Review article

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Involvement of the secosteroid vitamin D in autoimmune rheumatic diseases and COVID-19

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Abstract

Evidence supporting the extra-skeletal role of vitamin D in modulating immune responses is centred on the effects of its final metabolite, 1,25-dihydroxyvitamin D_3 (1,25(OH)$_2$D$_3$, also known as calcitriol), which is regarded as a true steroid hormone. 1,25(OH)$_2$D$_3$, the active form of vitamin D, can modulate the innate immune system in response to invading pathogens, downregulate inflammatory responses and support the adaptive arm of the immune system. Serum concentrations of its inactive precursor 25-hydroxyvitamin D_3 (25(OH)D$_3$, also known as calcidiol) fluctuate seasonally (being lowest in winter) and correlate negatively with the activation of the immune system as well as with the incidence and severity of autoimmune rheumatic diseases such as rheumatoid arthritis, systemic lupus erythematosus and systemic sclerosis. Thus, a low serum concentration of 25(OH)D$_3$ is considered to be a risk factor for autoimmune rheumatic diseases and vitamin D_3 supplementation seems to improve the prognosis; moreover, long-term vitamin D_3 supplementation seems to reduce their incidence (i.e. rheumatoid arthritis). In the setting of COVID-19, 1,25(OH)$_2$D$_3$ seems to downregulate the early viral phase (SARS-CoV-2 infection), by enhancing innate antiviral effector mechanisms, as well as the later cytokine-mediated hyperinflammatory phase. This Review provides an update of the latest scientific and clinical evidence concerning vitamin D and immune response in autoimmune rheumatic diseases and COVID-19, which justify the need for monitoring of serum 25(OH)D$_3$ concentrations and for appropriate supplementation following clinical trial-based approaches.

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Key points

- Vitamin D_3 (cholecalciferol) is a prototypical secosteroid, a type of steroid with a 'broken' ring, which originates from cholesterol, as do all steroid hormones (including glucocorticoids, mineralocorticoids and sex hormones).

- The activation of vitamin D_3 is accomplished by sequential hydroxylations that produce $25(OH)D_3$ (calcidiol) in the liver and then the active metabolite $1,25(OH)_2D_3$ (calcitriol) in the kidney and at extra-renal sites (including immune cells).

- $1,25(OH)_2D_3$ modulates the innate immune response against pathogens and invading microorganisms, downregulates the inflammatory response and supports the adaptive arm of the immune system.

- Reduced serum concentrations of $25(OH)D_3$ are detected in autoimmune rheumatic diseases such as rheumatoid, systemic lupus erythematosus and systemic sclerosis; vitamin D supplementation seems to improve at least the prognosis.

- Serum concentrations of vitamin D fluctuate seasonally (being lowest in winter) and correlate negatively with the activation of the immune system and with the incidence and severity of autoimmune rheumatic diseases.

- $1,25(OH)_2D_3$ downregulates both the early viral phase of COVID-19, through the enhancement of innate antiviral effector mechanisms, and the later cytokine-mediated hyperinflammatory phase.

Introduction

Vitamin D_3 is derived from cholesterol and is in fact a prototypical secosteroid, a type of steroid with a 'broken' ring. Vitamin D can be obtained by exposure to sunlight, intake of certain foods and supplementation. The form of vitamin D derived from these sources is biologically inert, but its final metabolite 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$; also known as calcitriol) is a high-affinity ligand of the transcription factor vitamin D receptor (VDR)[1]. As the active form of vitamin D, $1,25(OH)_2D_3$ acts as an endocrine hormone and primarily maintains plasma concentrations of calcium and phosphate and preserves a healthy mineralized skeleton. In addition to these endocrine hormonal actions, $1,25(OH)_2D_3$ produced locally by inflammatory and immune cells (intracrine intracellular synthesis) also exerts paracrine and autocrine actions that are important, particularly for the local innate immune response[2].

The main indicator of vitamin D status is the serum concentration of the vitamin D precursor 25-hydroxyvitamin D_3 ($25(OH)D_3$, also known as calcidiol). According to Endocrine Society guidelines, serum $25(OH)D_3$ concentrations are defined as deficient when <20 ng/ml, insufficient when 20–29 ng/ml and sufficient when >29 ng/ml[3]. Like other steroid hormones, vitamin D consists of a large range of metabolites that comprise the 'vitamin D metabolome', including of course active $1,25(OH)_2D_3$; however, endogenous metabolites other than $25(OH)D_3$, are usually present at low concentrations and might only be functional in specific target tissues rather than in the general circulation[4]. Insufficiency or deficiency of $25(OH)D_3$ is prevalent in autoimmune rheumatic diseases (ARDs), including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and systemic sclerosis (SSc), as well as in COVID-19, and has been linked to disease risk as well as outcomes. Thus, assessment of serum $25(OH)D_3$ concentration and vitamin D supplementation are important considerations in these settings (Box 1).

In this Review, we provide an overview of the vitamin D endocrine system, the effects of vitamin D and its metabolites on the innate and adaptive immune systems, the biological and clinical effects of vitamin D in ARDs and COVID-19 and discuss the rationale for monitoring serum $25(OH)D_3$ concentrations and correcting them via vitamin D supplementation.

The vitamin D endocrine system

The label 'vitamin D' implies that individuals need to consume this micronutrient through their diet because it cannot be synthesized by the body[1], but in fact human skin can endogenously synthesize vitamin D_3 (cholecalciferol) from the cholesterol precursor 7-dehydrocholesterol (7-DHC) upon exposure to ultraviolet B (UVB) light. Therefore, vitamin D_3 is a prototypical secosteroid, arising from cholesterol like all steroid hormones (Fig. 1). Vitamin D can be derived in small amounts from some foods (for example, fatty fish and cod oil), and a diet rich in this micronutrient can support vitamin D status in individuals with deficient exposure to solar UVB, such as those residing at Northern latitudes and/or living primarily inside buildings, a practice that contributed to the misclassification of the secosteroid as a vitamin.

Synthesis and activation of vitamin D

The concept of the photochemical formation of vitamin D from cholesterol was first introduced almost 100 years ago, with evidence that a cholesterol preparation, when irradiated with UV light, was able to prevent rickets in rats even when administered in very small doses (0.5 mg per day)[5]. The animal-derived anti-rachitic molecule was soon identified as vitamin D_3 (cholecalciferol) and its skin precursor as 7-DHC; also identified was vitamin D_2 (ergocalciferol), which is not synthesized by the human body but originates from the UV irradiation of ergosterol obtained from yeast[6,7].

Nevertheless, several years later, it was suggested that the synthesis of vitamin D_3 starts in bowel epithelial cells with the oxidation of cholesterol from food or bile to 7-DHC, which is subsequently carried to the skin, mainly at the level of the epidermis, where it is subsequently isomerized to cholecalciferol by UV-B radiation (sunlight)[8]. However, it was debated that any 7-DHC originating from the gut would be rapidly converted to cholesterol in the liver and never access the systemic circulation. Therefore, as each molecule of cholesterol originates from its precursor, 7-DHC, the circulating 7-DHC cannot have a substantial role in the formation of vitamin D_3 (ref. 9).

Cholesterol functions as a precursor molecule in the synthesis of not only vitamin D but also glucocorticoids (steroid hormones including cortisol and aldosterone) and sex hormones (testosterone, oestrogens and progesterone)[10] (Fig. 1). The terminal enzyme of cholesterol synthesis, 7-DHC reductase, which converts 7-DHC to cholesterol, is an important regulator of the switch between the synthesis of cholesterol and of vitamin D[11]. In fact, the loss of the enzymatic activity of this reductase results in the accumulation of the substrate 7-DHC, which can lead to increased production of cholecalciferol[1]. The activation of biologically inert cholecalciferol is achieved by sequential hydroxylations: the first, which produces 25-hydroxyvitamin D_3 (calcidiol), occurs mainly in the liver, and the second, which produces 1,25-dihydroxyvitamin D_3 (calcitriol), occurs in the kidney as well as at extra-renal sites (including

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in immune cells)[12,13] (Fig. 1). These hydroxylations are controlled by several cytochrome P450 enzymes, such as CYP2R1, CYP27A1, CYP3A4 and CYP2D25, which are capable of 25-hydroxylation, and CYP27B1, which mediates the second, 1α-hydroxylation[14]. By contrast, the catabolic pathways deactivating calcidiol and calcitriol are principally controlled by 24-hydroxylase, the cytochrome P450 component of which is CYP24A1 (ref. 15). This catabolic enzymatic activity is tightly controlled in the kidney, but also occurs in several other tissues and cells, where it is differently regulated[16,17].

Mediators of the effects of vitamin D

Vitamin D_3 metabolites, including $1,25(OH)_2D_3$, are carried by serum vitamin D binding protein (DBP; also known as Gc-globulin)[18]. DBP also binds to both saturated and unsaturated fatty acids with moderate affinity. Most of the biological functions of $1,25(OH)_2D_3$ are achieved through VDR-mediated regulation of gene expression (genomic effects)[19]. Interestingly, nuclear VDR is expressed in most human tissues and cell types, in particular the pituitary gland, parathyroid gland, small intestine, colon, kidney, skin and most of the cells of the immune system, and controls the transcription of more than 1,000 genes[20]. Like all steroid hormones, vitamin D also has non-genomic effects, which occur within minutes of its interaction with VDR, for example, the effect of vitamin D on calcium regulation[21].

Selected VDR polymorphisms have been found to be associated with autoimmune connective tissue disorders, supporting the hypothesis that genetic variability of the VDR gene and vitamin D–cell interactions could be involved in susceptibility to ARDs such as RA[22]. Similarly, a number of interactions between long non-coding RNAs and VDR have been detected, and some long non-coding RNAs can alter VDR expression and/or transcriptional activity and vitamin D signalling[23].

Evidently, the vitamin D endocrine system is complex and includes the endocrine, intracrine and paracrine synthesis of the final active metabolite $1,25(OH)_2D_3$. This complex system warrants further investigation, particularly in the field of ARDs.

Evidence of vitamin D interactions with the innate immune system

One of the earliest pieces of indirect evidence of the potential involvement of vitamin D in immune regulation, at least in innate immunity, seems to have been published in 1849, in a manuscript reporting the beneficial effects of fresh cod liver oil in the treatment of 234 cases of tuberculosis[24]. In 1903, Niels Ryberg Finsen was awarded the Nobel Prize "in recognition of his contribution to the treatment of diseases, especially lupus vulgaris (lupus pernio), with concentrated light radiation"[25]; practically, Finsen had discovered that UVB radiation was beneficial in treating 'lupus vulgaris', a skin lesion caused by *Mycobacterium tuberculosis*, which remained the only effective approach to combatting this infection and the related immune-inflammatory reaction to *M. tuberculosis* until the introduction in the 1950s of antituberculous chemotherapy. When in 1958 it was demonstrated that the UVB radiation in Finsen's lamps can lead to vitamin D production, it was more clearly suspected that increased levels of vitamin D could be involved in the killing of bacteria (such as *M. tuberculosis*), which is considered one of the pillars of innate immunity[26].

Nearly 50 years later the physiological role of vitamin D in the regulation of the innate immune response was better clarified with the discovery that activated macrophages and dendritic cells express both *CYP27B1* (encoding the enzyme 1α-hydroxylase) and *VDR*, and can set up an intracrine (or autocrine) pathway leading to the synthesis of calcitriol[27,28].

In particular, it was found that Toll-like receptor (TLR) activation of human monocytes and macrophages with a synthetic *M. tuberculosis*-derived lipopeptide enhanced the expression of *CYP27B1* and *VDR* genes, with concomitant enhanced endogenous production of $1,25(OH)_2D_3$ from circulating $25(OH)D_3$ (intracrine synthesis)[13].

Neutrophils have been found to upregulate expression of *VDR* and *CYP27B1* after treatment with lipopolysaccharide and, in addition, expression of the gene encoding vitamin D 24-hydroxylase (*CYP24A1*) was found to be upregulated in milk somatic cells treated with calcitriol and lipopolysaccharide, suggesting that neutrophils can act as a hormonal target for $1,25(OH)_2D_3$ (endocrine effect)[29]. Further data indicate that vitamin D can induce the formation of neutrophil extracellular traps and act on primary human neutrophils as a modulator of the inflammatory response by reducing production of neutrophil-derived pro-inflammatory cytokines[30].

The addition of $25(OH)D_3$ to TLR-stimulated cultured human monocytes led to increased expression of cathelicidin, further indicating the influence exerted by vitamin D on the innate immune system[13] (Fig. 2). Cathelicidin is engaged in different functions of the innate immune response, including inducing chemotaxis of monocytes,

Box 1

Vitamin D supplementation

- Generally, it should be considered that 100 IU per day of vitamin D is necessary to achieve an increase of 1 ng/ml in the serum concentration of $25(OH)D_3$ (ref. 232).

- The supplementation of vitamin D under conditions of deficiency seems advantageous and safe even in the long term, as evidence reveals that vitamin D toxicity is one of the rarest medical conditions and is typically attributable to intentional or inadvertent intake of extremely high daily doses of vitamin D over a long period (usually within the range >50,000–100,000 IU daily for months to years)[3].

- Since the 1930s, supplementation with vitamin D_2 (ergocalciferol) has been considered beneficial for bone health, although it has been demonstrated to be less efficacious than vitamin D_3 supplementation for achieving optimal calcifediol serum concentrations (net changes in serum concentrations of vitamin D_3 are lower with ergocalciferol than cholecalciferol) and for this reason it is not currently used for supplementation[233].

- Regarding the ideal route of administration (daily oral or single bolus administration) to achieve increased serum concentrations of $25(OH)D_3$, compared with daily vitamin D supplementation (5,000 IU) a single high-dose bolus of vitamin D (150,000 IU) was found after a 14-day lag to lead to greater production of the vitamin D catabolism product $24,25(OH)_2D_3$ (presumably via induction of the catabolic enzyme 24-hydroxylase) relative to $25(OH)D_3$ concentrations, and this effect persisted for at least 28 days after vitamin D administration[234]. Together with other evidence, it seems that a daily dose of vitamin D (average 1,000–2,000 IU) could have more lasting effectiveness in increasing $25(OH)D_3$ serum concentrations than high-dose bolus dosing as it induces less diversion of $25(OH)D_3$ to the $24,25(OH)_2D_3$ catabolite.

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macrophages, neutrophils and T cells into the site of infection, as well as promoting the clearance of pathogens (for example, from the respiratory system) by stimulating apoptosis and autophagy of infected epithelial cells[31]. In fact, the expression of cathelicidin is induced by $1,25(OH)_2D_3$ in both myeloid and epithelial cells[32,33].

Another antimicrobial peptide induced by calcitriol, albeit in a less direct manner, is defensin β4A[34]. In fact, vitamin D induces expression of NOD2, a pattern-recognition receptor for bacterial muramyl dipeptide. NOD2, in turn, stimulates the transcription factor nuclear factor κB (NF-κB), which directly induces expression of defensin β4A, leading to the recognition of bacterial molecules (peptidoglycans) and stimulation of the immune innate response[35]. Therefore, defensin β4A, similar to cathelicidin, is engaged in innate immune defence by contributing to the recruitment of monocytes and macrophages, natural killer cells, neutrophils, T cells and dendritic cells, as well as inducing the production of antiviral cytokines (such as IFNβ) and immune response-inducing molecules (that is, NOD2)[36].

Indeed, one of the anti-inflammatory actions of $1,25(OH)_2D_3$ is the blockade of NF-κB translocation to the nucleus, where it can stimulate the expression of a panel of pro-inflammatory cytokines. In practice, $1,25(OH)_2D_3$ directly enhances the prompt innate immune response to infection by modulating the expression of several cytokines, as demonstrated by the induction of IL-1β and IL-8 expression in co-cultures of infected macrophages, or by downregulation of the expression of other pro-inflammatory cytokines such TNF, IL-6 and interferon in infected

human peripheral blood mononuclear cells[37,38] (Fig. 2). $1,25(OH)_2D_3$ has also been found to inhibit, in a dose-dependent manner, the expression of cyclooxygenase-2, which is an important promotor of inflammation[39].

Similarly critical is that vitamin D regulates the maturation and activation of both macrophages and dendritic cells, altering their function as effective antigen-presenting cells[40]. In fact, following TLR engagement, macrophages and dendritic cells express several surface molecules, such as MHC-II, CD80–CD86, CD40 and selected cytokines, which have a critical role in the process of antigen presentation to T cells (Fig. 2); vitamin D suppresses these molecules by promoting macrophages and dendritic cells that are immature and to a certain extent tolerogenic[13,41]. The suppression of macrophages and dendritic cells mediated by $1,25(OH)_2D_3$ seems to be attributable to the suppression of TLRs or the inhibition of IL-12, always via NF-κB[42].

In the past few years, it has been shown that $1,25(OH)_2D_3$ interferes with macrophage polarization, namely the balance between classically activated macrophages (known as M1 macrophages), which are implicated in virus clearance and inflammatory reactions, and alternatively activated macrophages (known as M2 macrophages), which mainly inhibit inflammatory responses[43,44]. The balance between M1 and M2 macrophages is important in ARDs such as RA[45].

In a 2016 study in which human microglial HMO6 cells were treated with $1,25(OH)_2D_3$, an evaluation of M1 and M2 cytokine expression showed that vitamin D exerted anti-inflammatory effects by facilitating
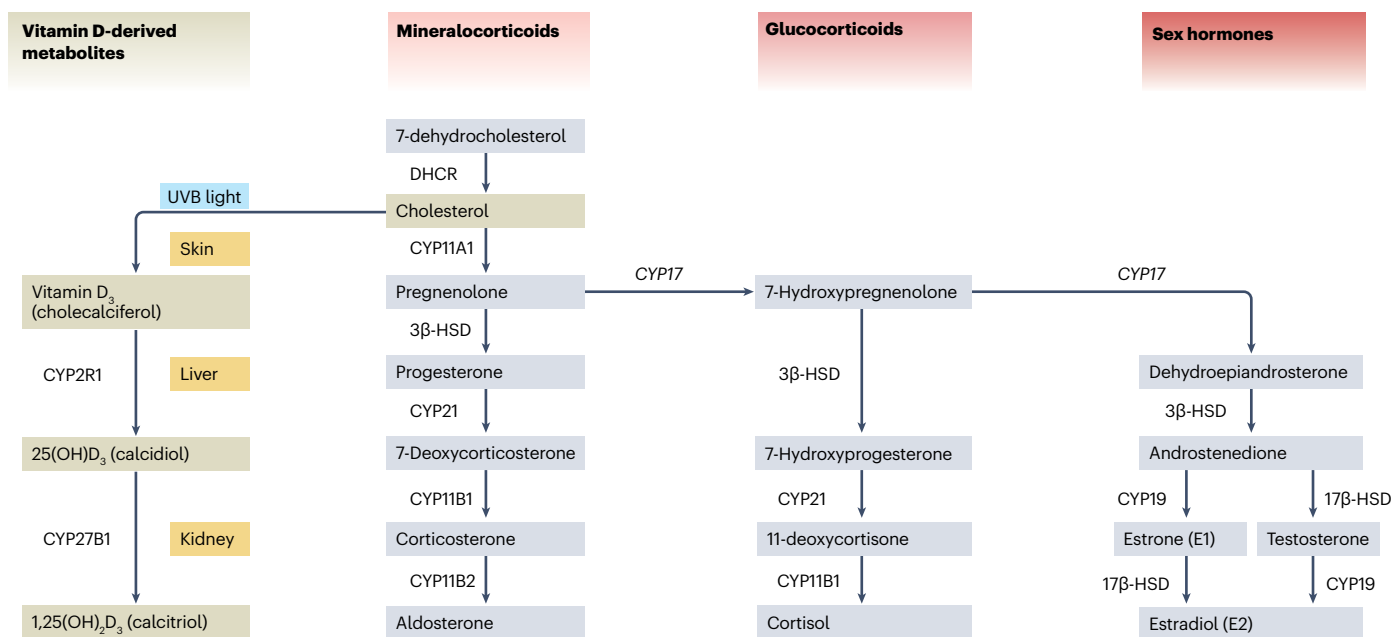

Fig. 1 | Metabolic pathways of vitamin D_3-derived molecules, mineralocorticoids, glucocorticoids and sex hormones. Vitamin D is a prototypical secosteroid, which, like all steroid hormones (including mineralocorticoids, glucocorticoids and sex hormones) arises from cholesterol. Vitamin D_3 (cholecalciferol) is synthesized from the cholesterol precursor 7-dehydrocholesterol (7-DHC) upon exposure to UVB light. Biologically inert cholecalciferol is further metabolized via sequential hydroxylations, under the control of cytochrome P450 enzymes, to produce 25-hydroxyvitamin D_3 (25(OH)D_3, also known as calcidiol) and then the active metabolite 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$, calcitriol). Also shown are the metabolic steps and corresponding enzymes involved in the derivation of other steroidal families from cholesterol. In addition, vitamin D_3 acting as an endocrine hormone, its active form, $1,25(OH)_2D_3$, is also produced locally by inflammatory and immune cells. 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; CYP2R1, vitamin D_3 25-hydroxylase; CYP11A, cholesterol side chain cleavage enzyme; CYP11B1, steroid 11-β-hydroxylase; CYP11B2, aldosterone synthase; CYP17, steroid 17α-hydroxylase/17,20 lyase; CYP19, aromatase; CYP21, steroid 21-hydroxylase; CYP27B1, 25-hydroxyvitamin D_3 1α-hydroxylase; DHCR, 7-dehydrocholesterol reductase.
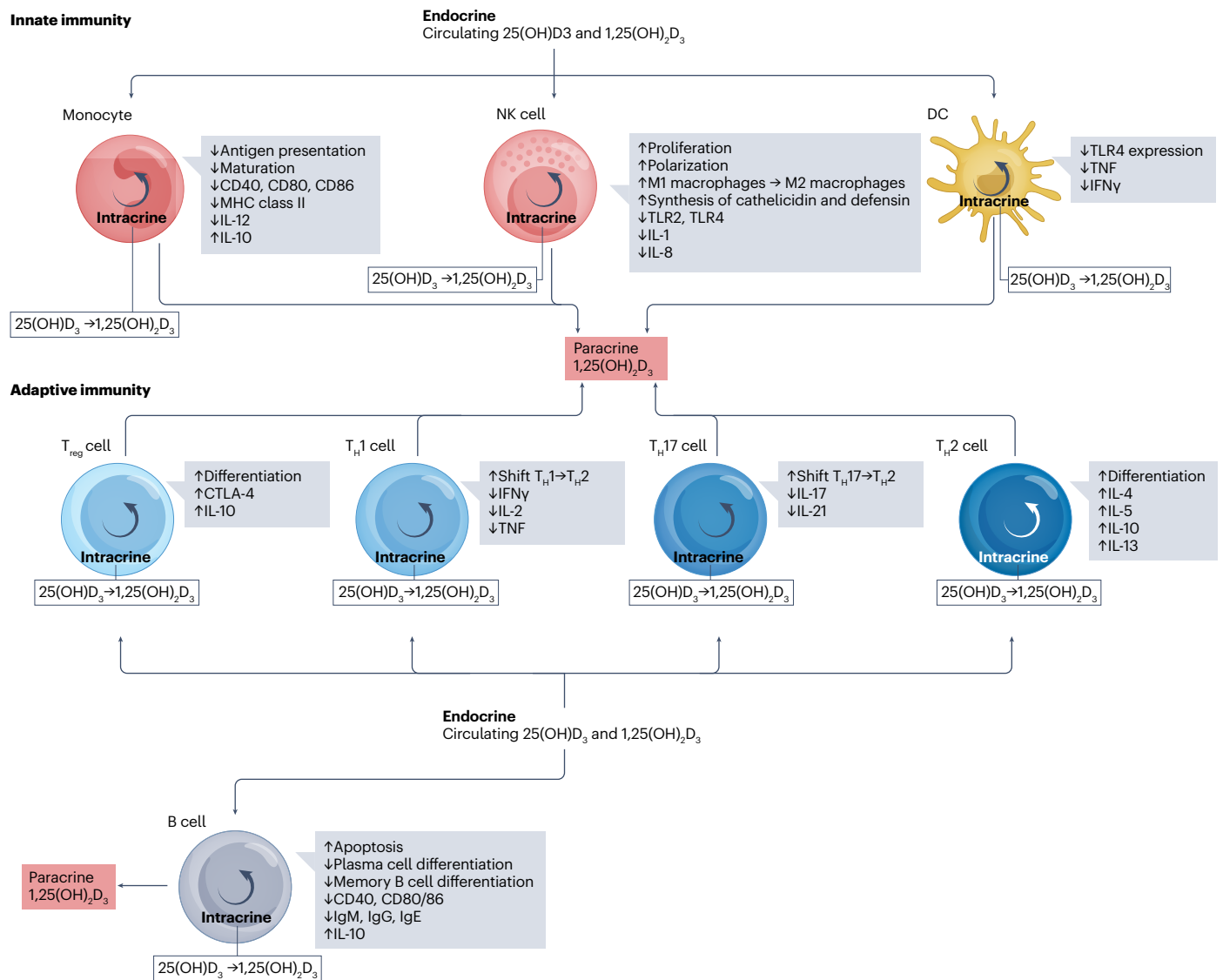
Fig. 2 | The vitamin D₃ endocrine system and the effects of vitamin D₃ metabolites on innate and adaptive immunity. Schematic representation of endocrine, paracrine and intracrine (autocrine) functions of vitamin D_3 and its metabolites 25-hydroxyvitamin D_3 (25(OH)D_3) and 1,25-dihydroxyvitamin D_3 (1,25(OH)$_2D_3$), and the effects of 1,25(OH)$_2D_3$ on cells of the innate and adaptive immune systems. In addition, vitamin D_3 acting as an endocrine hormone, its active form, 1,25(OH)$_2D_3$, is also produced locally by inflammatory and immune cells (intracrine intracellular synthesis) and exerts paracrine and autocrine actions that are important for local innate and adaptive immune responses. 1,25(OH)$_2D_3$, 1,25-dihydroxyvitamin D_3; 25(OH)D_3, 25-hydroxyvitamin D_3; CTLA-4, T lymphocyte-associated protein 4; DC, dendritic cell; M1 macrophage, pro-inflammatory macrophage; M2 macrophage, anti-inflammatory macrophage; NK cell, natural killer cell; T_H1 cell, T helper 1 cell; T_H17 cell, T helper 17 cell; T_H2 cell, T helper 2 cell; TLR, Toll-like receptor; T_{reg} cell, regulatory T cell.

the M2 polarization of these cells[46]. A subsequent study reported that M2 macrophages showed slightly higher phagocytic activity than M1 cells, and that phagocytosis was not changed by treatment with 1,25(OH)$_2D_3$ (ref. [47]).

However, the results of an investigation published in 2020 revealed that 1,25(OH)$_2D_3$-polarized monocyte-derived M2-like cells were most effective at controlling intracellular *M. tuberculosis* growth than cells not treated with active vitamin D[48]. Thus, macrophage polarization in the presence of vitamin D might provide the capacity to exert a superior antimicrobial response against *M. tuberculosis* infection (Fig. 2).

Another study from the past few years seems to suggest that hepatitis A virus cellular receptor 2 (also known as T cell immunoglobulin mucin receptor 3 (TIM-3)) can induce macrophage polarization to an M2 phenotype, and that 1,25(OH)$_2D_3$ can have immunosuppressive effects solely by increasing the expression of this receptor[49].

Chronic activation of the innate immune response can cause damage. Of note, 1,25(OH)$_2D_3$ inhibits the expression of several TLRs (TLR2, TLR4 and TLR9) in monocytes in the later stages of activation and consequently limits the excessive production of cytokines (such as TNF and IL-12) that are involved in chronic innate immune activity[50].

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In summary, interactions of active vitamin D with the innate immune system represent in clinical settings the first level of defence against invading pathogens, with initiation of the inflammatory response and activation of the adaptive immune response[51].

Evidence of vitamin D interactions with the adaptive immune system

The adaptive immune response is initiated when antigen presented by dendritic cells and macrophages activates antigen recognition by T and B lymphocytes[52]. The recruitment of T and B cells, which occurs during their priming period and at sites (such as lymph nodes) far from the site of the initial presence of the involved antigens, is characterized by post-translational modifications of immunoglobulin production that enable the immune response to adapt to specific antigens[53].

When dendritic cells are activated by a virus or bacterium, they show increased expression of CYP27B1 (1α-hydroxylase), but also a decreased appearance of VDR, expression of which is down-regulated by $1,25(OH)_2D_3$ as monocytes differentiate into immature dendritic cells[54]. Dendritic cells can produce $1,25(OH)_2D_3$ in an intracrine manner, especially following stimulation; terminal maturation renders these cells unresponsive to the effects of $1,25(OH)_2D_3$, whereas they are able to suppress in a paracrine way (through the production of $1,25(OH)_2D_3$) the differentiation of their own precursor cells (monocytes)[55] (Fig. 2). Therefore, $1,25(OH)_2D_3$ has been identified as a major factor in inhibiting the differentiation and maturation of dendritic cells and has also been shown to repress the expression of the co-stimulatory molecules HLA-DR, CD40 and CD80–CD86, thereby reducing the antigen-presenting function of these cells in the activation of T cells[56] (Fig. 2).

Additionally, $1,25(OH)_2D_3$, by suppressing macrophage production of IL-12, which is crucial for T helper 1 (T_H1) cell development, and of IL-23 and IL-6, which are crucial for the development and function of T_H17 cells, reduces the production of IFNγ (from T_H1 cells) and IL-17 (from T_H17 cells) and favours the shift from a T helper T_H1–T_H17 profile to a T_H2 and regulatory T (T_{reg}) cell profile[57] (Fig. 2). The suppression of IL-12 also promotes the development of T_H2 cells, leading to increased production of IL-4, IL-5 and IL-13, which further suppress T_H1 cell development, shifting the balance to a T_H2 cell-prevalent profile[58].

In particular, $1,25(OH)_2D_3$ increases the ratio of anti-inflammatory T_H2 cells to pro-inflammatory T_H1 and T_H17 cells by stimulating the STAT6–GATA3 cell signalling pathway[59,60]. One of the translational pieces of evidence is that $25(OH)D_3$ (optimal concentrations) has been shown to promote a more favourable environment for pregnancy, for example, with the enhancement of the shift towards T_H2 cells and the regulation of immune cell differentiation, as well as the modulation of cytokine secretion[61].

Treatment of dendritic cells with $1,25(OH)_2D_3$ can in fact induce $CD4^+CD25^+$ T_{reg} cells that synthesize the anti-inflammatory cytokine IL-10 and can inhibit the development of the other T helper cell subclasses (for instance, T_H17 cells)[58,62,63] (Fig. 2). Indeed, inhibition of dendritic cell differentiation and maturation, as well as the modulation of their survival leading to T cell hyporesponsiveness, has been suggested to explain the immunosuppressive activity of $1,25(OH)_2D_3$[64].

Regarding B cells, their differentiation and maturation into plasma cells is also regulated by VDR, and consequently antibody production is influenced. In addition, vitamin D downregulates several co-stimulatory molecules expressed on B cells (HLA-DR, CD40 and CD80–CD86), similar to what is observed in macrophages and dendritic cells; therefore, optimal vitamin D concentrations might also compromise the ability of B cells to function as antigen-presenting cells and thereby influence adaptive immune responses[65,66] (Fig. 2).

Vitamin D seems to affect the proliferation and differentiation of activated but not resting B cells; in fact, $1,25(OH)_2D_3$ blocked the proliferation of activated B cells and induced their apoptosis in culture, whereas initial B cell division persisted unaffected[67] (Fig. 2). Interesting, in an in vivo study in humans it was observed that following oral treatment with cholecalciferol (the serum concentration of $25(OH)D_3$ obtained was over 70 nmol/l), the number of B cells expressing CD38 (a molecule that regulates B cell differentiation and the response to inflammation) increased, suggesting that $25(OH)D_3$ can increase B cell differentiation and augment their response to the inflammatory reaction[68,69].

The bioactive form of vitamin D, $1,25(OH)_2D_3$, was shown to inhibit IgE production in human peripheral B cells stimulated with anti-CD40 antibodies and IL-4, by triggering the VDR to inhibit NF-κB activation[70]. As $1,25(OH)_2D_3$ can promote IL-10 production by B cells, and IL-10 is an anti-inflammatory cytokine that suppresses T cell activation by inhibiting antigen presentation (by dendritic cells, monocytes and macrophages), the production of antibodies such as IgE is consequently affected, and in that way $1,25(OH)_2D_3$, through the VDR, could represent an additional factor that helps to maintain low serum IgE responses[71,72].

It is likely that the intracrine production of $1,25(OH)_2D_3$ in the cells of the human immune system, including monocytes and macrophages, is what is really required for vitamin D to exert its immunomodulatory functions[73].

In summary, low availability of $1,25(OH)_2D_3$ in the clinical setting could enhance the ability of B cells to drive the activation and differentiation of T cells and thus increase the risk of developing several ARDs.

Vitamin D interactions with steroidal hormones and immune response

By considering the common origin of vitamin D, cortisol and sex hormones from cholesterol (Fig. 1), reports of an interaction between the secosteroid $1,25(OH)_2D_3$ and the other steroidal hormones is not unexpected, in particular, regarding common metabolic and cellular effects on the immune system response and autoimmunity[74,75] (Fig. 2).

The classes of steroid ligands and their nuclear receptors interact with each other at different levels in an integrated and complex network, rather than as individual systems. For example, interactions between vitamin D_3 and glucocorticoids have been widely reported. Treatment with dexamethasone increased VDR expression in squamous cell carcinoma and adipocytes, and induced up-regulation of CYP24A1 (which is involved in the catabolic pathways deactivating vitamin D3) in kidney cell lines and in osteoblasts[76–78].

At the level of the central nervous system the interactions seem to be dependent on the concentrations of glucocorticoids; for example, treatment of rodents with high doses of dexamethasone decreased the expression of VDR, CYP27B1 and CYP24A1 in the pre-frontal cortex and hippocampus, whereas treatment with low doses increased the expression of VDR in the pre-frontal cortex[79]. However, the distribution of the VDR in the human brain is remarkably similar to that reported in rodents. Many brain regions seem to contain equivalent amounts of VDR and 1α-hydroxylase[80]. These observations might suggest that $1,25(OH)_2D_3$ could have autocrine and/or paracrine properties in the human brain.

Interestingly, both $1,25(OH)_2D_3$ and dexamethasone similarly abolished the age-related increase in microglial expression of MHC-II, pro-inflammatory cytokines, stress-activated protein kinase and c-Jun

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N-terminal kinase, among other mediators, in the rat hippocampus, suggesting that both steroids downregulate microglial activation[81].

It has been shown that the combination of 1,25(OH)$_2$D$_3$ with dexamethasone synergistically skewed T cell differentiation towards a more anti-inflammatory profile in vitro by inducing the development of IL-10-producing T cells[63]. Of interest, the addition of 1,25(OH)$_2$D$_3$ to dexamethasone in cultures of CD4$^+$ T cells from patients with glucocorticoid-resistant asthma enhanced IL-10 production to levels observed in cells from patients with glucocorticoid-sensitive asthma cultured with dexamethasone alone[82]. This very important observation seems to suggest that vitamin D$_3$ could potentially increase the therapeutic response to glucocorticoids in patients with steroid-resistant disease and might overcome poor glucocorticoid sensitivity.

Interactions between vitamin D and sex steroids have been also reported in several investigations and under different immune-mediated conditions including multiple sclerosis[83]. A seasonal increase in serum concentrations of testosterone and luteinizing hormone in summertime has been described in healthy young males, similar to the observed circannual variations of serum vitamin D concentrations[84]. Furthermore, 1,25(OH)$_2$D$_3$, in a way almost similar to dexamethasone, induced expression of *CYP19* (encoding aromatase, which is involved in peripheral oestrogen synthesis) in human glioma cells, human osteoblasts and prostate cancer cell lines, but not in neuroblastoma or breast cancer cells[85–87].

Under inflammatory conditions, 1,25(OH)$_2$D$_3$ was found to downregulate the production of pro-inflammatory cytokines in activated human macrophages by decreasing aromatase activity, especially in an oestrogenic environment such as in RA synovial tissue[88]. In fact, treatment with oestradiol (E2) and 1,25(OH)$_2$D$_3$ (individually and in combination) reduced P450-aromatase synthesis and *CYP19A1* gene expression in cultured macrophages, as well as production of pro-inflammatory cytokines (IL-1β, IL-6 and TNF)[88].

Of note, synergy between E2 and 1,25(OH)$_2$D$_3$ was shown in an animal model of experimental autoimmune encephalomyelitis[89]. In fact, dietary vitamin D$_3$ supplementation was more efficient at protecting against disease in intact female mice than in ovariectomized female rats or male rats. Therefore, the 1,25(OH)$_2$D$_3$-mediated protection was found to be female specific and E2 dependent, suggesting that decreased vitamin D3 supplies due to reduced exposure to solar UV light might contribute to the increased prevalence of autoimmune diseases in women.

The interesting interaction between vitamin D and female sex steroids might be related to an E2-mediated decrease in CYP24A1 and an increase in VDR expression within the central nervous system, at least in the experimental autoimmune encephalomyelitis model. Oestrogen-mediated upregulation of VDR has also been shown in osteoblasts, breast cancer cells, duodenal cells and colon mucosa cells[90–93]. Conversely, pregnant women, who physiologically have high circulating concentrations of oestrogen and progesterone, can also show low circulating concentrations of 25(OH)D$_3$ but higher levels of 1,25(OH)$_2$D$_3$ than non-pregnant women[94,95].

Overall, the available data, especially regarding autoimmune responses, suggest that vitamin D-based therapeutic approaches have a greater protective effect in women than in men, at least during the years of fertility[96].

Finally, concerning progesterone, VDR expression was induced in activated T cells in a dose-dependent manner, and then both progesterone and 1,25(OH)$_2$D$_3$ induced T cells from a pro-inflammatory (T$_H$1 and T$_H$1) cell profile to a less inflammatory and regulatory profile[97].

Vitamin D and interconnections with autoimmune rheumatic diseases

Epidemiological evidence has long indicated a strong association between vitamin D deficiency and an increased incidence of autoimmune diseases, which, as discussed, is mainly linked to the immunomodulatory and anti-inflammatory activities of 1,25(OH)$_2$D$_3$[98–101]. Substantially reduced concentrations of vitamin D have been detected in several ARDs, such as RA, SLE and SSc[102]. It is also evident that the risk of selected immune-mediated diseases (for instance, type 1 diabetes mellitus, as well as multiple sclerosis and RA) is increased in high-latitude areas, where the quantity of UVB radiation is relatively low and as a consequence the prevalence of vitamin D deficiency is high[103,104]; as discussed in more detail in a separate section, seasonal rhythms of sun exposure influence concentrations of vitamin D and ARDs. To underline the role of sunlight in vitamin D skin synthesis, it is anticipated that daily UVB exposure of 7–30 min duration (depending on skin phototype, latitude and season of the year) is required to obtain vitamin D supplementation doses[105].

In this section, we discuss the evidence from studies reporting biological and clinical effects of vitamin D in RA, SLE and SSc.

Vitamin D and rheumatoid arthritis

In view of the immunomodulatory effects exerted by 1,25(OH)$_2$D$_3$ on T$_H$1 cell subpopulations, it is interesting to consider that increased activity of T$_H$17 and T$_H$1 cells and dysregulation of T$_{reg}$ cells also contributes to the pathogenesis of RA, suggesting a role for vitamin D in both pathogenesis and disease progression, as well as in the triggering of chronic synovial inflammation and symmetrical polyarthritis in RA[106–109]. Consistent with this idea, an interesting study evaluating 100 patients with RA showed a negative correlation between serum concentrations of 25(OH)D$_3$ and of pro-inflammatory cytokines (TNF, IL-1β, IL-6 and IL-17), as well as a positive correlation between the aforementioned pro-inflammatory cytokines and reactive oxygen species[110].

In addition, polymorphisms of the gene encoding VDR and DBP seem to be linked to predisposition to the development of RA, and further suggest that the vitamin D signalling pathway might be implicated in the pathogenesis of RA[111,112].

Interestingly, in the Nurses' Health Study I (NHSI, involving a cohort of 106,368 female nurses aged 30–55 years in 1976), higher cumulative average UVB exposure was associated with a decreased risk of RA, although UVB exposure was not associated with RA risk among women in the NHS II study (involving 115,561 female nurses aged 25–42 years in 1989); notably, the mean age at the time of RA diagnosis was 59 years in NHSI, compared with 47 years in NHSII[113]. Differences in sun-protective behaviours (for example, increased use of sunblock by younger generations and less sun exposure in the NHSII cohort), could explain the different results in the two studies and seem to be consistent with those of previous studies[114].

In the 2017 Comorbidities in RA (COMORA) study, which evaluated vitamin D status in 1,413 patients with RA from 15 countries, serum concentration of 25(OH)D$_3$ was found to be inversely correlated with RA disease activity, as assessed by 28-joint Disease Activity Score (DAS28), after adjustment for potential confounders[115]. A more recent investigation showed that in 645 patients with early RA, vitamin D deficiency correlated with more active and severe disease and the results indicated the usefulness of serum vitamin D concentration as a biomarker to predict progression of disease disability, at least over 1 year[116].

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All these observations further support the clinical and epidemiological evidence that increased vitamin D, obtained from food, supplementation or (mainly) sunlight exposure, could be at least partially protective against RA.

In a prospective cohort study of women 55–69 years old who were followed up for 11 years, total vitamin D intake from both diet and oral supplement use was found to be associated with a reduced risk of RA, although patients were not clinically assessed and sunlight exposure was not registered[117]. The study showed that the risk of developing RA was 33% lower for women within the highest tertile of vitamin D intake when compared with those in the lowest tertile. No particular food with high vitamin D content was found to be significantly associated with incident RA; however, a combined evaluation of different milk products was suggestive of an inverse association with the risk of developing RA. Interestingly, the authors commented that the evident correlation found between calcium and vitamin D from milk products and the moderate correlation of total calcium intake with total vitamin D intake, which included supplemental vitamin D, might have been related to vitamin D fortification of the foods.

Results from different clinical trials have suggested that the administration of vitamin D to patients with RA could help to reduce disease activity, as reported in a 2020 meta-analysis of six studies that included a total of 438 patients with RA[118]. Vitamin D supplementation induced a significant improvement in erythrocyte sedimentation rate-based DAS28 and tender joint count, but not in pain as assessed by visual analogue scale (VAS). However, another meta-analysis of two studies published the same year showed that vitamin D supplementation did not significantly reduce RA flares (DAS28 >3.2)[119], although a high risk of bias was observed in one of the studies[120].

It should nevertheless be mentioned that most of the individual studies included in these meta-analyses did not demonstrate a statistically significant clinical benefit from vitamin D supplementation. This finding could be attributable to differences in the characteristics of the patients with RA, the small sample sizes and limited statistical power of the individual studies, and the amounts and duration of vitamin D supplementation (which were too low in some investigations).

On the other hand, other studies have reported that supplementation with $1,25(OH)_2D_3$ can help to improve RA outcomes, such as in a 2011 open-label randomized clinical trial (RCT) in which the administration of $1,25(OH)_2D_3$ together with DMARDs and calcium (to 59 patients with RA) induced significantly higher pain relief than DMARDs and calcium alone (62 patients with RA)[121]. Likewise, interesting results from a more recent phase II clinical trial, in which 369 patients with RA were treated with $1,25(OH)_2D_3$, 22-oxa-$1,25(OH)_2D_3$ (an analogue designed to be less hypercalcaemic than $1,25(OH)_2D_3$) or placebo, showed a significant reduction in the number of swollen joints and improved Health Assessment Questionnaire (HAQ) Disease Activity Index scores in the patients receiving the active vitamin D treatment ($1,25(OH)_2D_3$ or 22-oxa-$1,25(OH)_2D_3$) compared with the placebo group[122].

Another RCT involving 150 patients with early, treatment-naive RA showed that weekly supplementation with 60,000 IU of $1,25(OH)_2D_3$ plus calcium for 2 months resulted in greater pain relief, as assessed by VAS, than calcium alone[123]. Similarly, in a subgroup analysis within a systematic review and meta-analysis, a significant improvement in VAS score was observed with $1,25(OH)_2D_3$ supplementation at a dose of more than 50,000 IU weekly for more than 3 months[118]. Interestingly, in an RCT of patients with early RA with low serum $25(OH)D_3$ concentrations, treatment with a single dose of cholecalciferol (300,000 IU) combined with standard treatment ($n = 18$) significantly improved global health after 3 months compared with those who did not receive vitamin D ($n = 18$)[124]; no significant effect of cholecalciferol on T helper cells was observed.

Furthermore, in a small cohort of 61 patients with RA, supplementation with vitamin D (100,000 IU per month) resulted in a significant reduction in DAS28 in patients with sufficient basal serum concentrations of vitamin D and VAS in patients with deficient basal serum concentrations of vitamin D[125]. Conversely, some investigations have found no significant clinical benefit after vitamin D supplementation[126–130].

Finally, the very recent vitamin D and omega 3 trial (VITAL), an RCT that enrolled and followed 25,871 participants from throughout the USA for a median of 5.3 years, investigated whether the administration of vitamin D (2,000 IU daily) or matched placebo, and omega 3 fatty acids (1,000 mg daily) or matched placebo, reduces the risk of autoimmune disease, including RA[131]. The important result (with some limitations) was that vitamin D supplementation for 5 years, with or without omega 3 fatty acid supplementation, reduced the incidence of autoimmune disease by 22% (statistically significant) with an effect already apparent after 2 years, whereas omega 3 fatty acid supplementation with or without vitamin D reduced the autoimmune disease rate by 15% (not statistically significant). However, both treatment arms showed stronger effects than the reference arm (vitamin D placebo and omega 3 fatty acid placebo). The authors concluded that this trial is clinically relevant, as both supplements are well tolerated and non-toxic and other effective approaches to reducing the incidence of autoimmune diseases are lacking at present.

In conclusion, actual evidence, with correct interpretation, seems to suggest that increasing vitamin D intake to maintain serum $25(OH)D_3$ concentrations within the range 40–60 ng/ml (100–150 nmol/l) might reduce the risk of developing RA. However, from clinical trials, there is no firm demonstration that vitamin D supplementation can reduce the risk of incident RA. Some evidence exists that oral $1,25(OH)_2D_3$ supplementation can reduce the severity of disease in patients with RA, but there is still not enough evidence to warrant vitamin D supplementation to improve the RA outcomes. The in vitro and in vivo studies reporting the biological and clinical effects of vitamin D in RA are summarized in Table 1.

Vitamin D and systemic lupus erythematosus

Patients with SLE are affected by an altered and complex immune response, which is characterized by increased T_H17 cell and decreased T_{reg} cell activities, with variable T_H1 and T_H2 cell activation, as well as B cell activation and the production of several autoantibodies (antinuclear antibodies, anti-double-stranded DNA (anti-dsDNA) antibodies, anti-Sm/ribonucleoprotein antibodies and others)[132–135].

Low serum concentration of $25(OH)D_3$ has been reported in SLE in several investigations and there is evidence from genetic studies that individuals with VDR polymorphisms are at an increased risk of developing SLE[136–138]. The expression of VDR in 20 biopsy-obtained kidney tissue samples was negatively associated with renal activity and SLE disease activity index (SLEDAI) scores[139]. In fact, there was a reduction in anti-dsDNA production by peripheral human blood mononuclear cells derived from patients with SLE when the cells were incubated with $1,25(OH)_2D_3$ (refs. [140,141]). Other studies showed that $1,25(OH)_2D_3$ reduced in vitro the activation of antigen-presenting cells from patients with SLE, inhibiting the expression of CD40, MHC class II and CD86 molecules, and also decreased the activation of dendritic cells and the expression of genes related to the effects of IFNα[142,143].

An inadequate serum concentration of vitamin D seems to be prevalent among patients with SLE not receiving vitamin D supplementation

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Table 1 | Biological and clinical effects of vitamin D in RA in in vitro and in vivo studies

Study	Population(s)	Intervention	Vitamin D_3 regimen	Follow-up	Results
Gopinath and Danda[121]	121 patients with treatment-naive early RA	59 patients received triple-DMARD therapy (methotrexate, sulfasalazine and hydroxychloroquine) and 1,000 mg calcium carbonate plus calcitriol 62 patients received only triple-DMARD therapy and 1,000 mg calcium carbonate	Calcitriol 500 IU daily	3 months	Vitamin D supplementation induced an improvement in pain in patients with early RA after 3 months of daily intake
Salesi and Farajzadegan[126]	117 patients with active RA receiving treatment with methotrexate (98 completed follow-up)	50 patients received vitamin D_3 treatment in addition to stable background methotrexate therapy 48 patients received stable background methotrexate therapy plus placebo	Cholecalciferol 50,000 IU per week	12 weeks	After 12 weeks, DAS28 did not differ between the two groups of patients
Dehghan et al.[127]	80 patients with RA in remission and with serum concentration of vitamin D_3 <30 ng/ml	40 patients received vitamin D_3 treatment 40 patients received placebo	Oral vitamin D_3 50,000 IU per week	6 months	Low vitamin D_3 concentrations did not seem to be a risk factor for disease flare in patients with RA in remission
Matsumoto et al.[128]	176 patients with moderately active RA 186 age- and sex-matched individuals without RA	None All patients self-assessed their dietary intake of vitamin D with a questionnaire	Dietary vitamin D intake only; pharmacological vitamin D supplementation was not allowed	N/A	Serum vitamin D_3 concentration was significantly lower in patients with RA than in matched individuals Serum vitamin D_3 concentration did not seem to correlate with disease activity in patients with RA
Yang et al.[119]	168 patients with RA in remission and serum vitamin D_3 concentration >30 ng/ml, 192 patients with RA in remission and serum vitamin D_3 concentrations <30 ng/ml	Of the 192 patients with serum vitamin D_3 concentrations <30 ng/ml, 84 received vitamin D_3 supplementation; the remaining 88 continued their current treatment (as did the 168 patients with vitamin D_3 sufficiency)	Alfacalcidol 0.25 μg twice daily	24 months	The rate of RA recurrence was significantly lower in patients with normal serum vitamin D_3 concentrations compared with those with vitamin D deficiency
Buondonno et al.[124]	39 patients with early RA 31 age-matched healthy individuals	18 of the patients with RA were randomly assigned to treatment with glucocorticoids, methotrexate and vitamin D_3, and 21 were randomly assigned to treatment with glucocorticoids, methotrexate and placebo	Cholecalciferol 300,000 IU monthly	3 months	Vitamin D_3 serum levels were significantly lower in patients with early RA than in healthy individuals Vitamin D_3 supplementation ameliorated global health in patients with early RA (evaluated on a numeric scale)
Hajjaj-Hassouni et al.[115]	521 patients with RA and serum vitamin D_3 concentrations >30 ng/ml 772 patients with RA and serum vitamin D_3 concentrations between 10 ng/ml and 30 ng/ml 120 patients with RA and serum vitamin D_3 concentrations <10 ng/ml	608 patients were receiving vitamin D_3 supplementation (at physician's discretion)	No standardized supplementation regimen	N/A	Low serum concentrations of vitamin D_3 were associated with patient characteristics (age, BMI), disease characteristics (disease activity, glucocorticoid dosage) and clinical comorbidities (lung disease, osteoporosis)
Mateen et al.[110]	100 patients with RA 50 healthy age- and sex-matched individuals	None	Vitamin D_3 supplementation was not allowed	N/A	Serum concentrations of vitamin D_3 were significantly lower in patients with RA than in healthy individuals Serum concentrations of vitamin D_3 were negatively associated with plasma concentrations of pro-inflammatory cytokines and nitric oxide
Li et al.[122]	369 patients with RA	123 patients received 22-oxa-calcitriol, 123 patients received calcitriol and 123 patients received placebo	50,000 IU per week of oxa-calcitriol or calcitriol	6 weeks	Vitamin D_3 supplementation significantly decreased swollen joint counts and HAQ-DAI scores in patients with RA

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Table 1 (continued) | Biological and clinical effects of vitamin D in RA in in vitro and in vivo studies

Study	Population(s)	Intervention	Vitamin D$_3$ regimen	Follow-up	Results
Adami et al.[125]	35 patients with RA and serum vitamin D$_3$ concentrations >20 ng/ml, 26 patients with RA and serum vitamin D$_3$ concentrations <20 ng/ml	Supplementation with cholecalciferol	Cholecalciferol 100,000 IU per month	3 months	Vitamin D$_3$ supplementation significantly decreased pain (measured on VAS) in patients with RA Vitamin D$_3$ serum concentrations >20 ng/ml led to a decrease in DAS28-CRP
Mukherjee et al.[123]	150 patients with early RA	75 patients received supplementation with calcium carbonate (1,000 mg per day) plus calcitriol 75 patients received supplementation with calcium carbonate only	Calcitriol 60,000 IU once weekly	8 weeks	Serum vitamin D$_3$ concentration <20 ng/ml was associated with an increased risk of developing active RA Weekly supplementation with vitamin D$_3$ relieved pain in patients with early RA
Mouterde et al.[116]	114 patients with RA and serum vitamin D$_3$ concentration >30 ng/ml 415 patients with RA and serum vitamin D$_3$ concentration between 10 ng/ml and 30 ng/ml 114 patients with RA and serum vitamin D$_3$ concentration <10 ng/ml	7 patients received vitamin D$_3$ supplementation	Vitamin D$_3$ supplementation according to physician's prescription	12 months	Serum vitamin D$_3$ concentration <10 ng/ml was associated with higher activity and severity of early RA at baseline and might predict disability and radiographic progression over 12 months
Wu et al.[130]	1,180 patients with RA	712 patients received vitamin D$_3$ supplementation together with standard RA therapy (DMARDs with or without glucocorticoids) 468 patients received only standard RA therapy	Calcitriol (0.25–0.5 µg per day) or calcium carbonate plus cholecalciferol (200–400 IU per day)	>12 months	Vitamin D$_3$ supplementation did not improve DAS28 when administered together with standard therapy
Hahn et al.[131]	25,871 individuals from the general population (men ≥50 years and women ≥55 years)	12,927 individuals received vitamin D$_3$ supplementation 12,933 individuals received omega 3 fatty acid supplementation 25,882 individuals received placebo	2,000 IU per day cholecalciferol	5 years	Vitamin D$_3$ supplementation reduced the risk of developing autoimmune diseases, including RA

CRP, C-reactive protein; DAI, Disease Activity Index; DAS28, 28-joint Disease Activity Score; HAQ, Health Assessment Questionnaire; N/A, not applicable; RA, rheumatoid arthritis.

and who live at a latitude north of 37°N[137]. Reduced exposure to the sun and the use of photoprotection, which are measures recommended to avoid photosensitivity reactions, as well as altered renal function, are further potential contributors to vitamin D deficiency in patients with SLE[144].

In a 2021 investigation of vitamin D status in 4,046 African Caribbean individuals in the UK, logistic regression showed that brown or black skin phenotype, together with blood draw (for serum 25(OH)D measurement) being carried out in winter or autumn and non-use of vitamin D supplements, predicted significantly increased odds of vitamin D deficiency[145]. On the other hand, a large body of evidence, as synthesized in a review analysing 36 studies, has confirmed that African American patients experience more severe SLE and a wider range of adverse outcomes than patients of other racial/ethnic groups[146]. The association of very low vitamin D concentrations in African Caribbean individuals and a greater severity of SLE in the same racial/ethnic group seems expected.

In a large study involving 763 patients with SLE and low serum vitamin D concentrations who were treated with 50,000 IU of vitamin D$_2$ per week together with calcium–vitamin D$_3$ (200 IU twice per day), an increase of 20 ng/ml in serum 25(OH)D concentration correlated with a 21% decrease in the odds of having a high disease activity score and a 15% decrease in the odds of having clinically relevant proteinuria[147].

By contrast, in a 24-month prospective study on the efficacy and safety of two different monthly regimens of vitamin D supplementation in pre-menopausal women with SLE (34 patients) no significant changes were found concerning SLE disease activity or serology (including anti-dsDNA antibody and complement protein measurements)[148]. Furthermore, no significant effects on clinical condition (evaluated by SLEDAI), were observed after administration of high weekly dosages of cholecalciferol in 90 patients with SLE and vitamin D deficiency[149].

However, in a previous prospective study, the safety and immunological effects of supplementation with the 25(OH)D$_3$ precursor cholecalciferol (100,000 IU per week for 4 weeks followed by 100,000 IU per month for 6 months) were tested in 20 patients with SLE and hypovitaminosis[141]. Cholecalciferol supplementation induced an increase in naive CD4$^+$ T cells and T$_{reg}$ cells and a decrease in effector T$_H$1 and T$_H$17 cells, as well as a decrease in memory B cells and anti-dsDNA antibodies.

A further investigation of 40 female patients with juvenile-onset SLE showed that treatment with high doses of cholecalciferol

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(50,000 IU per week for 24 weeks) decreased SLE disease activity (as assessed using SLEDAI and the European Consensus Lupus Activity Measurement) and improved fatigue (assessed using the Kids Fatigue Severity Scale)[150].

Evidence shows that serum $25(OH)D_3$ concentrations are substantially lower in patients with active disease and lupus nephritis than in patients with inactive SLE or without lupus nephritis[151]. Notably, vitamin D deficiency or insufficiency was found in 93% of patients with lupus nephritis with proteinuria ≥500 mg per day and 86% of those with proteinuria <500 mg per day. Therefore, the presence of lupus nephritis seems to be an important predictor of vitamin D deficiency.

Interestingly, vitamin D was found to protect against podocyte injury induced by autoantibodies in patients with nephritis[152]. Some investigations have reported that vitamin D supplementation could be a factor in decreasing urine protein-to-creatinine ratio and probably clinical proteinuria. Indeed, the activation of the vitamin D signalling pathway might contribute to reducing the autoinflammatory reactivity and functional damage in lupus nephritis (direct action on kidney tissue) through immunomodulatory effects on T_H17, T_{reg} and B cells[153]. In fact, vitamin D supplementation has shown modulatory effects on T and B cell homeostasis during the treatment of patients with SLE, by increasing T_H2 and T_{reg} cells and decreasing T_H17, T_H1 and memory B cells[141,154].

Low serum vitamin D concentrations seem to be associated with more severe SLE activity at baseline and several extra-musculoskeletal complications (such as cardiovascular risk and fatigue), as well as a trend towards more severe disease flares[155–160].

Regardless of whether low serum concentrations of $25(OH)D_3$ might be a cause or consequence of SLE, vitamin D supplementation should be regarded as a fundamental component of management strategies for SLE[161].

A meta-analysis of five RCTs including 490 patients with SLE concluded that vitamin D deficiency, insufficiency and sufficiency could significantly elevate, slightly decrease (not significantly) or significantly decrease SLE risk, respectively[162]. Serum $25(OH)D_3$ concentrations were found to be negatively correlated with SLEDAI, whereas vitamin D supplementation was found to decrease fatigue but no significant changes in SLEDAI or the presence of anti-dsDNA antibodies were detected.

In conclusion, vitamin D supplementation is strongly recommended for patients with SLE, at least to prevent bone loss and fractures, and serum concentrations of vitamin D should be frequently evaluated throughout the year (avoidance of UV exposure is commonly prescribed in SLE patients, particularly in summer, to prevent photo-induced DNA damage).

The in vitro and in vivo studies reporting the biological and clinical effects of vitamin D in SLE are summarized in Table 2.

Vitamin D and systemic sclerosis

Reduced serum concentrations of vitamin D are regularly recognized in patients with SSc, irrespective of clinical and/or serological phenotype. However, compared with what has already been determined and suggested in RA and SLE, the involvement of vitamin D in the pathogenesis of SSc remains less well defined, as are the effects of low serum concentrations on disease activity and of vitamin D supplementation on patients with SSc.

For example, serum concentrations of $25(OH)D_3$ have been shown to be significantly lower in patients with diffuse cutaneous SSc (dcSSc) than in those with limited cutaneous SSC (lcSSc), despite the fact that $25(OH)D_3$ concentration by itself does not seem to be associated with disease severity in either phenotype[163]. Similar results were obtained in another investigation that found significantly lower $25(OH)D_3$ concentrations in patients with dcSSc than in those with lcSSc[164].

Of note, in a study of 51 consecutive patients with SSc, 92% of whom had vitamin D insufficiency or deficiency, serum concentrations $25(OH)D_3$ correlated with the diffusing capacity of the lung for carbon monoxide ($P = 0.019$), diastolic dysfunction ($P = 0.033$), digital contractures ($P = 0.036$) and muscle weakness ($P = 0.015$)[165].

However, an updated systematic literature review published in 2021 that included 40 eligible articles (of >428 evaluated) confirmed that low serum concentrations of vitamin D are common in patients with SSc and seem to be associated with different serological and clinical phenotypes of the disease[166]. The majority of the investigations showed no significant association between vitamin D serum concentrations and overall disease activity or severity (evaluated by the Medsger disease severity score)[166].

Nevertheless, some clinical studies have found significant correlations with specific complications of SSc, such as a longitudinal analysis that evaluated the relationship between the variation over time of $25(OH)D_3$ serum concentrations and the incidence of digital ulcers in 65 patients with SSc[167].

A decrease in serum concentration of $25(OH)D_3$ occurred in 48% of the patients during a 5-year follow-up period and was associated with an increased risk of developing digital ulcers. In particular, in a univariate analysis, patients with incident digital ulcers had a significant decrease in $25(OH)D_3$ compared with patients with no incident digital ulcers ($P = 0.018$), and in a multivariate analysis corrected for previous digital ulcers and modified Rodnan Skin Score (mRSS) at baseline, those with a decrease in $25(OH)D_3$ were again at an increased risk of developing digital ulcers ($P = 0.017$). No differences in $25(OH)D_3$ variations were found for other disease complications.

In fact, lower serum $25(OH)D_3$ concentrations were found to be negatively correlated with the magnitude of skin fibrosis in a retrospective cohort study of 327 patients with SSc, and higher mRSS scores were observed in patients with SSc who had vitamin D insufficiency[168]. A subsequent study confirmed higher mRSS scores in patients with SSc and vitamin D insufficiency[169]. However, some other studies were apparently not able to detect significant associations between serum vitamin D concentrations and the extent of skin involvement or mRSS, or with other clinical complications[170–172].

In SSc, during any period of the disease, peripheral skin microvasculopathy is linked to skin fibrosis. Nailfold videocapillaroscopy (NVC) is widely used to evaluate microvascular changes in SSc; specific capillaroscopic patterns not only reflect the clinical diagnosis, but also predict internal organ involvement and prognosis[173]. Some studies have evaluated the potential association between vitamin D and NVC-SSc patterns in patients with SSc, and it was found that diffuse loss of capillaries (avascular areas; 'late' NVC pattern) were associated with lower vitamin D concentrations[174]. In addition, in another important study of 65 consecutive patients with SSc who underwent evaluation of serum $25(OH)D_3$ concentrations, a 'late' NVC pattern (the most severe pattern) was found more frequently in the presence of vitamin D deficiency ($P = 0.013$); low serum $25(OH)D_3$ concentration was also associated with longer disease duration ($P = 0.026$), lower diffusing lung capacity for carbon monoxide ($P = 0.014$) and higher estimated pulmonary artery pressure ($P = 0.037$)[175].

A further 2019 study in 62 consecutive patients with SSc, all with vitamin D deficiency, found significant correlations between sarcopenia

Table 2 | Biological and clinical effects of vitamin D in SLE in in vitro and in vivo studies

Study	Population(s)	Intervention	Vitamin D₃ regimen	Follow-up	Results
Linker-Israeli et al.[140]	8 patients with active SLE (SLAM index ≥10) never supplemented with vitamin D₃ 8 healthy age- and sex-matched individuals	In vitro treatment of SLE PBMCs with calcitriol and synthetic analogues	Calcitriol, 1,25-(OH)₂-16-ene-D₃ (HM), 1,25-(OH)₂-16-ene-23-yne-D₃ (V), 20-epi-1,25(OH)₂D₃ (MC1288 and KH1060) at different concentrations	N/A	Calcitriol and synthetic analogues inhibited polyclonal and anti-dsDNA IgG production by PBMCs from patients with SLE
Ruiz-Irastorza et al.[155]	18 patients with SLE and serum vitamin D₃ concentration >30 ng/ml 24 patients with SLE and serum vitamin D₃ concentration between 20 ng/ml and 30 ng/ml 25 patients with SLE and serum vitamin D₃ concentration between 10 ng/ml and 20 ng/ml 13 patients with SLE and serum vitamin D₃ concentration <10 ng/ml	60 patients received oral vitamin D₃ supplementation	Oral cholecalciferol or calcidiol (dosages at the discretion of the physician)	2 years	Increasing serum vitamin D₃ concentration improved fatigue but not SLEDAI or SDI in patients with SLE
Ben-Zvi et al.[143]	198 consecutively recruited patients with SLE of different ethnic groups (Asian, African American, white, Hispanic)	Monocyte-derived dendritic cells from patients with SLE were cultured with or without calcitriol and stimulated with LPS	Cell culture with 10 nM concentration calcitriol	N/A	Vitamin D₃ deficiency correlated with increased SLEDAI Serum vitamin D₃ concentration did not affect numbers of circulating myeloid and plasmacytoid dendritic cells Treatment of cells with calcitriol did not affect the expression of activation markers (HLA-DR, CD40, CD86)
Lerman et al.[142]	15 paediatric patients with SLE (median vitamin D₃ concentration 16 ng/ml) 5 healthy individuals (all vitamin D₃ concentration >20 ng/ml)	Monocytes were cultured in RMPI and treated for 3 days with calcitriol, IFNα, GM–CSF and IL-4	Cell culture with calcitriol	N/A	Calcitriol limited the activation of SLE monocytes, downregulating the expression of MHC class II, CD40 and CD86 and increasing the expression of CD14
Terrier et al.[141]	20 patients with SLE and serum vitamin D₃ concentrations <30 ng/ml	Vitamin D₃ supplementation	Cholecalciferol 100,000 IU per week for 4 weeks, followed by cholecalciferol 100,000 IU per month for 6 months	~7 months	Cholecalciferol supplementation significantly increased numbers of naive CD4⁺ T cells and regulatory T cells, and decreased numbers of T_H1 cells, T_H17 cells, memory B cells and anti-DNA antibodies
Sumethkul et al.[151]	36 patients with inactive SLE (SLEDAI <3) 36 patients with active SLE (SLEDAI ≥3) without active lupus nephritis 36 patients with active SLE (SLEDAI ≥3) and lupus nephritis	N/A	Vitamin D₃ supplementation was not allowed	N/A	Patients with active SLE and active lupus nephritis had lower serum vitamin D₃ serum concentrations than both patients with inactive SLE and patients with active SLE but without lupus nephritis Lupus nephritis predicts vitamin D₃ deficiency in patients with SLE
Petri et al.[147]	243 patients with SLE and serum vitamin D₃ concentrations >40 ng/ml 763 patients with SLE and serum vitamin D₃ concentrations <40 ng/ml	Vitamin D₃ supplementation for patients with serum concentrations <40 ng/ml	Vitamin D₂ 50,000 IU per week together with calcium plus vitamin D₃ 200 IU twice per day	128 weeks	In patients with low serum concentrations of vitamin D₃ at baseline, an increase of 20 ng/ml in serum vitamin D₃ concentration correlated with a 21% decrease in the odds of having a high activity score index and a 15% decrease in the odds of having clinically relevant proteinuria
Sahebari et al.[136]	82 patients with SLE not receiving more than 800 UI per day of vitamin D₃ supplementation as standard therapy 49 healthy age- and sex-matched volunteers	All patients with SLE received hydroxychloroquine (6 mg/day) and supplementation with calcium carbonate (1,500 mg per day) and vitamin D₃ (800 UI per day)	Vitamin D₃ (800 IU per day)	At least 3 months	No correlation was found between serum vitamin D₃ concentration levels and disease duration and SLEDAI
Dall'Ara et al.[195]	50 patients with SLE assessed twice for serum vitamin D₃ concentration during disease remission, once in summer and once in winter; 30 of these patients were also assessed during a disease flare 170 healthy volunteers	24 patients received supplementation with vitamin D₃ drops 12 patients received supplementation with calcium carbonate and cholecalciferol tablets 14 SLE patients did not receive vitamin D₃ supplementation	Vitamin D₃ drops (calcidiol or cholecalciferol; average dose 6,250 IU weekly) Or cholecalciferol in tablets (average dose 4,560 IU weekly)	~1 year	Winter SLE flares were associated with lower serum vitamin D₃ concentrations in comparison with samples collected during the same season when the patient was in remission

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Table 2 (continued) | Biological and clinical effects of vitamin D in SLE in in vitro and in vivo studies

Study	Population(s)	Intervention	Vitamin D_3 regimen	Follow-up	Results
Andreoli et al.[148]	34 premenopausal patients with SLE and SLEDAI-2k score <6 in the year prior to enrolment and without vitamin D_3 supplementation	In addition to their ongoing treatment, patients received either a standard regimen or intensive regimen of vitamin D_3 supplementation for 1 year, and for the 2nd year switched to the other regimen	Intensive regimen: cholecalciferol 300,000 IU initial bolus then maintenance dosing with 50,000 IU per month to 12 months. Standard regimen: cholecalciferol 25,000 IU per month to 12 months	2 years	Neither the standard nor the intensive vitamin D_3 supplementation regimen changed SLE disease activity or serology (anti-dsDNA antibodies, C3, C4, CH50)
Piantoni et al.[154]	34 premenopausal patients with SLE	Patients received either a standard regimen or intensive regimen of vitamin D_3 supplementation for 1 year, and for the 2nd year switched to the other regimen	Intensive regimen: cholecalciferol 300,000 IU initial bolus then 50,000 IU per month as maintenance. Standard regimen: cholecalciferol 25,000 IU per month	2 years	Cholecalciferol supplementation increased the number of T_{reg} cells and the production of T_H2 cytokines
Lima et al.[150]	40 patients with juvenile-onset SLE and SLEDAI <12; vitamin D_3 supplementation was discontinued 3 months before the study	20 patients received supplementation with cholecalciferol and 20 patients received placebo	Cholecalciferol 50,000 IU per week	24 weeks	Cholecalciferol supplementation decreased SLE disease activity (assessed with SLEDAI and ECLAM) and improved fatigue (assessed with K-FSS)
Karimzadeh et al.[149]	90 patients with SLE (mean SLEDAI-2K score 3.09) and serum vitamin D_3 concentrations <30 ng/ml	45 patients received vitamin D_3 supplementation 45 patients received placebo	Vitamin D_3 50,000 IU weekly for 12 weeks and subsequently 50,000 IU monthly for 3 months	6 months	SLEDAI did not significantly improve after vitamin D_3 supplementation
Mok et al.[159]	55 patients with SLE and serum vitamin D_3 concentrations >30 ng/ml. 150 patients with SLE and serum vitamin D_3 concentrations between 15 ng/ml and 30 ng/ml. 71 patients with SLE and serum vitamin D_3 concentrations <15 ng/ml	Usual treatment plus vitamin D_3 supplementation according to physician's prescription	Vitamin D_3 supplementation according to physician's prescription	32.5±5.5 months	Serum vitamin D_3 concentration <15 ng/ml was associated with more active SLE disease at baseline and with the risk of future disease flares
Yu et al.[152]	25 patients with SLE and lupus nephritis (5 patients within each of the following histological classes of lupus nephritis: III, IV, V, III+V, IV+V). 7 individuals with renal carcinoma without features of kidney dysfunction or altered glomeruli as the control group	Treatment of SLE renal biopsy-obtained specimens with calcitriol	Calcitriol 100 nM	N/A	Among patients with SLE and lupus nephritis, those with serum vitamin D_3 concentrations <20 ng/ml showed more autophagosomes in podocytes than those with serum vitamin D_3 concentrations between 20 ng/ml and 30 ng/ml. Treatment with vitamin D_3 reduced autophagic activity and alleviated podocyte injury
Dutta et al.[161]	10 patients with SLE and serum vitamin D_3 concentrations >30 ng/ml. 38 patients with SLE and serum vitamin D_3 concentrations between 20 ng/ml and 30 ng/ml SLE. 61 patients with SLE and serum vitamin D_3 concentrations <20 ng/ml. 109 healthy individuals	N/A	Vitamin D_3 supplementation was not allowed	N/A	Serum vitamin D_3 concentrations were significantly lower in patients with SLE than in healthy individuals. Serum vitamin D_3 concentrations were inversely associated with SLEDAI scores. Serum vitamin D_3 concentrations <30 ng/ml increase the risk of high or very high SLE disease activity. High SLEDAI score (>10) correlated with an increased risk of having serum vitamin D_3 concentrations <20 ng/ml

Anti-dsDNA, anti-double-stranded-DNA antibodies; BILAG, British Isles Lupus Assessment Group of SLE Clinics; ECLAM, European Consensus Lupus Activity Measurement; GM–CSF, granulocyte-macrophage colony-stimulating factor; K-FSS, Kids Fatigue Severity Scale; N/A, not applicable; PBMC, peripheral blood mononuclear cell; SDI, Systemic Lupus International Collaborating Clinics/American College of RheumatologyACR Damage Index; SELENA, Safety of Estrogens in Systemic Lupus Erythematosus; SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

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(according to the Relative Skeletal Mass Index) and the 'late' NVC pattern as well with higher mRSS[176]. Similarly, in 154 patients with SSc recruited in all seasons, vitamin D deficiency correlated with peripheral vascular involvement, as evaluated by NVC, as well as with kidney, lung involvement and gastrointestinal parameters of the Medsger disease severity score[177]. Because low 25(OH)D$_3$ serum concentrations failed to be corrected by oral cholecalciferol administration, the authors suggested that serum 25(OH)D$_3$ concentrations should be monitored 3–6 months from the start of treatment, and if they remain low then supra-physiological oral doses of vitamin D should be considered in patients with SSc.

Interestingly, a high prevalence of 25(OH)D$_3$ insufficiency was also found in patients with juvenile onset SSc and, as expected, serum 25(OH)D$_3$ concentrations correlated with values of bone mineral apparent density[178]. The in vitro and in vivo studies reporting biological and clinical effects of vitamin D in SSc are summarized in Table 3.

Vitamin D and seasonal rhythms of autoimmune rheumatic diseases

The 25(OH)D$_3$ circulating in serum as a precursor of the active secosteroid 1,25(OH)$_2$D$_3$ derives for the most part (80%) from skin cholesterol (7-DHC) following exposure to UVB (sunlight); as a consequence, serum concentrations of vitamin D fluctuate according to the seasonal rhythm of sun exposure (circannual rhythm)[179]. VDR mRNA expression is at its lowest during late winter and early spring and correlates negatively with mRNA expression and serum concentrations of inflammatory cells and biomarkers (Fig. 3a), increased disease activity and clinical severity, as well as relapse rates in several disease autoimmune diseases including RA, SLE and SSc[180,181] (Fig. 3b).

Seasonal changes in the transcriptional landscapes of peripheral blood mononuclear cells, which seem to be driven predominantly by circannual fluctuations in the cellular composition of blood, are expected to influence various functions of the human immune response[182]. In fact, seasonal variations (lower in summer, higher in winter) have been found for dendritic cells, monocytes/neutrophils, natural killer cells, activated CD4$^+$ T cells and activated B cells in healthy volunteers[183]. Accordingly, the concentration of soluble IL-6 receptor protein was found in the same investigation to be increased in winter, in full agreement with the increased winter expression of *IL6R* mRNA, as well as of the acute phase reactant C-reactive protein (mRNA and protein), whereas expression of *VDR* was found to be increased in summer[183] (Fig. 3a). B cell receptor signalling and FcR-γ-associated processes were also strongly associated with winter expression (Fig. 3a).

A detailed ex vivo study in 15 healthy volunteers over the course of the four seasons of the year clearly showed that serum concentrations of 25(OH)D$_3$ and 1,25(OH)$_2$D$_3$ were significantly higher during summer ($P < 0.05$), and TLR-4-mediated production of IL-1β, IL-6, TNF, IFNγ and IL-10 was significantly down-regulated in summer compared with winter ($P < 0.05$)[184].

Furthermore, another key report that evaluated peripheral blood mononuclear cells and whole blood from 15 healthy participants every 3 months for 1 year showed that elevated serum 25(OH)D$_3$ and 1,25(OH)$_2$D$_3$ concentrations in summer were associated with a higher number of peripheral CD4$^+$ and CD8$^+$ T cells[185]. In addition, the percentage of T$_{reg}$ cells, but not their absolute numbers, decreased in summer. Of note, within the T$_{reg}$ cell population, the levels of FOXP3 expression increased in summer; also, CD4$^+$ and CD8$^+$ T cells showed a decreased capacity to produce pro-inflammatory cytokines in summer. All these observations linked to seasonal variations in vitamin D might explain why ARDs have peak incidences in winter.

Interestingly, in the northern hemisphere, spring (after winter) has been associated with worsening of inflammatory articular symptoms in patients with RA, whereas autumn (after summer) has been associated with their improvement, as evaluated by patient VAS and HAQ as well as tender joint count, swollen joint count and measurement of inflammatory markers (C-reactive protein and erythrocyte sedimentation rate)[186,187]. Seasonal variations of fatigue have similarly been described in patients with RA, using both reported VAS and the Bristol Rheumatoid Arthritis Fatigue Multidimensional Questionnaire, and with the worst results evident during winter[188].

In addition, the season of clinical onset of RA, according to data from 736 patients with RA in a multicentre study, seems to predict the severity of the disease. In fact, RA onset during spring or winter (with low exposure to solar UVB and hence low synthesis of vitamin D) rather than summer or autumn was reportedly associated with faster erosive radiographic progression (already at 6 months), together with a lower probability of disease remission at 1 year[189,190].

To avoid seasonal interference with vitamin D concentrations, in a large European cross-sectional study involving 625 individuals with RA from 13 different countries, data were collected only in winter (lowest concentrations), and the study confirmed significant correlations between low serum 25(OH)D$_3$ concentrations and quality of life, disease activity and disability of patients with RA[191].

Patients with RA are also characterized by a circadian (24-h) rhythm of morning time clinical inflammatory symptoms that are related to the night-time activation of inflammatory processes, but no circadian variations of vitamin D serum concentrations have been found in these patients[192].

An interesting 2021 study showed that children with juvenile idiopathic arthritis ($n = 164$) were more often born in the fall or winter than healthy children, and among the children with juvenile idiopathic arthritis low levels of vitamin D were associated with indicators of more intense inflammation[193].

However, in autoimmune diseases such as SLE, it was unclear if low 25(OH)D$_3$ status is simply a chronic condition associated with severe disease or if it is linked to SLE flare onset. A study on SLE flare rates in a large cohort of patients with SLE (106 patients, 201 flares) showed a tendency towards higher flare rates during low-daylight months (October through March) compared with high-daylight months (April through September) and only in non-African American (European American, Asian or Hispanic) patients ($P = 0.060$). SLE flare rates were lower during high-daylight months for non-African American patients than for African American patients ($P = 0.028$)[194]. The conclusion is that in patients with SLE who are not African American, large declines in serum concentrations of 25(OH)D$_3$ during low-daylight months seem to be mechanistically associated with SLE flare, whereas relatively high 25(OH)D$_3$ concentrations during high-daylight months seem to protect against flares.

In a further study, serum 25(OH)D$_3$ concentrations were evaluated longitudinally in 50 patients with SLE from northern Italy at two timepoints (summer and winter) and during disease remission[195]. Interestingly, winter SLE flares were associated with lower 25(OH)D$_3$ levels in comparison with remission during the same season for each patient.

Investigations on seasonal variations of vitamin D concentration in patients with SSc are very limited; however, a detailed study in 53 female patients with SSc during winter found that 60% of the patients with SSc had vitamin D insufficiency compared with 38% of age-matched healthy individuals (19.3 ± 12.3 ng/ml versus 32.1 ± 14.1 ng/ml; $P < 0.001$). During summer, 64% of the patients with SSc and 36% of controls had

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Table 3 | Biological and clinical effects of vitamin D in SSc in in vitro and in vivo studies

Study	Population(s)	Intervention	Vitamin D$_3$ regimen	Follow-up	Results
Humbert et al.[170]	11 patients with SSc (3 with the CREST syndrome variant and 8 with dcSSc)	Treatment with oral calcitriol	Oral calcitriol, mean dose 1.75 µg per day	6 months to 3 years	Vitamin D$_3$ supplementation seemed to improve skin thickness and extensibility (assessed with a twistometer)
Caramaschi et al.[175]	65 patients with SSc, 40 of whom had lcSSc and 25 had dcSSc (43 had serum vitamin D$_3$ concentrations between 10 ng/ml and 30 ng/ml and 19 had serum vitamin D$_3$ concentrations <10 ng/ml)	N/A	No vitamin D$_3$ supplementation was allowed	N/A	SSc patients with serum vitamin D$_3$ concentrations <10 ng/ml more frequently showed a 'late' NVC pattern, lung involvement (low DLCO) and higher estimated artery pressure than those with serum vitamin D$_3$ concentrations between 10 ng/ml and 30 ng/ml
Shinjo et al.[178]	10 patients with juvenile-onset SSc with serum vitamin D$_3$ concentrations <20 ng/ml 10 healthy children	N/A	No data about vitamin D$_3$ supplementation	N/A	Patients with SSc had significantly lower serum vitamin D$_3$ concentrations than healthy individuals Bone mineral apparent density (assessed with DXA) in total femur and femoral neck correlated with serum vitamin D$_3$ concentration in juvenile-onset SSc
Seriolo et al.[196]	53 patients: in winter, 32 of these patients had serum vitamin D$_3$ concentrations between 10 ng/ml and 30 ng/ml and 5 had serum vitamin D$_3$ concentrations <10 ng/ml; in summer, 34 had serum vitamin D$_3$ concentrations between 10 ng/ml and 30 ng/ml and 13 had serum vitamin D$_3$ concentrations <10 ng/ml 35 healthy individuals	N/A	No data about vitamin D$_3$ supplementation	1 year	Patients with SSc had significantly lower serum vitamin D$_3$ concentrations than healthy individuals Patients with SSc show circannual variations in serum vitamin D$_3$ concentrations, with a peak in late summer and a decrease in late winter
Arnson et al.[168]	327 patients with SSc 141 healthy individuals	N/A	Incomplete data about vitamin D$_3$ supplementation	N/A	Patients with SSc had significantly lower serum vitamin D$_3$ concentrations than healthy individuals Serum vitamin D$_3$ concentration inversely correlated with skin fibrosis (assessed with mRSS) and lung DLCO
Gambichler et al.[171]	137 patients with SSc; at baseline, 14 of these patients had serum vitamin D$_3$ concentrations >30 ng/ml, 74 had serum vitamin D$_3$ concentrations between 10 ng/ml and 30 ng/ml, and 49 had serum vitamin D$_3$ concentration <10 ng/ml	N/A	No vitamin D$_3$ supplementation was allowed	2 years	No significant correlations were found between serum vitamin D$_3$ concentration and clinical features of SSc
Corrado et al.[164]	64 consecutive post-menopausal patients with SSc (33 with lcSSc and 31 with dcSSc) 35 healthy, age-matched post-menopausal women		No vitamin D$_3$ supplementation was allowed	N/A	Bone mineral density (assessed with DXA) at the femoral neck, total hip and spine was significantly lower in dcSSc than in lcSSc Serum vitamin D$_3$ concentrations were significantly lower in dcSSc than in lcSSc, and inversely correlated with the extent of skin thickness (assessed with mRSS)
Atteritano et al.[169]	40 patients with SSc (12 with lcSSc and 28 with dcSSc); at baseline, 20 of these patients had serum vitamin D$_3$ concentrations >30 ng/ml and 20 had serum vitamin D$_3$ concentrations between 10 ng/ml and 30 ng/ml 40 healthy individuals	N/A	No vitamin D$_3$ supplementation was allowed	N/A	Patients with SSc had significantly lower serum vitamin D$_3$ concentrations than healthy individuals Cutaneous involvement (assessed with mRSS) and systolic pulmonary artery pressure >35 mmHg (assessed with echocardiography) correlated with low serum concentrations of vitamin D$_3$
Sampaio-Barros et al.[174]	38 patients with dcSSc (5 with serum vitamin D$_3$ concentration >30 ng/ml, 14 with serum vitamin D$_3$ concentration between 20 ng/ml and 30 ng/ml, 15 with serum vitamin D$_3$ concentration between 10 ng/ml and 20 ng/ml and 4 with serum vitamin D$_3$ concentration <10 ng/ml)	N/A	No data about vitamin D$_3$ supplementation	N/A	Low serum concentration of vitamin D$_3$ was associated with worse quality of life (assessed with SF-36 and HAQ) and severe NVC alterations (avascular areas) in patients with SSc
Groseanu et al.[165]	24 patients with lcSSc 27 patients with dcSSc		No data about vitamin D$_3$ supplementation	N/A	Low serum concentration of vitamin D$_3$ correlated with pulmonary fibrosis, low DLCO and diastolic cardiac dysfunction

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Table 3 (continued) | Biological and clinical effects of vitamin D in SSc in in vitro and in vivo studies

Study	Population(s)	Intervention	Vitamin D$_3$ regimen	Follow-up	Results
Trombetta et al.[177]	92 patients with lcSSC 39 patients with dcSSc 23 patients with SSc sine scleroderma	N/A	22 patients were routinely supplemented with 1,000 IU/day of oral cholecalciferol	N/A	Serum vitamin D$_3$ concentration <20 ng/ml correlated with SSc lung fibrosis and some Medsger disease severity scale parameters (peripheral vascular, kidney and gastrointestinal), and also followed seasonal fluctuations
Zhang et al.[172]	60 patients with dcSSc (35 with serum vitamin D$_3$ concentration between 10 ng/ml and 30 ng/ml and 10 with serum vitamin D$_3$ concentration <10 ng/ml) 60 healthy individuals	N/A	No vitamin D$_3$ supplementation was allowed	N/A	Patients with SSc had significantly lower serum vitamin D$_3$ concentrations than healthy individuals No statistical differences in SSc clinical manifestations were seen between patients with serum vitamin D$_3$ concentrations above or below 30 ng/ml
Caimmi et al.[167]	65 consecutive patients with SSc (45 with lcSSc and 20 with dcSSc); at baseline, 17 of these patients had serum vitamin D$_3$ concentrations between 20 ng/ml and 30 ng/ml and 16 had serum vitamin D$_3$ concentrations <20 ng/ml	Vitamin D$_3$ supplementation was being received by 39 (60%) patients at baseline and 45 (69%) patients by the end of the follow-up period.	Cholecalciferol 8,750 IU per week, according to physician choice	5 years	Low serum concentrations of vitamin D$_3$ are a risk factor for the development of digital ulcers in patients with SSc

CREST: calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly and telangiectasia; dcSSc, diffuse cutaneous systemic sclerosis; DLCO, diffusing lung capacity for carbon monoxide; DXA, dual-energy X-ray-absorptiometry; HAQ, Health Assessment Questionnaire; lcSSc, limited cutaneous systemic sclerosis; mRSS, modified Rodnan Skin Score; NVC, nailfold videocapillaroscopy; SF-36, Short-Form-36 Questionnaire; SSc systemic sclerosis.

vitamin D insufficiency (21.7 ± 13.4 ng/ml versus 39.4 ± 15.4 ng/ml; $P < 0.001$)[196]. Interestingly, both the patients with SSc and the matched healthy individuals showed seasonal variations in serum 25(OH)D$_3$ concentrations, probably owing to different exposure to sunlight (UVB) according to the study period, even though the average serum 25(OH)D$_3$ concentrations were significantly lower in the patients with SSc than in the controls throughout the year. In a large multicentre investigation involving 154 consecutive patients with SSc from Italy and Belgium, serum concentrations of vitamin D differed significantly by season in the patients overall ($P = 0.032$) and particularly when winter 25(OH)D$_3$ serum concentrations were compared with summer ones ($P = 0.0086$)[177].

A 2020 study in a large cohort of Thai patients with SSc ($n = 2,480$) concerning the influence of the seasons on clinical manifestations of SSc found that the rate of admission to the health care system was highest in the rainy season (between mid-May and mid-October) followed by the cool dry season (between mid-October and mid-February) and then the hot season (between mid-February and mid-May), although no vitamin D values were reported[197].

In conclusion, seasonal changes in vitamin D concentrations seem to correlate negatively with both the onset and the severity of ARDs.

Vitamin D involvement in immune response and therapeutic effects in COVID-19

The COVID-19 pandemic has had important effects on complex ARDs, including the difficult management of care and treatment during (and after) SARS-CoV-2 infection[198]. In a multicentre cross-sectional study, symptomatic COVID-19 occurred in 122 of 3,028 patients (4.0%) affected by ARDs, of whom 24 (19.7%) were admitted to hospital and 4 (3.3%) died[199]. By considering the increased risk of infections in vitamin D-deficient patients with ARDs, from the beginning of the COVID-19 pandemic in 2020 it was evident that generally individuals with low vitamin D concentrations were more prone to experiencing SARS-CoV-2

infection and disease progression, and in addition they had worse disease prognosis and higher mortality rates[200-203]. A growing number of new original research articles, meta-analyses, systematic reviews and narrative reviews emphasize these findings (368 publications in 2020, 780 in 2021 and 1,488 in total to date)[204-207].

Even before the COVID-19 pandemic, a systematic review and meta-analysis of individual participant data from RCTs clearly suggested the use of vitamin D supplementation to prevent acute respiratory tract infections[208]. Interestingly, a 2021 meta-analysis of studies that included ~2 million participants concluded that low serum 25(OH)D$_3$ concentrations (<20 ng/ml) increased the risk of SARS-CoV-2 infection by 1.46-fold[209].

Although SARS-CoV-2 infects many different cells in the body, its infection at the level of the upper and lower respiratory tract (upper airway epithelia, pulmonary alveolar pneumocytes and macrophages) is the major cause of morbidity and mortality, at least in men, as women (because of oestrogens) have a more efficient immune response against infections[210,211].

Airway epithelium constitutively expresses CYP27B1, the enzyme involved in the synthesis of 1,25(OH)$_2$D$_3$, and VDR, for which vitamin D is the ligand. Pulmonary alveolar macrophages are also induced to express both CYP27B1 and VDR by various pathogens, including viruses[52].

Viruses (possibly including SARS-CoV-2) and other respiratory tract pathogens induce the activation of innate immunity and as a consequence the increased local production of 1,25(OH)$_2$D$_3$, which enhances viral neutralization and clearance but also downregulates the subsequent pro-inflammatory response[212] (Fig. 4). T$_{reg}$ cells provide a principal system of defence against inflammation and against viral infection in general: their levels have been found to be low in many patients with COVID-19 and can be increased by vitamin D supplementation[213]. Therefore, it is biologically conceivable that 1,25(OH)$_2$D$_3$ could exert immunomodulatory effects in patients with COVID-19, by regulating both innate and adaptive immunity[214].

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By considering what we have discussed in the early sections of this Review regarding the interactions of the vitamin D endocrine system with the immune system, there are solid reasons to consider that stimulation of the VDR signalling pathway could also have multiple functional effects in patients with COVID-19 (ref. 209). Indeed, $1,25(OH)_2D_3$ is strongly involved in the early reactivity against SARS-CoV-2 infection, through innate antiviral effector mechanisms that involve the induction of antimicrobial peptides (such as cathelicidin and defensin) and autophagy, as well as in the later hyperinflammatory phase of COVID-19, by downregulating the cytokine storm[215] (Fig. 4).

In particular, effective concentrations of $1,25(OH)_2D_3$ might produce beneficial effects in patients with COVID-19 by dampening the cytokine/chemokine storm, inducing a transition from a T_H1 and T_H17 cell phenotypes towards adaptive immune responses characterized by a T_{reg} and T_H2 profile, modulating neutrophil activity and regulating the renin–angiotensin–aldosterone system, in addition to maintaining the integrity of the pulmonary epithelial barrier, directly and indirectly reducing hypercoagulability and attenuating lung fibrosis, all conditions associated with severe COVID-19 (refs. 216–219) (Fig. 4).

A 2022 multicentre, single-blind, prospective RCT in patients with COVID-19 pneumonia and with very low levels of $25(OH)D_3$ (14.8 ng/ml; SD 6.18) tested the antiviral efficacy, tolerance and safety of 10,000 IU per day versus 2,000 IU per day of cholecalciferol[220]. Treatment with cholecalciferol 10,000 IU per day did not modify levels of pro-inflammatory cytokines; however, an increase in the anti-inflammatory cytokine IL-10 and of $CD4^+$ T cells was noted. The cytotoxic response against SARS-CoV-2-infected cells was increased more than four-fold in patients with COVID-19 who were treated with cholecalciferol 10,000 IU per day in comparison with those treated with 2,000 IU per day, accompanied by significantly higher levels of IFNγ. Finally, clinical benefit of supplementation with cholecalciferol 10,000 IU per day was observed in patients with COVID-19 who developed acute respiratory distress syndrome, as they stayed in hospital for an average of 8.0 days, compared with the average stay of 29.2 days for patients treated with 2,000 IU of cholecalciferol per day ($P = 0.0381$). In agreement with these findings, and in line with what is done in ARDs, mounting evidence suggests that consideration be given to vitamin D supplementation in order to rapidly correct $25(OH)D_3$ deficiency and related complications in all patients with COVID-19, especially in the early stages of the disease[209].

Generally, calcifediol ($25(OH)D_3$) might have some advantages over native vitamin D (cholecalciferol), as it induces a more rapid increase in circulating $25(OH)D_3$ and has a higher rate of intestinal absorption than oral cholecalciferol[221]. In a very interesting and paradigmatic study, a total of 8,076 patients affected by advanced chronic kidney disease and hypoparathyroidism were selected for treatment with $1,25(OH)_2D_3$ treatment. The use of $1,25(OH)_2D_3$ was associated with reductions in the risk of SARS-CoV-2 infection ($P = 0.010$), severe COVID-19 and COVID-19-related mortality ($P = 0.001$)[222].

The results of a more recent double-blind RCT in which frontline health care workers were treated with 4,000 IU of cholecalciferol daily or placebo for 30 days confirmed that the risk of acquiring SARS-CoV-2 infection was lower in the group that received cholecalciferol (although the loss of one-third of the patients during follow-up undermined the
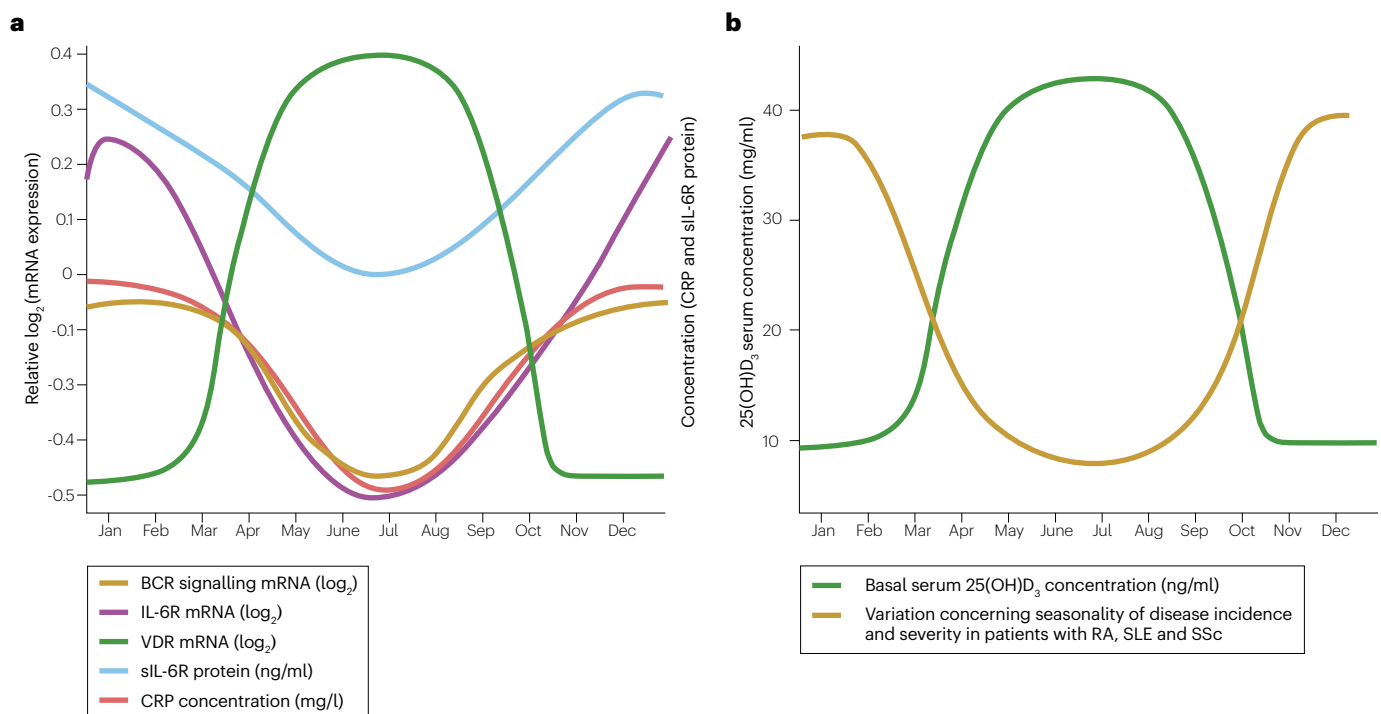

Fig. 3 | Seasonal trends in vitamin D status, expression of inflammatory mediators and incidence and severity of autoimmune rheumatic diseases. **a**, Serum concentrations of mediators of inflammation (measured as either mRNA or protein expression), vary seasonally in healthy individuals[183]. Whereas expression of pro-inflammatory markers increases in winter, that of vitamin D receptor (VDR) peaks in summer. **b**, The incidence and severity of disease in patients affected by rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) also vary seasonally and correlate negatively with average serum concentrations of $25(OH)D_3$. BCR, B cell receptor; CRP, C-reactive protein; IL-6R, IL-6 receptor; sIL-6R, soluble IL-6 receptor.
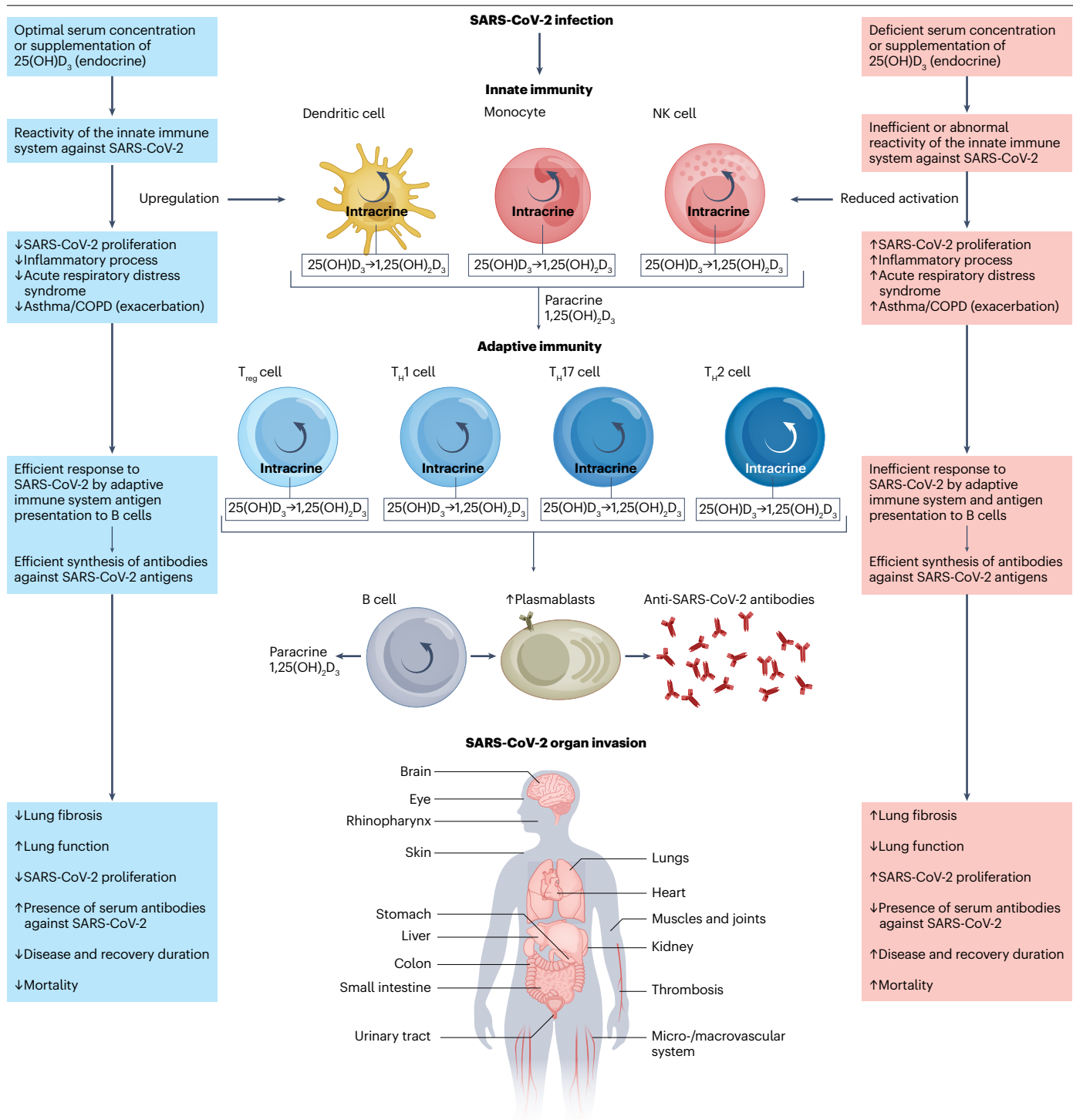
SARS-CoV-2 infection

| Optimal serum concentration or supplementation of $25(OH)D_3$ (endocrine) | | Deficient serum concentration or supplementation of $25(OH)D_3$ (endocrine) |

Innate immunity

Dendritic cell   Monocyte   NK cell

| Reactivity of the innate immune system against SARS-CoV-2 | | Inefficient or abnormal reactivity of the innate immune system against SARS-CoV-2 |

Upregulation → Intracrine (Dendritic cell) | Intracrine (Monocyte) | Intracrine (NK cell) ← Reduced activation

$25(OH)D_3 \rightarrow 1,25(OH)_2D_3$ $25(OH)D_3 \rightarrow 1,25(OH)_2D_3$ $25(OH)D_3 \rightarrow 1,25(OH)_2D_3$

| ↓SARS-CoV-2 proliferation ↓Inflammatory process ↓Acute respiratory distress syndrome ↓Asthma/COPD (exacerbation) | | ↑SARS-CoV-2 proliferation ↑Inflammatory process ↑Acute respiratory distress syndrome ↑Asthma/COPD (exacerbation) |

Paracrine $1,25(OH)_2D_3$

Adaptive immunity

T_{reg} cell T_H1 cell T_H17 cell T_H2 cell

Intracrine   Intracrine   Intracrine   Intracrine

$25(OH)D_3 \rightarrow 1,25(OH)_2D_3$ $25(OH)D_3 \rightarrow 1,25(OH)_2D_3$ $25(OH)D_3 \rightarrow 1,25(OH)_2D_3$ $25(OH)D_3 \rightarrow 1,25(OH)_2D_3$

| Efficient response to SARS-CoV-2 by adaptive immune system antigen presentation to B cells | | Inefficient response to SARS-CoV-2 by adaptive immune system and antigen presentation to B cells |
| Efficient synthesis of antibodies against SARS-CoV-2 antigens | | Efficient synthesis of antibodies against SARS-CoV-2 antigens |

B cell   ↑Plasmablasts   Anti-SARS-CoV-2 antibodies

Paracrine $1,25(OH)_2D_3$

SARS-CoV-2 organ invasion

Brain
Eye
Rhinopharynx
Skin
Stomach
Liver
Colon
Small intestine
Urinary tract

Lungs
Heart
Muscles and joints
Kidney
Thrombosis
Micro-/macrovascular system

| ↓Lung fibrosis ↑Lung function ↓SARS-CoV-2 proliferation ↑Presence of serum antibodies against SARS-CoV-2 ↓Disease and recovery duration ↓Mortality | | ↑Lung fibrosis ↓Lung function ↑SARS-CoV-2 proliferation ↓Presence of serum antibodies against SARS-CoV-2 ↑Disease and recovery duration ↑Mortality |

Fig. 4 | Effects of vitamin D in patients with COVID-19. This schematic depicts the effects exerted by 25-hydroxyvitamin D_3 ($25(OH)D_3$) on innate and adaptive immune responses following SARS-CoV-2 infection, as well as the associated clinical manifestations. In the setting of optimal serum concentrations or supplementation of $25(OH)D_3$, the endocrine, paracrine and autocrine effects of active $1,25(OH)_2D_3$ serve to augment both innate antiviral effector mechanisms following SARS-CoV-2 infection and also adaptive immune responses in the later cytokine-mediated hyperinflammatory phase of COVID-19, thereby influencing clinical outcomes. COPD, chronic obstructive pulmonary disease.

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estimate of the effectiveness of vitamin D supplementation in reducing the risk of infection)[223].

A strategy of daily administration of low-dose 25(OH)D$_3$ has been evaluated in a double-blind, placebo-controlled, multicentre RCT including 106 hospitalized patients with COVID-19 who had low serum 25(OH)D$_3$ concentrations (<30 ng/ml) when admitted, with the first results showing a significant increase in the percentage of lymphocytes as well as an overall trend towards a reduced need for hospitalization, duration of intensive care, need for respiratory support and mortality, but these differences were not statistically significant[224].

Following these encouraging results, new placebo-controlled, double-blind, multi-centre RCTs with the aim of assessing whether vitamin D supplementation has a therapeutic effect in vitamin D-deficient patients with COVID-19 have been launched (NCT04525820, NCT04780061)[225,226].

However, a study that investigated the production of SARS-CoV-2 IgG and the neutralization potency of serum antibodies in response to mRNA vaccination in relation to serum 25(OH)D$_3$ concentrations over 24 weeks in a cohort of healthy adults[227] found no significant differences in the dynamic increase or decrease of SARS-CoV-2 IgG as a consequence of 25(OH)D$_3$ status. Furthermore, 25(OH)D$_3$ status was not linked to the response to the first or second vaccination over time, the peak SARS-CoV-2 IgG concentrations achieved or the reduction of SARS-CoV-2 IgG concentrations over time.

A 2021 study confirmed that serum 25(OH)D$_3$ deficiency was associated with more severe lung involvement, longer disease duration and an increased risk of death in elderly patients with COVID-19 (ref. [228]). Indeed, at present there is strong evidence suggesting a significant association between vitamin D insufficiency or deficiency and COVID-19 susceptibility and severity, as well as data from various RCTs demonstrating the beneficial effects of vitamin D supplementation on generally improving all respiratory infections[200,229]. A 2022 systematic review and meta-analysis concluded that in the face of ongoing vaccinations, keeping serum 25(OH)D$_3$ concentrations above 50 ng/ml is recommended in order to prevent or mitigate new outbreaks due to new mutations or a decrease in antibody activity[230]. As a final interesting note, statistical correlation analyses and a panel data fixed-effects model showed that UVB radiation coefficients correlate negatively in a significant manner with COVID-19 transmission rates[231].

In conclusion, therapeutic improvement in 25(OH)D$_3$ serum concentrations up to 80–100 ng/ml has been found to significantly decrease serum concentrations of the inflammatory markers associated with COVID-19, and vitamin D supplementation at doses recommended by the Endocrine Society can help to reduce the risk of not only SARS-CoV-2 infection but also its severe outcomes, including mortality[207,229].

Conclusions

Vitamin D is a prototypical secosteroid rather than a vitamin and should be regarded like all steroidal hormones, including mineralocorticoids, glucocorticoids and sex hormones. The vitamin D endocrine system is complex and synthesis of the final active metabolite 1,25(OH)$_2$D$_3$ involves endocrine, intracrine and paracrine modalities.

Both innate and adaptive immune responses are modulated by calcitriol and serum deficiency of this secosteroid is considered a risk factor for several ARDs, including RA, SLE and SSc. Circannual rhythms of vitamin D concentrations seem to correlate negatively with both the onset and the severity of ARDs. In the setting of vitamin D deficiency, supplementation with 25(OH)D$_3$ seems an efficient approach to better

manage ARDs at the onset of symptoms and also to reduce the severity of the disease progression.

Vitamin D also seems to have a crucial role in the prevention of severe SARS-CoV-2 infections, as well as in reducing the intensity of the innate immune system response, as shown by an increasing number of RCTs.

Considering the emerging evidence of the actions exerted by vitamin D as a modulator of immune system reactivity, its serum concentrations should be regularly evaluated, and 25(OH)D$_3$ supplemented until acceptable values for immune system balance are reached.

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Author contributions

M.C. and E.G. researched data for the article. V.S. and S.P. made a substantial contribution to discussion of the content. M.C. and E.G. wrote the manuscript. All authors reviewed and/or edited the manuscript before submission.

Competing interests

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