by inflammation of the synovium that leads to the destruction of joint bone and cartilage. The cellular and molecular processes that characterize the pathology of RA have been the focus of intense research that enabled the design of specific and efficacious therapies. Genetic factors are known to contribute to the risk of RA;[1] additionally, a range of environmental factors that include tobacco smoking, alcohol intake[2,3] and dietary factors[4–6] have also been shown to contribute to the risk of RA. Interestingly, it has been suggested that some genetic and environmental risk factors interact to increase the risk of RA.[7] One potential environmental factor for RA that has been studied extensively in the past decade is vitamin D. This focus is due, in part, to the accumulating evidence suggesting that a worldwide deficiency in vitamin D might be linked with common health problems in humans.[8] These epidemiological observations have been supported by *in vitro* studies in which the active form of vitamin D 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) has potent antiproliferative, antibacterial and anti-inflammatory properties.[9] Immunomodulatory responses to $1,25(OH)_2D$ are likely to be physiologically important owing to several cells from the immune system expressing the vitamin D receptor (VDR), the mitochondrial enzyme 25-hydroxyvitamin D-1α-hydroxylase (also known as 1α-hydroxylase, which catalyses the synthesis of the active form of vitamin D $1,25(OH)_2D$ from its precursor 25-hydroxyvitamin D (25(OH)D), or both.[10]

The self-contained intracrine or paracrine model for vitamin D metabolism and signalling in the immune system provides a mechanism by which the (apparently) inactive metabolite 25(OH)D can influence both innate and adaptive immune responses by means of localized metabolism to $1,25(OH)_2D$.[11,12] As 25(OH)D is the major form of vitamin D in circulation and the principal determinant of patient vitamin D 'status', 25(OH)D deficiency has been suggested to lead to impaired immune system-related synthesis of $1,25(OH)_2D$ and consequential suboptimal antibacterial and anti-inflammatory immune responses.[13] Consistent with this hypothesis, some epidemiological studies have reported an inverse association between serum 25(OH)D concentrations and RA disease activity and severity.[14] Nevertheless, the effect of vitamin D in RA causality and progression is still undefined. The role of vitamin D in RA will be assessed in part by new placebo-controlled supplementation trials, but it should be recognized that a link between vitamin D and RA is supported by mechanistic data showing effects of 25(OH)D and $1,25(OH)_2D$ on immune system function.

The existence of a preclinical phase in RA was first described in the 1980s, with the detection of rheumatoid factor often several years before the onset of symptomatic arthritis.[15] The presence of antibodies to citrullinated matrix proteins (anti-citrullinated protein antibodies, ACPAs) before the onset of RA was subsequently reported.[16,17] As research in this area grew exponentially,[18]

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Key points

- Nonclassical effects of vitamin D have been recognized for many years, but were only accepted as an important component of vitamin D physiology in the past decade
- Prominent among the nonclassical effects of vitamin D are its anti-inflammatory properties
- Vitamin D deficiency is prevalent worldwide and has been linked to several chronic inflammatory diseases, including rheumatoid arthritis (RA)
- Randomized controlled trials of vitamin D supplementation in prospective at-risk cohorts and in patients with active RA are needed to assess the effect of vitamin D on disease initiation and progression

five discrete phases—from phase A (genetic risk factors of RA) to phase E (unclassified arthritis)—were defined to describe the progress towards established RA.[19] In this Review, we explore the links between vitamin D and RA from a clinical and mechanistic perspective, with particular emphasis on the role of vitamin D in the progression from preclinical to clinical disease.

Vitamin D metabolism

Endocrine pathway

Vitamin D is produced after UV light stimulation of skin or is acquired from dietary sources, and is subsequently activated in sequential metabolic steps by specific cytochrome P450 enzymes. In the first of these steps, the enzyme vitamin D 25-hydroxylase, expressed primarily in the liver, generates the principal circulating form of vitamin D, 25(OH)D. In the second step, 25(OH)D is metabolized to the active form $1,25(OH)_2D$ by 1α-hydroxylase, which can then exert biological effects on target cells expressing the VDR. In this setting, circulating $1,25(OH)_2D$ acts as a hormone to maintain intestinal uptake of minerals—notably calcium—and optimal bone function (Figure 1).[20] While this endocrine model of vitamin D requires 25(OH)D for activation by 1α-hydroxylase, the efficacy of renal synthesis of $1,25(OH)_2D$ is also dependent on other hormonal factors such as parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23), which positively and negatively regulate 1α-hydroxylase activity, respectively.[20]

Intracrine and paracrine pathways

In contrast to the endocrine regulation of calcium homeostasis, the immunomodulatory actions of vitamin D seem to be dependent on localized intracrine or paracrine conversion of 25(OH)D to $1,25(OH)_2D$, an effect mainly involving dendritic cells (DCs) and macrophages expressing 1α-hydroxylase[11,12] (Figure 2). *In vitro* studies have shown that the ability of DCs and macrophages to synthesize $1,25(OH)_2D$ is increased by immune factors such as cytokines, and by pathogen-associated molecular patterns.[10] Furthermore, unlike the endocrine effects of vitamin D on the skeleton that are tightly regulated by peptides such as PTH and FGF23,[20] the immunomodulatory actions of vitamin D seem to be far more dependent on the availability of precursor 25(OH)D for conversion to $1,25(OH)_2D$.[21] As such, innate and adaptive immune functions associated with intracrine and paracrine activity of 1α-hydroxylase might be compromised under conditions of 25(OH)D deficiency,[22] or might be, conversely, enhanced by vitamin D supplementation.[21]

Linking immune disorders and vitamin D

The substrate dependency model for 1α-hydroxylase in DCs and macrophages is a pivotal component in the proposed benefits of vitamin D supplementation for immune disorders. However, this model has probably been oversimplified, particularly when considering immune disorders in which expression of 1α-hydroxylase might be dysregulated.

One of the first observations linking vitamin D and the immune system was the increase in circulating levels of $1,25(OH)_2D$ associated with hypercalcaemia in some patients with granulomatous diseases such as sarcoidosis.[23] Later studies showed that this systemic effect resulted from dysregulated 1α-hydroxylase activity in disease-associated macrophages, with locally-generated $1,25(OH)_2D$ spreading into the circulation.[24] Similar disease-associated, extra-renal 1α-hydroxylase activity has also been reported in patients with RA, in whom synovial macrophages had an increased capacity for $1,25(OH)_2D$ synthesis.[25] However, similarly to the observations in granulomatous diseases, the underlying basis for dysregulated extra-renal vitamin D metabolism in patients with RA remains unclear.

In both sets of observations, the systemic consequences of macrophage 1α-hydroxylase activity seem to be highly dependent on substrate availability, but elevated serum $1,25(OH)_2D$ has only been associated with hypercalcaemia in patients with RA who received vitamin D or 25(OH)D supplementation.[26,27] Conversely, in patients with RA who did not receive these supplements, circulating concentrations of $1,25(OH)_2D$ were negatively correlated with disease activity,[28] with levels of the metabolite being lower than normal in many patients.[29] Although these initial studies were carried out in patients who had established disease, a subsequent report described similar suppression of circulating $1,25(OH)_2D$ at baseline and during the first year of disease in a polyarthritis cohort that included patients with RA.[30] Notably, this later study showed that progression of RA was associated with decreased levels of circulating 25(OH)D and $1,25(OH)_2D$, and low levels of both metabolites predicted worse Health Assessment Questionnaire scores.[30] These effects of RA on vitamin D metabolism are likely to be further complicated by the therapeutic use of anti-inflammatory steroids, which can supress circulating concentrations of $1,25(OH)_2D$ in patients with RA via effects on extra-renal 1α-hydroxylase activity.[31]

The apparent reduction of serum 25(OH)D and $1,25(OH)_2D$ levels in patients with RA raises important questions concerning the importance of renal (endocrine) versus extra-renal (intracrine or paracrine) vitamin D metabolism in the pathophysiology of this disease (Figure 1). Even though renal and extra-renal $1,25(OH)_2D$ are the product of *CYP27B1* (which encodes 1α-hydroxylase), this gene seems to be differentially regulated at renal and extra-renal sites. Notably, inflammatory cytokines such as TNF, IL-15 and IL-1β—which are

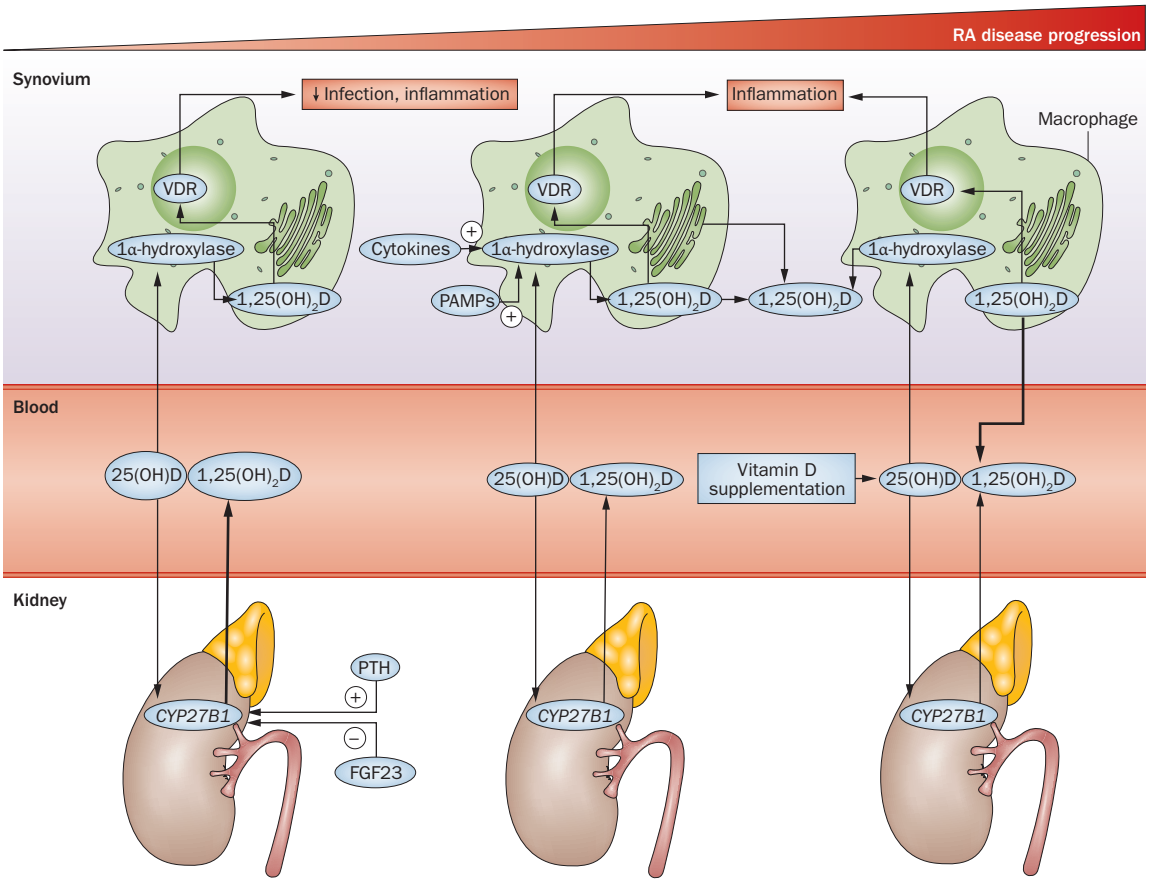

Figure 1 | Renal and extra-renal vitamin D metabolism in RA progression. In healthy individuals, metabolism of the precursor 25(OH)D to active $1,25(OH)_2D$ occurs primarily in the kidneys, catalyzed by the product of *CYP27B1*, 1α-hydroxylase. In this setting, the synthesis of $1,25(OH)_2D$ is regulated in an endocrine manner: positively by PTH and negatively by FGF23. Extra-renal synthesis of $1,25(OH)_2D$ by synovial macrophages is restricted to the local tissue microenvironment, and facilitates antimicrobial and anti-inflammatory immune responses; in this case, the synthesis of $1,25(OH)_2D$ is stimulated by PAMPs and inflammatory cytokines. RA progression has been linked to lower concentrations of serum 25(OH)D, impaired renal *CYP27B1* expression and decreased serum $1,25(OH)_2D$, but also to increased extra-renal expression of *CYP27B1* in disease-associated macrophages. In patients with RA who received vitamin D supplements the resulting increased synovial concentrations of $1,25(OH)_2D$ could have spread to the general circulation. VDR activation in macrophages has a dampening effect on inflammation. The effect of enhanced localized synthesis of $1,25(OH)_2D$ on synovial immune cell function remains unclear. Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; FGF23, fibroblast growth factor 23; PAMP, pathogen-associated molecular pattern; PTH, parathyroid hormone; RA, rheumatoid arthritis; VDR, vitamin D receptor.

characteristic of RA—are known to stimulate expression of *CYP27B1* in monocytes,[32] DCs[33] and endothelial cells.[34] By contrast, *CYP27B1* promoter analyses suggest that inflammatory mediators, such as NFκB, suppress expression of *CYP27B1* in a possible mechanism for inflammatory inhibition of renal synthesis of $1,25(OH)_2D$.[35] Additionally, as low serum $1,25(OH)_2D$ levels can occur in RA before steroid therapy, they might be a cause for the reduced bone mineral density frequently observed in patients with this disorder. In this setting, supplementation with vitamin D to elevate serum concentrations of 25(OH)D might overcome the inflammatory suppression of renal 1α-hydroxylase activity and restore normal serum levels of $1,25(OH)_2D$. However, as previously mentioned,[26,27] these strategies can lead to exaggerated extra-renal synthesis of $1,25(OH)_2D$ by synovial macrophages in patients with advanced RA, with potential spreading into the circulation. With this in mind, it will be important that future studies explore the effects of vitamin D supplementation in early stage RA, in which the dysregulation of synovial macrophage *CYP27B1* expression might be minimal. In these patients, increased availability of 25(OH)D might enhance intracrine synovial production of anti-inflammatory $1,25(OH)_2D$ without affecting the systemic levels of this metabolite. At the same time, increased availability of substrate 25(OH)D in early RA might help skeletal homeostasis by normalization of renal synthesis of $1,25(OH)_2D$.

Cellular targets for vitamin D in RA

The finding that specific receptors for $1,25(OH)_2D$ were expressed by lymphocytes from patients with RA was an early piece of evidence linking vitamin D and immune function.[36] VDR expression has since been described

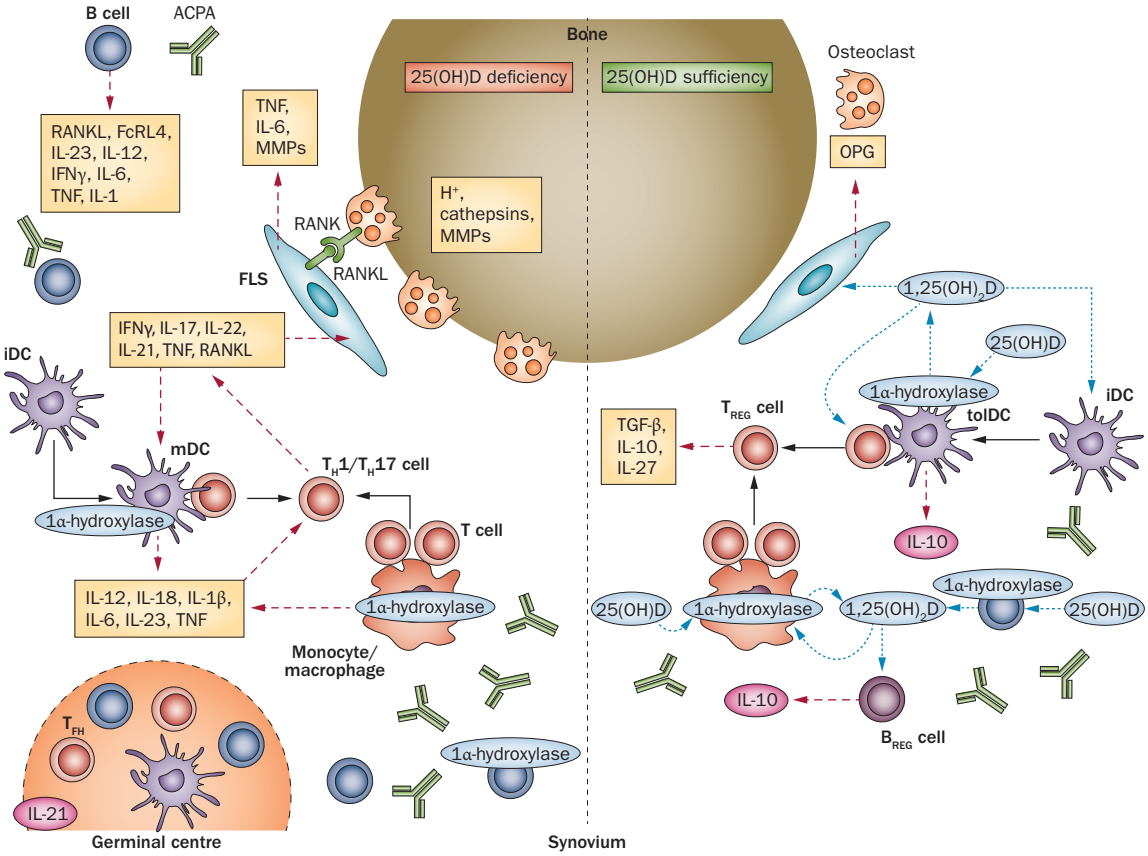

Figure 2 | Vitamin D status and synovial immune cell function. In RA, vitamin D deficiency favours inflammatory responses and osteoclast-mediated bone loss. Autoantigens such as citrullinated matrix proteins are presented to CD4$^+$ T cells by professional APCs such as DCs or monocytes and macrophages. DCs, monocytes, macrophages and B cells express the vitamin D-activating enzyme 1α-hydroxylase and can generate $1,25(OH)_2D$ from 25(OH)D under conditions of vitamin D sufficiency. Although expression of *CYP27B1* is enhanced in mDCs, these cells promote a T_H1 cell or T_H17 cell phenotype in the absence of sufficient 25(OH)D. IFNγ$^+$ T_H1 cells promote APC maturation, whereas T_H17 cells act on FLSs to promote release of inflammatory cytokines and MMPs and to express RANKL. RANKL expression by FLSs, T_H17 cells and FCRL4$^+$ B cells drives osteoclastogenesis and bone erosion. Vitamin D deficiency also favours the production of IL-21 and generation of T_{FH}-mediated germinal centres for B-cell activation and differentiation. By contrast, in conditions of vitamin D sufficiency, 25(OH)D is metabolized to $1,25(OH)_2D$ by 1α-hydroxylase-expressing APCs, promoting the differentiation of tolDCs, which favour the differentiation of T_{REG} cells and B_{REG} cells. $1,25(OH)_2D$ produced by APCs or B_{REG} cells might also act directly on T cells and B cells to promote an anti-inflammatory phenotype, and enhances expression of OPG over RANKL by FLSs, thereby reducing osteoclastogenesis. Cell differentiation pathways (black arrows), cytokine production and function (red arrows) and vitamin D metabolism (blue arrows). Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; ACPA, anti-citrullinated protein antibody; APC, antigen-presenting cell; B_{REG}, B regulatory cell; DC, dendritic cell; FCRL4, Fc receptor-like protein 4; FLS, fibroblast-like synoviocyte; iDC, immature dendritic cell; mDC; myeloid dendritic cell; MMP, matrix metalloproteinase; OPG, osteoprotogerin; RA, rheumatoid arthritis; RANK: tumor necrosis factor receptor superfamily member 11A; RANKL, tumor necrosis factor ligand superfamily member 11; T_{FH}, T follicular helper cell; T_H, T helper cell; tolDC; tolerogenic dendritic cell; T_{REG}, T regulatory cell.

in many other cells of the immune system,[37] supporting a wide array of potential actions for $1,25(OH)_2D$. These include innate antibacterial responses in monocytes and neutrophils, effects on antigen presentation by DCs, and modulation of T-cell and B-cell phenotype and function.[11,12] Multiple immune-cell and stromal-cell interactions are involved in RA pathogenesis, with persisting inflammatory connections culminating in joint destruction as the balance between bone-degrading osteoclast and bone-forming osteoblast activities shifts in favour of bone destruction. T cells, synovial macrophages and fibroblast-like synoviocytes (FLSs) seem to be the major cellular players in RA, but B cells and DCs also have important roles (Figure 2).[38] When assessing the potential relevance of vitamin D in the pathology of RA, it is therefore important to consider the roles of these different cell types in RA pathogenesis—and the effect of vitamin D on each of them.

Antigen-presenting cells and T helper cells

CD4$^+$ T helper (T_H) cells are the key orchestrators of antigen-specific adaptive immune responses. Although no unique T-cell receptor (TCR) antigen has been identified in RA, citrullinated matrix proteins, as well as proteins with other post-translational modifications, are implicated as drivers of disease.[39] In addition to TCR

activation, T cells require a generic co-stimulatory signal to become fully active and to avoid anergy and cell death. This signal is typically provided by the engagement of CD28 and CD80 (B7-1) or CD86 (B7-2) ligands on antigen-presenting cells (APCs). Within the inflamed synovium, both macrophages and DCs can function as 'professional' APCs, but FLSs, B cells and osteoclasts can also process and present antigen to T cells.[38,40]

APCs and FLSs also direct T-cell differentiation via the cytokines they secrete. T_H1 cells (which produce mainly IFNγ) and T_H17 cells (which produce IL-17A and IL-17F, as well as TNF, IL-21, IL-22 and IL-26) have established pathogenetic roles in RA. IL-17 in particular has pleiotropic effects within the joint, driving FLSs to release proinflammatory cytokines, such as IL-1β, IL-6 and TNF, and chemokines such as neutrophil-attracting IL-8 and CCL20, which attracts $CCR6^+$ T_H17 cells and DCs.[41] IL-17 produced by T_H17 cells can also promote osteoclast activity and RA-associated bone resorption by inducing expression of RANK ligand (also known as tumour necrosis factor ligand superfamily member 11) on FLSs and osteoblasts.[42]

While excess T_H1 cell and T_H17 cell activity can contribute to RA pathology, reduced frequencies of tolerogenic regulatory T (T_{REG}) cells are also a feature of autoimmune disease that could similarly contribute to the pathology of RA. Consistent with this hypothesis, levels of anti-inflammatory cytokines such as IL-10, transforming growth factor β[43] and the novel IL-12-family cytokine IL-35[44,45] are reduced in serum from patients with RA. However, findings have been contradictory regarding the frequency of peripheral T_{REG} cells in patients with RA relative to healthy controls,[46] and no difference in T_{REG} cell frequency has been observed between patients with RA or osteoarthritis.[47] Nonetheless, an imbalance in the T_H17/T_{REG} populations has been suggested for RA.[48]

In the context of RA, APCs might be particularly important targets for vitamin D. *In vitro* treatment of DCs with $1,25(OH)_2D$ was shown to decrease expression of antigen-presenting MHC class II molecules and co-stimulatory molecules such as CD40, CD80, CD86 and CD83, resulting in an inhibition of antigen presentation and T-cell activation.[49,50] This tolerogenic response to $1,25(OH)_2D$ seems to be specific to myeloid DCs (mDCs), although mDCs and plasmacytoid DCs (pDCs) express similar levels of VDR.[51]

$1,25(OH)_2D$ and its synthetic analogues can also act on DCs to suppress T_H1-inducing IL-12[49,52,53] and pro-T_H17 IL-23[54,55] while promoting expression of tolerogenic IL-10.[56] In peripheral blood mononuclear cells and cultured monocytes/macrophages from patients with RA, $1,25(OH)_2D$ has been shown to suppress TNF, IL-17, IL-1 and IL-6 production,[57–59] highlighting the potential for vitamin D responsiveness in disease-affected leukocytes. However, the fact that lymphocytes express VDR, and upregulate its expression upon activation,[60] suggests that the effects of intracrine $1,25(OH)_2D$ in RA could also include direct effects on lymphocytes. Studies by several groups have shown that $1,25(OH)_2D$ can act directly on T cells to suppress T_H1-associated and T_H17-associated cytokines such as IL-17, IFNγ, IL-21, IL-22 and TNF,[57,61,62] as well as reduce expression of receptors for pro-T_H17 cytokines such as IL-21, IL-1β and IL-23, and downregulate the T_H17-cell hallmark transcription factors RAR-related orphan receptor C (RORC) and aryl hydrocarbon receptor (AHR).[63] In this way, $1,25(OH)_2D$ can inhibit $CD4^+$ T-cell differentiation towards T_H1 cell or T_H17 cell lineages, which are pathogenic in RA. By contrast, $1,25(OH)_2D$ can directly promote a T_{REG} cell phenotype by enhancing T_{REG} cell markers such as IL-10 and the co-inhibitory protein CTLA-4, as well as the T_{REG} cell lineage determinant FoxP3.[61,64,65] Thus, sufficient vitamin D could prevent or correct the imbalance in the T_H17/T_{REG} cell populations, as suggested for RA,[48] and restore functionality to T_{REG} cells.

Although *in vitro* data have clearly demonstrated that $1,25(OH)_2D$ can drive leukocyte activity towards protection (i.e. to reduce disease severity), the extent to which improved vitamin D status (increased serum 25(OH)D) can fulfil a similar function *in vivo* is less clear. Serum concentration of 25(OH)D is negatively associated with serum levels of IL-23[14] and IL-17[66] and with disease activity in patients with RA. While these data are consistent with the localized activation of 25(OH)D to $1,25(OH)_2D$, it is also possible that the positive correlation of IL-23/IL-17 with disease activity observed in this study is independent of serum 25(OH)D levels. However, the fact that studies of healthy individuals who received vitamin D supplementation show reduced frequencies of IFNγ and/or IL-17-expressing $CD4^+$ T cells in the periphery,[67] and increased numbers of T_{REG} cells,[68,69] supports a functional response to vitamin D supplementation and increased serum 25(OH)D *in vivo*. Similar induction of T_{REG} cells after vitamin D supplementation has also been reported for patients who underwent a renal transplant.[70]

Senescent T cells

In addition to imbalances in T_H cells, RA is also characterized by accumulation of senescent T cells as a consequence of loss of telomerase expression. Senescent T cells (which lack expansion potential) are characterized by loss of CD28 and gain of killer immunoglobulin molecules, such as NKG2D and CX3C chemokine receptor 1, which make them sensitive to activation by induced self-antigens that are abnormally expressed in the joint.[71] Although these T cells are senescent, they continue to function as a source of proinflammatory cytokines. Some evidence suggests that $1,25(OH)_2D$ might help reduce the rate of T-cell senescence and RA pathology given that higher levels of vitamin D are associated with increased telomere length.[72] Likewise, vitamin D supplementation *in vivo* has been shown to increase telomerase activity in peripheral blood mononuclear cells considerably.[73] $1,25(OH)_2D$ was also shown to increase CD28 expression and inhibit the emergence of $CD28^-$ T cells even in the presence of TNF,[74] and vitamin D supplementation reduced $CD28^-$ T-cell frequencies in patients with primary sclerosing cholangitis who, similarly to patients with RA, have increased frequencies of these cells.[74]

Interestingly, even naive $CD4^+$ T cells from patients with RA seem to have defects in telomerase activity[75] independently of disease activity and disease duration.[71] Therefore, telomerase deficiency could be an important preclinical marker for RA,[71] and vitamin D supplementation, by increasing telomerase activity, might help reduce RA risk and slow disease progression.

B cells

The observed therapeutic benefit of depleting B cells in patients with RA highlights the significance of these cells in RA pathology.[76] Evidence suggests that B cells contribute to RA pathology by producing autoantibodies that initiate the complement cascade and promote selective antigen uptake.[77] In addition, B cells also present antigen to T cells, provide co-stimulation, release inflammatory cytokines[78,79] and contribute to RA-associated osteoclast activation and bone destruction through expression of RANKL.[80,81] Lack of IL-10^+ regulatory B (B_{REG}) cell activity, through low frequencies or defective function, has also been suggested to contribute to RA.[82]

B cells express VDR; direct effects of $1,25(OH)_2D$ include suppression of plasma cell and class-switched memory-cell differentiation.[83] $1,25(OH)_2D$ might also favour the B_{REG} cell phenotype, increasing the production of B-cell-derived IL-10.[84] By inhibiting IL-21 production by T follicular helper cells,[61,62] $1,25(OH)_2D$ might also inhibit the formation of germinal-centre-like structures in the synovium, where B-cell activation, differentiation and class switching occurs.[38] Together, these findings imply that vitamin D could be beneficial in RA through its effects on B cells. Interestingly, B cells have been reported to express *CYP27B1*,[83] suggesting that they can act as an alternative source of locally-synthesized $1,25(OH)_2D$.

Fibroblasts

Historically, the synovial tissue stroma composed of FLSs was viewed as the stage upon which leukocyte reactions take place in the joint.[85] However, it is now recognized that FLSs also have an active role in the pathogenesis of RA, releasing inflammatory cytokines, leukocyte-attracting chemokines and leukocyte survival factors.[86] FLSs also directly mediate joint destruction by secretion of cartilage-degrading matrix metalloproteinases (MMPs), cathepsins and RANKL (promoting osteoclastogenesis) and Dickkopf-related protein 1 (DKK-1, which inhibits osteoblast function).[86] In *ex vivo* studies of FLSs, $1,25(OH)_2D$ has been shown to inhibit the release of TNF, IL-6 and MMPs; additionally, by impeding actin rearrangement and lamellipodia formation, $1,25(OH)_2D$ might also reduce FLS invasiveness.[87] In contrast to the reduction of RANKL expression by $1,25(OH)_2D$ on leukocytes,[58] the same metabolite was shown to increase RANKL expression in FLSs.[88] However, $1,25(OH)_2D$ was also shown to enhance FLS-derived production of the RANKL decoy receptor osteoprotogerin (OPG) and increase the OPG:RANKL ratio.[88] Thus, $1,25(OH)_2D$ might affect RA pathology by reducing osteoclast activation by FLSs.

Effects of vitamin D on RA

Cell culture studies have shown that cells critical for RA pathology are sensitive to $1,25(OH)_2D$, promoting anti-inflammatory responses predicted to limit FLS-mediated and osteoclast-mediated cartilage and bone destruction. In support of these data, *in vivo* studies have shown that deletion of the *VDR* gene in a transgenic mouse model of RA exacerbated inflammation and associated bone loss.[89] However, the extent to which low vitamin D status is a driving factor for RA disease, and not merely a consequence of the condition, remains unclear. The impact of inflammation itself on vitamin D can be observed in studies of patients undergoing elective arthroplasty, which reported a short-term post-operative decrease in serum concentrations of 25(OH)D associated with the acute inflammatory stress of surgery.[90] Importantly, RA is a highly heterogeneous condition, with some individuals having high levels of disease activity and a poor outcome characterized by rapid radiological progression, while others show a milder disease course. Thus, whereas elevated levels of vitamin D might reduce the risk of disease, they might also influence the course of the disease by creating a more tolerogenic environment.

Risk of RA

Although vitamin D-deficiency seems to be prevalent in many patients with autoimmune disease, including those with RA,[91] the contribution of low serum 25(OH)D to RA risk is less clear. Arguably, the most important limitation has been the use of food frequency questionnaires to assess vitamin D intake in prospective cohorts,[92–94] which do not give an accurate indication of total vitamin D status, especially when there is no adjustment for sunlight exposure.[94] Even when serum 25(OH)D measurements have been used, sample timing might be a considerable confounder.[95,96] One study which reported no overall association between serum concentrations of 25(OH)D and RA showed a negative association in a small subset of patients whose serum levels had been measured 3 months to <4 years before diagnosis.[95] However, in a different study no difference in vitamin D status was observed between patients with preclinical RA and controls when blood samples were taken 1, 2 or >5 years before symptom onset.[96] Similarly, no difference in serum 25(OH)D was observed between autoantibody-positive individuals at-risk of RA and matched autoantibody-negative controls.[97]

Future vitamin D supplementation trials will be the most effective way to test whether vitamin D sufficiency can reduce the incidence of RA. In the Women's Health Initiative Calcium plus Vitamin D trial, the treatment group showed no significant effect of vitamin D supplementation on RA risk, and suggestions were made that multiple exposures to vitamin D might be associated with worse RA risk.[98] By contrast, a negative linear relationship between vitamin D intake (estimated from sunlight exposure and dietary vitamin D) and RA incidence was observed in the placebo group.[98] In studies carried out using patients with early RA, vitamin D deficiency (<20 ng/ml or 50 nM serum 25(OH)D) was

common but not statistically different to controls.[99] However, severe vitamin D deficiency (<10 ng/ml or 25 nM serum 25(OH)D) was significantly higher in patients with early RA relative to controls.[99] Other meta-analyses with large sample sizes have shown an association between vitamin D intake and RA disease risk.[100]

Disease progression

By far the strongest evidence linking RA and vitamin D relates to its effect on disease progression. A 2015 study showed that vitamin D deficiency in patients with early RA was associated with lower rates of remission, higher disease activity and poorer response to treatment compared with patients with RA who were vitamin D sufficient (>20 ng/ml, 50 nM) at diagnosis.[101] Similar observations of prevalent vitamin D deficiency, and inverse correlations between serum 25(OH)D concentration and RA-associated functional impairment and disease activity, have also been reported in large Japanese[102] and European[103] cohorts. In addition to conventional RA disease activity and functional outcome scores, some studies have reported inverse correlations between serum 25(OH)D and circulating inflammatory markers.[14] Direct correlation between serum 25(OH)D concentrations and RA-associated systemic bone loss has also been reported in some studies,[14,104] whereas in others no association between serum 25(OH)D and focal bone erosion was observed.[105]

Although these data suggest that low vitamin D status, in this case low levels of serum 25(OH)D, is associated with RA disease progression, inferring causality is difficult because disease severity can also restrict patient mobility, limit access to UV light and thus diminish conventional epidermal synthesis of parental vitamin D. Some cross-sectional analyses have shown a strong inverse association between serum 25(OH)D concentrations and disease activity in patients with RA, suggesting that disease severity can contribute to low vitamin D status in these patients.[106]

The functional role of vitamin D in RA risk and progression will only be resolved with new placebo-controlled trials. However, it is important to recognize that this approach might be complicated by dysregulation of the vitamin D metabolic system in disease-affected cells from patients with RA (Figure 1). Inflammatory diseases such as RA seem to be associated with resistance to steroid hormones such as $1,25(OH)_2D$,[107] and thus the therapeutic doses of $1,25(OH)_2D$ required to overcome this block might promote hypercalcaemic adverse effects. Whether local intracrine synthesis of $1,25(OH)_2D$ after supplementation with parental vitamin D can circumvent these adverse effects is currently unknown, and might be dependent on the renal and/or extra-renal 1α-hydroxylase activity associated with particular stages of disease. One approach to this problem has been to use 1α-hydroxyvitamin D (alfacalcidol), which can be metabolized to $1,25(OH)_2D$ without 1α-hydroxylase. In studies of patients with RA, alfacalcidol was shown to be more effective than parental vitamin D in promoting suppression of PTH, decreasing inflammation and decreasing bone resorption.[108] Another therapeutic strategy might be to utilize $1,25(OH)_2D$ or vitamin D supplementation as an adjunct to existing conventional treatments for RA. Studies *in vitro* have shown that $1,25(OH)_2D$ can improve tolerogenic DC function and IL-10$^+$ T_{REG} cell differentiation in response to dexamethasone therapy,[109–111] as well as suppress the increase in T_H17 cell frequency induced by anti-TNF therapy.[112] In this way, low doses of $1,25(OH)_2D$ or supplementary vitamin D, in combination with standard treatments, might help reduce the dose of conventional therapies and their associated adverse effects.

RA genetic associations and vitamin D

Genetic variations associated with vitamin D metabolism and function have been studied extensively, focusing on both skeletal and extra-skeletal factors. The most commonly reported single nucleotide polymorphisms (SNPs) in the vitamin D pathway have been those in *VDR*, *CYP27B1* and *GC* (the gene encoding vitamin D-binding protein [DBP]). For *VDR*, the rs2228570 (*Fok1*)[113–115] and rs731236 (*Taq1*)[115] SNPs seem to be associated with RA risk, although the functional outcome of these genetic variations is yet to be defined. SNPs in the *GC* gene have also been linked with RA risk, and seem to be associated with decreased expression of DBP in patient synovial tissue.[116] As with *VDR* SNPs, the precise mechanism by which *GC* variants and DBP concentration affect RA pathophysiology is difficult to determine. *GC* SNPs are major contributors to genetic-related variations in serum 25(OH)D levels,[117] but serum DBP concentrations can also influence the amount of 'free' or 'unbound' 25(OH)D that is available to immune cells.[118] Although SNPs in *CYP27B1* are associated with decreased expression of this gene in DCs[119] and have been linked with other autoimmune diseases,[119] their role on RA remains unclear.

While studies of vitamin D-associated SNPs have focused primarily on RA risk, some reports have highlighted the effect of genetic variations on disease severity and progression. Subdividing RA patients according to seropositivity for rheumatoid factor has revealed a link with the DBP *GC2* phenotype,[120] which comprises the rs7041T and rs4588A *GC* SNPs,[121] suggesting that this gene contributes to some of the genetic variation in RA severity. In the case of *VDR* SNPs, the bb genotype of rs1544410 (*Bsm1*) has been linked to decreased disease severity,[122] while the Ff rs2228570 (*Fok1*) *VDR* genotype has been linked to RA-associated bone loss.[123] These cohort studies provide a genetic perspective on how specific components of the vitamin D system might affect RA severity. Using a different approach, genome-wide strategies have demonstrated enrichment for vitamin D response elements in RA susceptibility gene loci;[124] this profiling provides an unbiased overview of the effect of vitamin D in RA at the transcriptome level, and further highlights its therapeutic potential for the disease.

In the absence of reliable estimates of vitamin D status in the years before disease onset, SNPs associated with serum 25(OH)D concentrations have been used as genetic

markers of vitamin D status.[117] This strategy has linked genetic determinants of vitamin D status with overall human mortality[125] and has also been used to study RA outcomes, albeit with less consistent results.[126] One particular *GC* SNP (rs2282679) known to be associated with low serum 25(OH)D levels has been linked with an increased risk of osteoporotic fractures in patients with RA.[127]

Conclusions

Early studies of vitamin D in RA identified the enhanced capacity for synthesis of active $1,25(OH)_2D$ by immune cells in the joint, and the potential effect this production might have on immune function and on the potential development of hypercalcaemia. However, more recent reports have focused on the link between vitamin D status (as defined by serum concentrations of 25(OH)D) and RA risk and progression, leading to a large increase in the number of serum 25(OH)D assays carried out in patients with this disease. While some studies suggest a role for low serum 25(OH)D in RA development, the evidence remains contradictory, and is complicated by the many confounding factors detailed herein. Notably, changes in the activity of renal and extra-renal vitamin D metabolism associated with RA mean that the consequences of vitamin D supplementation or $1,25(OH)_2D$ therapy is likely to vary with disease progression.

Ultimately, randomized controlled trials of vitamin D supplementation in both prospective at-risk cohorts and in patients with active disease are required to address the effect of vitamin D on both the initiation and progression of RA. Studies performed to date have highlighted some of the difficulties associated with supplementation trials for vitamin D. For example, the Women's Health Initiative Calcium plus Vitamin D trial involved large numbers of individuals, enabling analysis of the impact of vitamin D on a small number of persons who went on to develop RA.[98] However, a key limitation of this study was that serum 25(OH)D concentrations were assessed in very few of the trial participants, so no conclusions could be made about baseline vitamin D status or whether supplementation actually raised serum 25(OH)D concentrations.

Implementing comprehensive analysis of vitamin D status in trial participants is difficult in the context of large trials and lengthy supplementation periods. Moreover, an additional complication arose from studies suggesting that serum concentrations of non-DBP-bound or 'free' 25(OH)D concentrations might be a more accurate marker of the immunomodulatory potential of vitamin D.[128] Despite these potential limitations, several large vitamin D supplementation trials are currently underway, including the VITamin D and OmegA-3 TriaL (VITAL), which has currently ~26,000 individuals enrolled and which will be completed in October 2017.[128] Although the primary endpoints for this trial are common malignancies and cardiovascular disease, the data from this study will also provide further insight into the effects of vitamin D on other common health problems.

The most persuasive evidence in favour of health benefits of vitamin D for patients with RA stems from the mechanistic studies of 25(OH)D and $1,25(OH)_2D$ in the context of immunomodulation. However, most of these studies were centred on *in vitro* experiments, and data from *in vivo* experiments in animal models is limited.[89] This area of research clearly needs to be expanded in future studies of vitamin D in the context of RA. It will be important to utilize animal models that explore global and tissue-specific knockout strategies for key genes in the vitamin D system, such as *CYP27B1* and *VDR*, to better understand the mechanisms by which vitamin D might impact on RA pathology. However, from a physiological point of view, it is also important to utilize animal models of vitamin D deficiency and supplementation, as these might help answer the key question of whether vitamin D deficiency has a causative role in RA, and whether vitamin D supplementation can prevent or treat the disease.

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