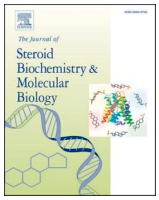
Journal of Steroid Biochemistry and Molecular Biology 249 (2025) 106710

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb



COVID-19 and our understanding of vitamin D and immune function

Martin Hewison [a,b,*]

[a] Department of Metabolism and Systems Science, School of Medical Sciences, University of Birmingham, Birmingham B15 2TT, UK
[b] Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Birmingham B15 2TT, UK

ARTICLE INFO

Keywords:
Vitamin D
COVID-19
Inflammation
adaptive immunity
innate immunity
antiviral
antibacterial
endometriosis
inflammation
immune
steroidogenic

ABSTRACT

The interaction between vitamin D and the immune system is perhaps the most well recognised extraskeletal facet of vitamin D, encompassing early studies of therapy for TB and leprosy through to more recent links with autoimmune disease. However, the spotlight on vitamin D and immune function has been particularly intense in the last five years following the COVID-19 pandemic. This was due, in part, to the many association studies of vitamin D status and COVID-19 infection and disease prognosis, as well as the smaller number of clinical trials of vitamin D supplementation. However, a potential role for vitamin D in COVID-19 also stemmed from the basic biology of vitamin D that provides a plausible mechanistic rationale for beneficial effects of vitamin D for improved immune health in the setting of respiratory infection. The aim of this review is to summarise the different strands of mechanistic evidence supporting a beneficial effect of vitamin D in COVID-19, how this was modified during the pandemic itself, and the potential new aspects of vitamin D and immune function that are likely to arise in the near future. Key topics that feature in this review are: antibacterial versus antiviral innate immune responses to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$); the function of immune 1α-hydroxylase (CYP27B1) activity and metabolism of 25-hydroxyvitamin D (25(OH)D) beyond antigen-presenting cells; advances in immune cell target gene responses to 1,25-dihydroxyvitamin D (notably changes in metabolic profile). Whilst much of the interest during the COVID-19 era has focused on vitamin D and public health, the continued evolution of our understanding of how vitamin D interacts with different components of the immune system continues to support a beneficial role for vitamin D in immune health.

1. Introduction

Extraskeletal actions have played a prominent role in the evolution of vitamin D research over the last 40 years [1], with the most well characterised of these being the interaction between vitamin D and the immune system [2]. A link between vitamin D and immunity initially arose from studies in the 19th century prior to the actual discovery vitamin D itself. In seminal studies carried out at the Royal Brompton Hospital in London, cod liver oil (a rich source of vitamin D) was shown to have positive effects in the treatment of tuberculosis (TB) (reviewed in [3]). In a similar fashion, in 1903 Niels Finsen won a Nobel Prize for his work on the use of sunlight for the treatment of lupus vulgaris (skin TB) [4]. Again, this was prior to the discovery of vitamin D, but the inference is that sunlight-generated vitamin D may have been a key factor in Finsen's observations, and exposure to sunlight became a key feature of the treatment of TB in sanatoria during the first half of the 20th century [5]. A specific immunological role for vitamin D was further endorsed by studies in the 1940s that used actual vitamin D as treatment for TB [6] and another mycobacterial disease, leprosy [7].

Further assessment of the therapeutic use of vitamin D in the setting of immunity was sidelined by the advent of antibiotics in the 1950s, and it was another 25 years before there was renewed interest in the

Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; CYP27B1, 25-hydroxyvitamin D-1α-hydroxylase; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; ACE2, angiotensin converting enzyme; APC, antigen presenting cell; B cells, B lymphocytes; CAMP, cathelicidin antimicrobial protein; LL-37, CAMP proteinase-cleaved; CTL, cytotoxic T cells; BD-2, β-defensin 2; DC, dendritic cell; DsRNA, double-stranded RNA; Th, helper T cells; HAMP, hepcidin antimicrobial protein; MTOR, mammalian target of rapamycin; *M. Tb*, *Mycobacterium tuberculosis*; NOD2, nucleotide-binding oligomerization domain 2; RBD, receptor binding domain; RAAS, renin-angiotensin-aldosterone system; SARS-CoV-2, SARS coronavirus-2; SINE, short interspersed nuclear element; S1, spike protein; T cells, T lymphocytes; TLR, toll-like receptor; VDR, vitamin D receptor.

* Correspondence to: Department of Metabolism and Systems Science, School of Medical Sciences, The University of Birmingham, Room 227, Level 2 IBR, Birmingham B15 2TT, UK.
E-mail address: m.hewison@bham.ac.uk.

https://doi.org/10.1016/j.jsbmb.2025.106710
Received 20 December 2024; Received in revised form 6 February 2025; Accepted 19 February 2025
Available online 20 February 2025

immunomodulatory actions of vitamin D. In this case, the link between vitamin D and immunity initially stemmed from observations of elevated circulating levels of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) in patients with the granulomatous disease sarcoidosis [8]. Whilst many subsequent studies focused on the role of vitamin D in the pathophysiology of hypercalcemia in patients with sarcoidosis [9], this era also marked the beginning of awareness that the link between vitamin D and immune function was much more than a simple pathophysiological phenomenon. The initial observation suggesting a broader role for vitamin D in the immune system arose from studies demonstrating that the source of elevated $1,25(OH)_2D$ in patients with sarcoidosis is the macrophages associated with the disease, rather than the classical renal location of the 1α-hydroxylase (CYP27B1) enzyme that converts 25-hydroxyvitamin D (25(OH)D) to $1,25(OH)_2D$ [10,11]. Macrophage metabolism of 25(OH)D provided the first example of extra-renal CYP27B1 activity, but initially it was unclear what function immune-synthesized $1,25(OH)_2D$ might have beyond the enhancement of renal CYP27B1-generated circulating levels of $1,25(OH)_2D$, and the hypercalcemic risks associated with this.

In parallel with studies of macrophage 25(OH)D metabolism, the other major observation linking vitamin D and immunity was the detection of intracellular receptors for $1,25(OH)_2D$ in immune cells. Many of these studies were carried out prior to cloning of the human vitamin D receptor (VDR) in 1988 [12], but nevertheless reported varying levels of intracellular $1,25(OH)_2D$ binding in a diverse array of immune cells including monocyte precursors to macrophages [13,14] and also T and B lymphocytes (T cells and B cells) [15–17], suggesting that cells from both the innate and adaptive (acquired) immune systems were potential targets for $1,25(OH)_2D$ [18]. The precise functional impact of $1,25(OH)_2D$ on these cells was somewhat less clear. For monocytes/macrophages the predominant effect of $1,25(OH)_2D$ appeared to be inhibition of proliferation and concomitant induction of cell differentiation towards a more mature macrophage-like phenotype [13,14,19,20]. Early studies of the functional response of lymphocytes to $1,25(OH)_2D$ also described potent anti-proliferative effects of $1,25(OH)_2D$ and related anologs [21–23]. These preliminary observations supported an initial hypothesis that vitamin D promoted innate immunity via enhanced monocyte differentiation, but suppressed adaptive immunity via decreased T and B cell proliferation [18]. This, in turn, lead to investigation of the potential use of 'non-calcemic' vitamin D analogs as therapeutic agents for immune disorders [24,25], whilst also providing the foundation for anti-cancer applications based on the antiproliferative/prodifferentiation effects of vitamin D analogs on monocytic cells of leukemic origin [26,27].

At the beginning of the 21st Century, 20 years after the first studies linking vitamin D and the immune system, the over-arching perspective was still pathophysiological, with aberrant vitamin D metabolism being a feature of granulomatous disorders [28], and immune actions vitamin D viewed in the setting of therapeutic responses to supplemental non-calcemic $1,25(OH)_2D$ analogs [29]. However, since then there has been a significant shift in our understanding of vitamin D and immune function, with localised synthesis of $1,25(OH)_2D$ by immune cells postulated as pivotal feature of normal immune physiology, and intracrine and paracrine responses to this immune-generated $1,25(OH)_2D$ described for both innate and adaptive arms of the immune system [30,31]. Crucially, this new perspective of vitamin D and immunity provided a mechanistic rationale for the increasing numbers of association studies linking vitamin D-deficiency with immune disorders. Specifically, decreased availability of circulating 25(OH)D was hypothesized to diminish immune synthesis of active $1,25(OH)_2D$ and thereby compromise intracrine/paracrine innate and/or adaptive immune function [32,33]. Conversely, enhanced serum 25(OH)D status following vitamin D supplementation might be expected to promote local immune synthesis of $1,25(OH)_2D$ and thus better promote effective antimicrobial innate and anti-inflammatory adaptive immune function.

This core concept of intracrine synthesis and action of $1,25(OH)_2D$ has underpinned the many association studies linking vitamin D status with human health issues, as well as the randomized control trials for vitamin D supplementation that have been carried out over the last 20 years. So far, this intracrine model for vitamin D has been based on studies *ex vivo* or *in vitro*, and it is only recently that new analytical technologies have confirmed the efficacy of localised extra-renal synthesis of $1,25(OH)_2D$. Specifically, the recently reported adoption of mass spectrometry imaging to 'map' the tissue distribution of $1,25(OH)_2D$ and other vitamin D metabolites has provided new evidence for tissue-specific synthesis of $1,25(OH)_2D$ [34]. This approach, coupled with ablation of CYP27B1 expression in classical renal tissue, has confirmed the capacity for synthesis of $1,25(OH)_2D$ at extra-renal sites, such as the spleen, that are enriched for immune cells [35]. In particular, these studies have shown that splenic production of $1,25(OH)_2D$ is strongly enhanced following *in vivo* vitamin D supplementation, supporting the core hypothesis that vitamin D status (25(OH)D availability) is a major driver of local synthesis of $1,25(OH)_2D$ in tissues exposed to infection, and associated immune responses [35].

The aim of this review is to firstly document the three fundamental mechanisms that define our current understanding of vitamin D and the immune system: 1) the importance of intracrine synthesis of $1,25(OH)_2D$; 2) identification of specific innate immune responses to locally synthesized $1,25(OH)_2D$; 3) identification of specific adaptive immune responses to $1,25(OH)_2D$. The second aim of this review is to detail each of these mechanisms linking vitamin D and immunity in the setting of developments that occurred during the COVID-19 pandemic. In the 5 years since SARS Coronavirus-2 (SARS-CoV-2) infections were first reported there have been almost 2000 published papers on vitamin D and COVID-19. Most of these publications have focused on associations between serum vitamin D status and risk and/or severity of COVID-19 [36], or the potential benefits of vitamin D supplementation in preventing or managing COVID-19 [37]. These studies have been well summarised in many other publications and the current review will not attempt to address epidemiological and clinical aspects of vitamin D and COVID-19. Instead, in common with other areas of vitamin D and the immune system, it is important to recognise that a plausible role for vitamin D in COVID-19 is supported by key underpinning mechanisms. The current review will focus on three of these pivotal mechanisms and how our perspective of these mechanisms and our broader view of vitamin D and immunity has been modified during the COVID-19 era.

2. Innate immune responses to vitamin D: from antibacterial to antiviral

2.1. Antiviral actions of antibacterial proteins

Perhaps the most pivotal observation linking vitamin D and innate immunity is the ability of $1,25(OH)_2D$ to promote antibacterial proteins such as cathelicidin antimicrobial protein (CAMP) (see Fig. 1A). The initial report detailing the direct regulation of CAMP expression by $1,25(OH)_2D$ [38] provided a platform for subsequent studies that placed vitamin D at the heart of innate immune responses to infection. The vitamin D responses element (VDRE) associated with $1,25(OH)_2D$-mediated induction of *CAMP* gene transcription was shown to be associated with a short interspersed nuclear element (SINE) transposable region of DNA [39]. The fact that this SINE and its associated VDRE were only detectable in higher primates strongly suggests that the ability of $1,25(OH)_2D$ to stimulate CAMP is a relatively recent genomic adaptation [39]. The potential importance of this evolutionary conservation for human health was then further underlined by seminal studies describing vitamin D induction of CAMP in monocytes following toll-like receptor (TLR) stimulus to mimic exposure to *Mycobacterium tuberculosis* (*M. Tb*) [40]. The crucial advance in this study was that activation of TLR signalling stimulated expression of both VDR and CYP27B1 in the monocytes so that *CAMP* gene transcription could be induced by addition of 25(OH)D, with intracrine production of $1,25(OH)_2D$ driving *CAMP*

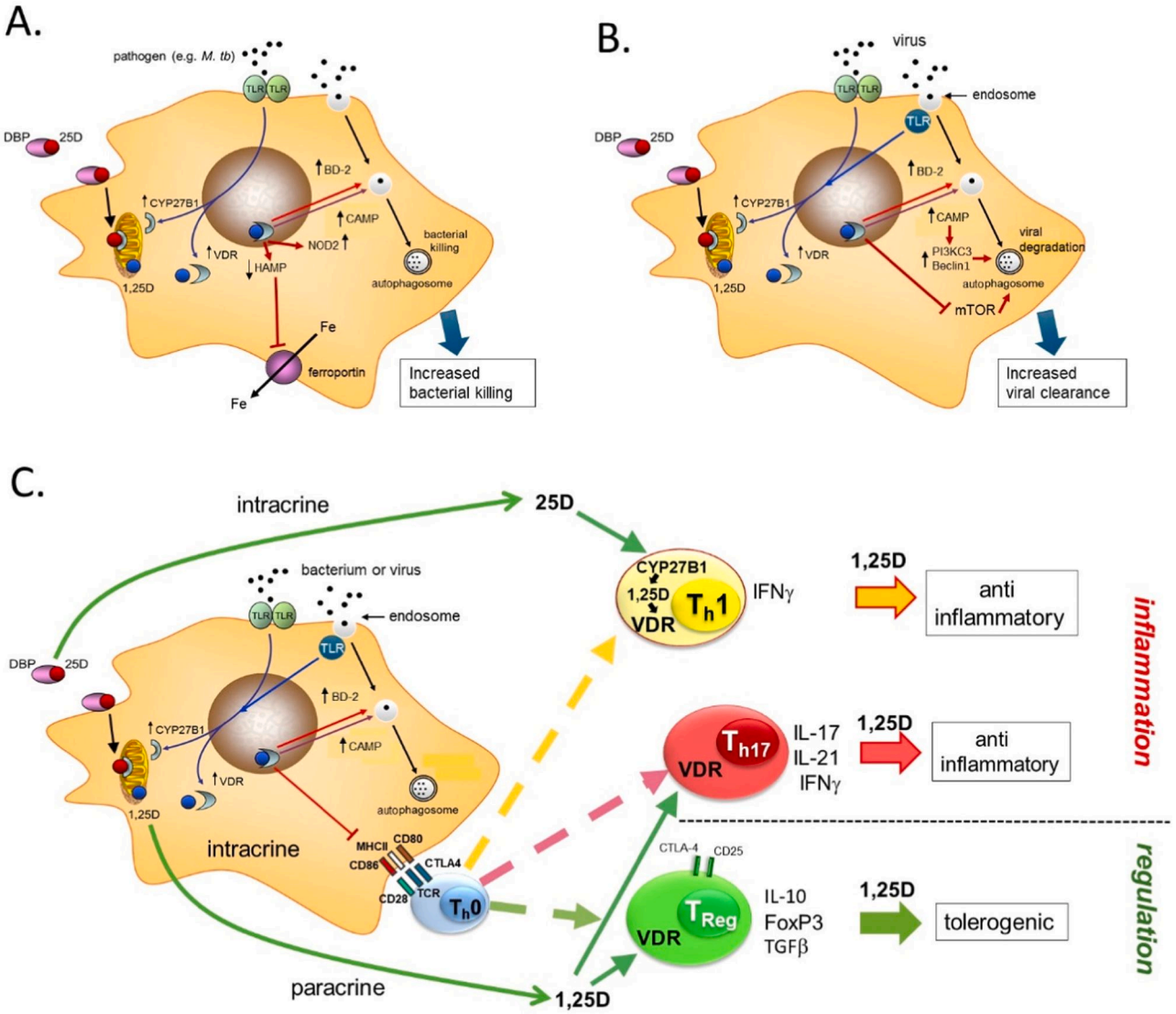


Fig. 1. Vitamin D and innate and adaptive immunity. A. *Vitamin D and monocyte/macrophage responses to a bacterial (e.g. M. tb) challenge*. *M. tb* phagocytosed but also signalling via membrane TLR to stimulate expression of VDR and CYP27B1. The resulting intracellular metabolism of 25(OH)D (transported by the vitamin D binding protein, DBP) to $1,25(OH)_2D$ enables intracrine transcriptional regulation of target genes such as the antibacterial proteins cathelcidin (CAMP) and β-defensin-2 (BD-2), the intracellular pattern recognition receptor NOD2 and the iron regulatory protein hepcidin (HAMP). Intracrine $1,25(OH)_2D$ also stimulates autophagy to promote further processing of the phagocytosed *M. tb*. B. *Vitamin D and responses to viral challenge.* Cellular uptake of virus is associated with both cell membrane and endosomal signalling via TLR to stimulate the same intracrine system observed for responses to bacterial infection. CAMP and BD-2 promote antiviral activity via effects on ACE2 receptor masking and potentiation of TLR signalling (see Fig. 2). Intracrine $1,25(OH)_2D$ also promotes autophagy by stimulating PI3KC3 and beclin and by inhibition of mTOR. C. *Vitamin D and adaptive immune responses to infection*. In addition to its innate immune activity, intracrine $1,25(OH)_2D$ inhibits expression of major histocompatibility complex II (MHCII) and T cell co-stimulators (CD80/CD86) to modulate antigen presentation to Th0 T cells expressing T Cell receptor (TCR), CTLA4 and CD28. Different types of T cells are induced following antigen presentation (dashed coloured arrows) – e.g. inflammatory Th1 and Th17 T cells and tolerogenic regulatory T cells (Treg), which each T cell type characterised by specific cytokines (to right of cell) but also induction of VDR expression. $1,25(OH)_2D$ synthesized by antigen-presenting cells (APC) can act in a paracrine fashion on T cells expressing VDR: inhibition of Th1 (suppressed IFNγ) and Th17 (suppressed IL-17, IL-21 and IFNγ) to promotes anti-inflammatory activity and enhanced Treg development (enhanced IL-10, FoxP3 and TGFβ) to promote tolerogenesis. In the setting of SARS-CoV-2 infection and T cell activation by complement, Th1 cells are also characterised by intracrine synthesis of $1,25(OH)_2D$ (in combination with VDR expression) as part of anti-inflammatory responses.

expression [40]. As well as suggesting that enhanced vitamin D metabolism is a key feature of immune responses to infection, this study also underlined the importance of $1,25(OH)_2D$ in driving antibacterial innate immunity. In the setting of *M. tb*, the ability of $1,25(OH)_2D$ to combat infection appears to be primarily due to transcriptional induction of the *CAMP* gene, with antisense knockdown of *CAMP* in monocytes compromising inhibition of the intracellular pathogen [41]. Nevertheless, $1,25(OH)_2D$ has also been shown to stimulate expression other antibacterial proteins such as β-defensin 2 (BD-2), and $1,25(OH)_2D$ induction of antibacterial proteins has also been described for pathogens other than *M. tb*, and in cells other than monocytes/macrophages [42].

Vitamin D can act to promote expression of antibacterial proteins and enhance bacterial killing but these responses may also be applicable in the setting of viral infection (Fig. 1B). This is due, in part, to direct antiviral activities of cathelicidins such as CAMP on a wide range of virus types to disrupt viral membranes, assembly of viral particles, viral load, and the replication and release of viruses [43]. The majority of these studies have been carried out on enveloped viruses such as respiratory syncytial virus, influenza A virus, herpes simplex virus, human acquired immunodeficiency virus (HIV), vaccina virus and dengue virus

[43], but there have also been reports of antiviral CAMP activity on the non-enveloped adenovirus [44]. Whilst none of these studies has specifically assessed direct effects of vitamin D, the ability of $1,25(OH)_2D$ to enhance CAMP and BD-2 expression suggests a possible role for vitamin D in facilitating this particular facet of cathelicidin/defensin antiviral activity.

In addition to effects on virus assembly, replication and release, CAMP and BD-2 can also influence the process of viral infection and action. This varies significantly for different types of viruses (the retrovirus HIV is quite distinct from SARS-CoV-2 coronavirus or the influenza A virus) so that a generalised mode of action for vitamin D can only be seen as hypothetical. Fig. 2 shows a schematic representation of viral infection using the SARS-CoV-2 virus (COVID-19) as an example (see blue arrows) of viral infection and associated immune responses. SARS-CoV-2 uses its obligate receptor, the angiotensin converting enzyme 2 (ACE2) to enter target host cells such as bronchial epithelia [45]. Following entry into ACE2-expressing tissue, replication of the SARS-CoV-2 virus is followed by release of infective virus, which can then trigger host innate immune responses [46]. This involves some of the cellular innate immune responses described earlier in this review but also includes the activation of complement [47].

Another facet of viral function that may be influenced by antibacterial proteins concerns the ability of these proteins to influence virus-receptor interactions. Using *in silico* analyses, the active peptide component of CAMP (generated by proteinase cleavage of CAMP and referred to as LL-37) and BD-2 were predicted to bind to the receptor binding domain (RBD) within the spike protein (S1) region of the SARS-CoV-2 [48,49] and thus have the potential to compromise binding to ACE2. Subsequent studies *in vivo* using mouse models showed that intranasal administration of LL-37 diminished lung epithelial cell uptake of a SARS-CoV-2 pseudoviron [50]. This was due, in part, to LL-37 binding to the SARS-CoV-2 RBD to prevent S1-ACE2 interaction, as predicted by the *in silico* studies. However, LL-37 was also shown to be able to bind directly to ACE2 and thereby 'cloak' the receptor against SARS-CoV-2 binding [50]. The inference from these studies is that proteins such as CAMP/LL-37 that have antibacterial properties can also act to supress infection by viruses. As such, the ability to upregulate peptides such as CAMP – with vitamin D being a prime candidate – may have significant benefits for diseases such as COVID-19 [51]. The red arrows shown in Fig. 2 highlight the putative mechanisms by which vitamin D could potentially interact with viral infection by SARS-CoV-2.

Specific data demonstrating CAMP and/or BD-2-modulated virus-receptor interaction in response to $1,25(OH)_2D$ have yet to be reported. However, somewhat paradoxically, $1,25(OH)_2D$ has been shown to enhance ACE2 expression. Whilst this would appear to support increased opportunity for SARS-CoV-2 infection, it should also be recognized that ACE2 plays a pivotal role in the renin-angiotensin-aldosterone system (RAAS) that controls blood pressure and fluid/electrolyte homeostasis. Within the RAAS ACE acts to generate angiotensin II, whilst ACE2 functions to attenuate levels of angiotensin II, with a balance maintained between these two key components of RAAS at both a systemic and tissue-specific level [52]. In particular, ACE2 protects against the vasoconstrictive, pro-inflammatory and pro-fibrotic effects of angiotensin II, and thus plays a key role in directing RAAS cardiovascular homeostasis. This is a pivotal feature of the development of COVID-19 disease, where impaired lung function leading to acute respiratory distress syndrome (ARDS) is a key contributor to COVID-19 mortality. It has been proposed that COVID-19 is a disease associated with ACE2 deficiency, with the resulting imbalance of angiotensin II contributing to the dysregulation of lung function that is central to the development of

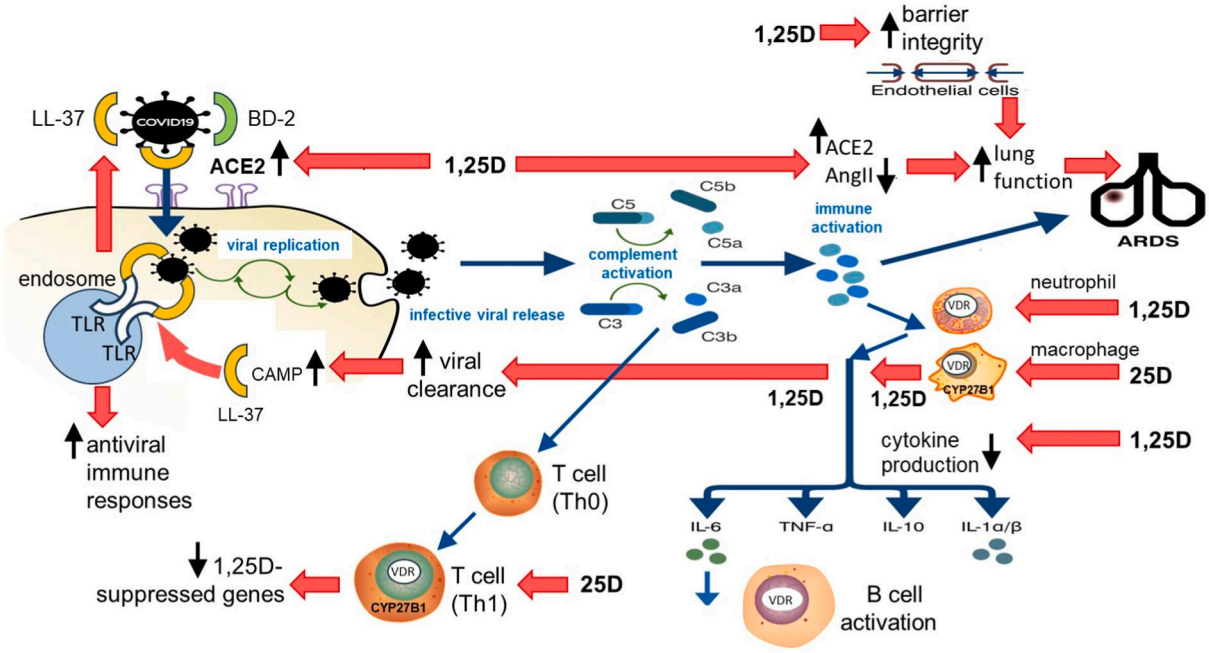


Fig. 2. Vitamin D and immune responses to SARS-CoV-2. Uptake of the SARS-CoV-2 by targets such as lung epithelial cells is facilitated by the receptor angiotensin converting enzyme 2 (ACE2). Following viral replication the release of infective virus promotes complement activation, leading to activation of innate and adaptive immune responses, including T and B cell responses (see blue arrows). Effects of vitamin D are shown as red arrows. The active peptide of cathelicidin (CAMP) referred to as LL-37 and β-defensin2 (BD-2) generated by these cells in response to intracrine synthesis of $1,25(OH)_2D$ (1,25D) from 25(OH)D (25D) by antigen presenting cells such as macrophages can i) bind to the SARS-CoV-2 spike protein or ii) bind and cloak ACE2, thus inhibiting cellular viral uptake. 1,25D also stimulates expression of ACE2 which suppresses angiotensin II (AngII). AngII is associated with impaired lung function and acute respiratory distress syndrome (ARDS). Thus, 1,25D can act to ameliorate this. 1,25D can also improve lung function through enhanced barrier through increased expression of junction proteins and other epithelial defence mechanisms. Endosomal internalization of virus incorporates toll-like receptor (TLR)-induced innate immune responses. These THR responses are enhanced by the action of LL-37. Other innate immune responses to viral infection include complement activation, leading to further innate and adaptive immune responses as shown in Fig. 1. This includes complement activation of Th1 cells to induce intracrine, 25D-dependent gene responses, including a specific group of 1,25D suppressed genes that are dysregulated in patients with COVID-19.

ARDS [53]. Thus, by stimulating ACE2 and generally promoting a shift away from angiotensin II, vitamin D may provide a more balanced RAAS to support normal lung function and prevent possible ARDS in diseases such as COVID-19 [54]. Previous studies using a lipopolysaccharide-induced sepsis model of acute lung injury (ALI) in mice showed that ALI was exacerbated in mice lacking the VDR, and this was due, in part, to the loss of $1,25(OH)_2D$-mediated down-regulation of angiotensin II in these mice [55]. The lung vascular protective effect of vitamin D is further endorsed by the established actions of $1,25(OH)_2D$ in maintaining tissue barrier integrity. This effect of vitamin D has been well documented in the gastrointestinal tract, where vitamin D has been shown to be a potent regulator of the gap junction proteins that are central to the maintenance of gut mucosal barrier integrity [56]. Similar effects of vitamin D have now been reported for lung epithelial mucosa [57], indicating that this is another mechanism by which vitamin D can influence lung function and ARDS.

The ability of the LL-37 peptide of CAMP to bind to viruses has the potential to promote immune responses beyond the inhibition of cellular uptake of virus described above for SARS-CoV-2 and its cognate ACE2 receptor. However, CAMP may also modulate the interaction between SARS-CoV-2 and pattern recognition receptors. Innate immune cells such as monocytes, macrophages and dendritic cells (DC) express several types of toll-like receptor (TLR) that recognise and respond to viruses. With respect to viral infections various TLR are expressed within locations such as endolysosomes that facilitate intracellular responses to nucleic acids such as the single- and double-stranded RNA and DNA that are characteristic of viruses [58]. Studies have shown that the LL-37 peptide of CAMP can bind to double-stranded RNA (dsRNA) to promote dsRNA signalling via TLR3 [59–61]. Conversely, LL-37 binding to lipopolysaccharide appears to inhibit signalling via TLR4 [60]. These observations indicate that LL-37 has the potential to augment virus signalling via intracellular (endosomal) pattern recognition receptors such as TLR (see Fig. 2**)**. In this way, antibacterial peptides such as CAMP may act to promote more effective innate and adaptive immune responses to viral infection [62].

2.2. Regulation of hepcidin and iron homeostasis

The ability of vitamin D to promote innate immune responses to infection is not restricted to induction of antibacterial proteins. As shown in Fig. 1A, $1,25(OH)_2D$ also stimulates expression of nucleotide-binding oligomerization domain 2 (NOD2), an intracellular pattern recognition receptor for the bacterial cell wall component muramyl dipeptide [63]. In this instance the $1,25(OH)_2D$-mediated bacterial surveillance by NOD2 was associated with enhanced antibacterial activity by β-defensin 2 [63]. Paradoxically, the antimicrobial protein hepcidin (HAMP) is supressed by $1,25(OH)_2D$ [64]. Expression of HAMP is induced as part of innate immune responses to bacterial infection but HAMP also functions as a key regulator of intracellular iron homeostasis [65]. Specifically, HAMP binds the iron-exporter protein ferroportin to promote its endocytosis and degradation [66]. Consequently, inflammatory immune responses following infection are associated with elevated hepcidin expression and increased intracellular accumulation and decreased export of iron, contributing to the so-called anaemia of inflammation or anaemia of chronic disease [67]. The resulting accumulation of intracellular iron in cells such as macrophages has also been linked to exacerbation of infection by pathogens such as *M. tb* [68]. In human monocytes and hepatocytes treatment with $1,25(OH)_2D$ results in transcriptional suppression of hepcidin [69]. This, in turn, is associated with enhanced ferroportin protein expression and decreased expression of ferritin, a marker of intracellular iron [69]. Suppression of serum hepcidin levels has also been reported for healthy subjects supplemented with vitamin D where concentrations of serum 25(OH)D, but not $1,25(OH)_2D$, were elevated [69]. In a similar fashion vitamin D supplementation decreased hepcidin expression in macrophages from the dialysis fluid of patients undergoing peritoneal dialysis following supplementation [70]. Thus, studies *ex vivo* and *in vivo* suggest that suppression of hepcidin by vitamin D supports cellular iron homeostasis. By promoting cellular iron export in this way, vitamin D may help to minimise intracellular bacterial survival through depletion of iron availability, whilst also helping to prevent the systemic anaemia commonly associated with infections [71].

The innate immune actions of vitamin D in maintaining intracellular iron export and restricting pathogen access to iron do not immediately appear to be transferable to viral immunity. Intracellular iron overload predisposes to viral infections and some viruses are able to hijack components of iron homeostasis to promote their replication [72]. Notably in a small number of case studies, infection with COVID-19 has been reported to be associated with increased expression of hepcidin and iron overload (particularly in elderly and obese subjects). Conversely ectopic expression of the iron exporter protein ferroportin in monocytes has been shown to promote intracellular iron export and reduce replication of another type of virus, human immunodeficiency virus-type 1 (HIV) [73]. As a naturally occurring regulator of intracellular iron, vitamin D may play a pivotal role in the link between iron homeostasis and viral infection and this connection has been expanded recently to include possible effects on ferroptosis [74]. Ferroptosis is a form of oxidative cell death that is associated with iron accumulation, lipid peroxidation and damage to cell membranes [75]. The broad applicability of oxidative damage to human life means that a wide range of human pathologies have been linked to ferroptosis, notably tumor suppression and tissue degenerative disorders [75]. Ferroptosis is also closely linked to immune surveillance, with ferroptotic cell death described for cells from both the innate and adaptive immune systems [74]. In common with many other cell processes, viruses are known to hijack facets of the ferroptotic process to support survival and promote replication [76]. In various model systems, notably in the setting of inflammation [77] and cognitive impairment [78] vitamin D has been shown to inhibit ferroptosis. It is therefore possible to predict that, in addition to established effects on intracellular iron levels, the broader actions of vitamin D on ferroptosis may act as an additional component of its antiviral immunoregulatory properties.

2.3. Regulation of autophagy and cell metabolism

In cells such as macrophages and neutrophils, the ability of proteins such as CAMP and DEFB4 to combat pathogens such as *M. tb* is dependent on internalization of the mycobacterium by phagocytosis and incorporation of the antibacterial agent into a fused phagosome-lysosome that provides an enhanced intracellular environment for bacterial killing. This process can be further embellished by the integration of the phagolysosomal machinery with elements of autophagy. Canonical autophagy facilitates the degradation of cellular components such a organelles and intracellular proteins, but it can also contribute to pathogen phagocytosis by incorporating the autophagy marker protein LC-3, into the phagosomal membrane and then fusing with a lysosome to form a phagolysosome, which is then able to utilize antibacterial proteins and lysosomal enzymes to degrade the internalized pathogen [79]. In human monocytes $1,25(OH)_2D$ was reported to promote autophagy indirectly via induction of CAMP [80], and subsequent studies showed that this response could be driven by intracrine metabolism of 25(OH)D following mycobacterial lipoprotein TLR activation [81].

As outlined in Fig. 2, many of the recent advances in our understanding of vitamin D and immunity arising from studies of viruses, and SARS-CoV-2 in particular, have stemmed from analysis of cell types outside the traditional immune system – notably lung epithelial cells [57]. Nevertheless, it should also be recognised that other facets of monocyte/macrophage/DC function previously described for antibacterial innate immunity, are also applicable for antiviral immunity. The potent induction of autophagy in monocytic cells by intracrine-generated $1,25(OH)_2D$ can also impact viruses. In previous studies of HIV, $1,25(OH)_2D$ inhibited growth and replication of HIV in a

similar fashion to *M. tb*, and this effect was shown to be dependent on beclin- and autophagy-related 5 homologue (ATG5)-mediated autophagy [82,83]. Autophagy is also strongly influenced by activity of mammalian target of rapamycin (mTOR), which acts to inhibit the initiation of autophagy [84]. As such, modulation of mTOR is a key pharmacological target in a variety of disease settings and it is interesting to $1,25(OH)_2D$ can act as an intracrine suppressor of mTOR by directly inducing the mTOR inhibitor DDIT4 [85]. Collectively these observations underline the importance of autophagy as a pivotal component of innate immune responses to vitamin D, comparable to the more well-characterised induction of antibacterial peptides by $1,25(OH)_2D$. Further studies are required to demonstrate the wider applicability of autophagy for antiviral responses to vitamin D beyond the existing studies of HIV [82,83]. Autophagy can be antiviral, with virophagy (also referred to as xenophagy) targeting the virus for degradation and subsequent antigen presentation, but viruses can also suppress or modify autophagy to promote viral survival [86,87]. SARS-CoV-2 is known to stimulate autophagy but is also able to hijack autophagy machinery to enhance replication of the virus [88]. In future studies it will be interesting to determine how the pro-autophagic effects of vitamin D specially influence innate immune responses to different types of viruses.

As well as regulating autophagy and ferroptosis, recent studies have demonstrated broader effects of vitamin D on cell metabolism in cells from the immune system. Monocytes/macrophages and DC treated with $1,25(OH)_2D$ show strong induction of genes associated with glycolysis, oxidative phosphorylation and the TCA cycle, indicating that changes in cell metabolism are crucial for innate immune responses to vitamin D [89,90]. In DC this was due in part to increased fatty acid synthesis [91]. The specific relevance of this to vitamin D-mediated regulation of DC function has yet to be determined and may simply reflect cell membrane morphological changes due to changes in DC maturation required for antigen presentation. However, changes in fatty acid metabolism may also contribute to ferroptosis as described in Section 2.2. In future studies it will be important to determine the relative impact of vitamin D on fatty acid synthesis, lipid peroxidation and iron accumulation in cells such as macrophages and DC, and the impact that has on viral and bacterial infection. Interestingly, in T cells, treatment with $1,25(OH)_2D$ has the opposite metabolic impact to that observed in DC. Activated CD4 + helper T cells (Th) showed decreased aerobic glycolysis when treated with $1,25(OH)_2D$ and this effect was strongly linked to the classical action of $1,25(OH)_2D$ in supressing IFNγ expression in these cells [92]. This effect is consistent with the role of glycolysis in inflammatory/memory T cell function and promotion of an immunosuppressive and regulatory T cell phenotype with decreased glycolysis [93].

3. Intracrine synthesis of $1,25(OH)_2D$ by innate and adaptive immune cells

3.1. *CYP27B1 expression by antigen presenting cells*

Tissue-specific localized synthesis of $1,25(OH)_2D$ is a pivotal feature of the interaction between vitamin D and the immune system. The most well-recognised example of this, in the setting of normal physiology, is the TLR-induced expression of CYP27B1 that is associated with monocyte/macrophage generation of $1,25(OH)_2D$ to stimulate expression of antibacterial proteins [40]. However, this was not the first example of an intracrine role for CYP27B1 in innate immunity. Earlier studies using monocyte-derived DC described increased expression of CYP27B1 and the capacity to generate $1,25(OH)_2D$ as DC differentiated towards a mature, antigen-presenting cell (APC) phenotype [94]. Interestingly, elevated levels of CYP27B1 were associated with a concomitant decrease in VDR expression, suggesting that mature DC actively metabolising 25(OH)D may not necessarily be the same cells that respond to the $1,25(OH)_2D$ product. An alternative scenario is that the $1,25(OH)_2D$ produced by mature DC, acts primarily on VDR-rich less mature DC [33]. This makes immunological sense in that mature DC would be able to fulfil their APC commitments to present antigen to T cells from the adaptive immune system, whilst rheostatically regulating APC activity through paracrine delivery of $1,25(OH)_2D$ to DC that are still in the process of differentiating [33]. Whatever the case, it is clear that within the immune system the capacity of moncoytes/macrophages and DC to synthesize $1,25(OH)_2D$ is a pivotal feature of not only the intracrine antibacterial/antiviral responses described in the previous section, but also the expansion of immune response into the adaptive immune system (Fig. 1**C**).

3.2. *CYP27B1 expression by lymphocytes*

Innate immunity provides a rapid but relatively non-specific response to bacterial or viral infection. A more sustained and targeted immune responses is provided by the adaptive immune system through lymphocytes such as T cells and B cells. As outlined earlier in this review, one of the initial observations linking vitamin D and adaptive immunity was the detection of VDR by activated (proliferating) lymphocytes [15,95]. Thus, following activation, both T and B cells are putative targets for $1,25(OH)_2D$, and studies *in vitro* and *ex vivo* have shown that T and B cells can respond directly to $1,25(OH)_2D$ [21,96,97]. The source of this $1,25(OH)_2D$ could either be endocrine (due to renal production of $1,25(OH)_2D$) or paracrine (due to synthesis of $1,25(OH)_2D$ by macrophages or DC within the local immune microenvironment). In addition, other studies *ex vivo* have shown that the availability of 25(OH)D and subsequent synthesis of $1,25(OH)_2D$ by DC defines the T cell phenotype during antigen presentation [98]. This supports an indirect model for regulation of T cell function by vitamin D via intracrine synthesis of $1,25(OH)_2D$ by APC. However, it does not negate the fact that activated T cells express VDR and can directly respond to $1,25(OH)_2D$ in an endocrine or paracrine fashion [96,97], and so it is possible that T cell responses to $1,25(OH)_2D$ involve both intracrine effects on APC and antigen presentation and paracrine effects on the resulting activated T cells. What is less clear is whether lymphocytes themselves express CYP27B1 and synthesise $1,25(OH)_2D$ to promote direct regulation of these adaptive immune cells in an intracrine fashion.

Previous studies have reported expression of CYP27B1 in B lymphocytes [99], and studies *in vivo* have described enhanced immunoglobulin responses in mice with T cell-specific knockout of *Cyp27b1* [100], suggesting a role for lymphocyte synthesis of $1,25(OH)_2D$ in the regulation of T and B cell function. Nevertheless, the functional importance of lymphocyte $1,25(OH)_2D$ production has only recently achieved prominence as a result of studies to specifically characterise T cell phenotypes associated with COVID-19. Single cell RNA seq analysis of lung bronchoalveolar lavage fluid cells showed that COVID-19 infection is characterised by an inflammatory Th1 T cell phenotype, with no significant difference for other inflammatory T cell markers such as IL-17 (Th17) [101]. As well as being enriched for Th1 genes, cells from COVID patients were also characterised by expression of genes associated with the complement immune pathway [101]. As shown in Fig. 2, activation of the complement system plays a key role in innate immune responses to bacterial or viral infection by tagging pathogens for subsequent phagocytosis and further immune responses [102]. Studies to mimic complement activation of T cells (CD3 and CD46 activation to generate intracellular complement C3b) showed enhanced expression of VDR and CYP27B1 consistent with an intracrine vitamin D metabolism system in Th1 cells [101]. Whilst the authors did not specifically analyse 25(OH)D to $1,25(OH)_2D$ conversion in complement-activated Th1 cells, the functionality of Th1 cell CYP27B1 and VDR expression was endorsed by data showing 25(OH)D-mediated regulation of Th1 cytokines, together with other novel T cell targets identified as part of the broader COVID-19 study (see next section) [101]. As shown in Fig. 1**C**, these observations support a role for intracrine 25(OH)D metabolism to $1,25(OH)_2D$ in driving specific T cell

responses.

As yet it is unclear whether the T cell-specific CYP27B1 expression in COVID-19-infected subjects is due exclusively to complement activation. Likewise, whilst this mechanism is sensitive to differences in 25(OH)D availability, it has yet to be determined how Th1 cell capacity for intracrine synthesis of $1,25(OH)_2D$ is integrated with the synthesis of $1,25(OH)_2D$ by APC, which is known to impact other T cells, including inflammatory Th17 cells and regulatory T cells (Treg) [33]. Nevertheless, it now appears that the capacity to synthesize $1,25(OH)_2D$ is common to a broader range of immune cells than previously thought, with CYP27B1 activated by a wider array of pathogenic stimuli.

4. Redefining adaptive immune responses to $1,25(OH)_2D$

As outlined in Section 3, the expression of VDR by cells from the adaptive immune system – T and B cells was one of the original observations linking vitamin D with the immune system. Initial functional responses were focused on the suppression of proliferation and production of cytokines such as interleukin-2 (IL-2) by $1,25(OH)_2D$ in activated but not resting T cells [103], and the inhibition of immunoglobulin production by B cells [17]. Since these early observations the functional impact of $1,25(OH)_2D$ on T and B cells has been greatly expanded, notably in the setting of inflammatory diseases such as rheumatoid arthritis [104]. As shown in Figs. 1**C**, $1,25(OH)_2D$ can act on Th1 and Th17 cells to inhibit inflammation through suppression of IFNγ, IL-17 and IL-21[2]. In the case of Treg, $1,25(OH)_2D$ acts to stimulate expression of IL-10, FoxP3, TGFβ, and the immune checkpoint protein CTLA-4[2]. In this setting, the over-arching action of $1,25(OH)_2D$ on adaptive immune function is to suppress inflammatory Th1 and Th17 function whilst promoting tolerogenic Treg [104]. Although most of the studies defining these responses have been carried out *in vitro* or *ex vivo* using normal peripheral blood-derived T cells, similar responses have been demonstrated using T cells from disease affected human tissues (e.g. synovial fluid), albeit with decreased sensitivity to $1,25(OH)_2D$ that was attributed, in part, to increased memory T cells in these tissues [105].

Vitamin D can also affect T cell function in the setting of infectious disease. Here APC present antigen to Th cells to promote an inflammatory response that includes activation of CD4 + Th cells but also CD8 + cytotoxic T cells (CTL) to lyse infected target cells and their intracellular pathogen [106]. As a counterpoint to this pathogen-driven inflammatory response, Treg can act to suppress both Th and CTL activity [106]. However, whilst there have been extensive studies of the effects of vitamin D on Th cells and associated Treg in relation to inflammation and autoimmune disease [104], much less is known about vitamin D and CTL function during infection despite this being an important facet of the immune response to both bacterial and viral infection [107]. Studies using *Vdr* knockout mice infected with a natural mouse viral pathogen have shown that lack of $1,25(OH)_2D$ signalling is associated with aberrant CTL differentiation, memory function and lymph localisation [108]. These observations support a possible role for vitamin D in regulating CTL function *in vivo*, although it is still unclear at what level this occurs – via intracrine regulation of DCs and antigen presentation, or through paracrine actions on CTL themselves. It seems likely that vitamin D-mediated regulation of adaptive immunity will involve effects on both CD4 + and CD8 + T cells. Recent studies in a mouse tumour model showed that enhanced bioavailability of 25(OH)D, as a consequence of knockout of the vitamin D binding protein gene, resulted in increased tumour accumulation of CD4 + and CD8 + T cells that was associated with diminished tumour size [109]. This effect was abrogated using an anti-CD8 antibody, underlining the importance of CD8 + CTL in driving antitumour effects of 25(OH)D [109]. Here the authors did not specifically measure $1,25(OH)_2D$ but the inference was that higher levels of 25(OH)D were associated with increased CYP27B1 activity within the tumour microenvironment. This study also showed that the ability of vitamin D to promote tumour immune surveillance is mediated via regulation of gut microbiome composition, specifically increased levels of the bacterium *Bacteroides fragilis* [109]. The link between vitamin D and microbiota has gained prominence in recent years, primarily in relation to established immune disorders associated with vitamin such as autoimmunity [110]. It is to be hoped that future studies will build on the new data linking vitamin D, microbiota and tumour surveillance to provide an entirely new perspective of vitamin D and adaptive immunity in the setting of cancer.

Another study that has provided a new perspective on vitamin D and adaptive immunity is the report by Chauss et al. detailing complement-induced expression of CYP27B1 by Th1 cells from patients with COVID-19 [101]. As outlined earlier in this review, this work described a new facet of immune intracrine vitamin D metabolism – the extension of CYP27B1 expression from the innate to the adaptive immune system. However, the report also revealed new facets of $1,25(OH)_2D$ adaptive immune cell function, particularly in relation to SARS-CoV-2 infection, T cell inflammation and COVID-19 disease severity. By carrying out extensive genomic and epigenetic analyses, Chauss et al. showed that some key effects of $1,25(OH)_2D$ on T cells, such as induction of IL-10, were dependent on paradoxical induction of another inflammatory cytokine, IL-6, and also the epigenetic induction of three key transcription factors – BACH2, c-JUN and STAT3- with BACH2 being particularly important for Th1 and Th17 responses to $1,25(OH)_2D$ [101]. Specific analysis of gene expression in lung Th1 cells from COVID-19 patients versus healthy controls showed that expression of a group of genes conventionally suppressed by $1,25(OH)_2D$ in Th1 cells from healthy controls was higher in Th1 cells from COVID-19 patients. By contrast, there was no significant difference in $1,25(OH)_2D$-induced genes between healthy controls and COVID-19 patients [101]. The set of $1,25(OH)_2D$-repressed genes identified in this study performed almost as well as signature Th1 genes in distinguishing Th cells from patients with COVID-19 from healthy controls [101]. Furthermore, modelling studies were carried out to predict the best therapeutic options for counteracting aberrant gene expression in Th1 cells from COVID-19 patients. Out of 461 possible drugs the top ten predicted to most effective included the vitamin D analog (Alfacalcidol) [101].

The studies outlined above provide a mechanistic rationale for the link between vitamin D and COVID-19 prognosis, with intracrine Th1 gene suppression by vitamin D being linked to attenuation of the inflammatory responses associated with disease progression after COVID-19 infection. Whilst much of our current understanding of vitamin D and the immune system stems from seminal studies of the intracrinology of vitamin D in antibacterial innate immunity [40] or antigen presentation [94], it is clear that anti-inflammatory adaptive immunity is also a crucial target for vitamin D. To date much of our knowledge of T cell responses to vitamin have arisen through analysis of dysregulated T cell function in autoimmune diseases, and the possible preventative or therapeutic application of vitamin D supplementation in resolving T cell dysfunction in these diseases [111]. However, the advent of SARS-CoV-2 infection has provided an entirely new perspective on vitamin D immunomodulation that is more focused on inflammation in the setting of acute viral infection. In this regard, vitamin D can be viewed in a similar fashion to the high profile analysis of glucocorticoids as therapy for patients hospitalized with COVID-19 [112]. Notably, one of the most successful applications of vitamin D in the setting of COVID-19 was the use of 25(OH)D (Calcifediol) as an anti-inflammatory agent in people hospitalized with COVID-19 [113]. In future studies it will be interesting to determine if Calcifediol is a more effective agent for correcting the aberrant Th1 cell gene sets that were so characteristic of COVD-19 patients. Furthermore, the growing recognition of Calcifediol as a more rapid and effective strategy for 'fuelling' intracrine and paracrine 25(OH)D metabolism within the immune system highlights an entirely new facet of vitamin D immunology, namely the potential impact of vitamin D in the setting of acute medicine. In this regard, vitamin D (including Calcifediol) has distinct advantages over more established anti-inflammatory therapeutics such as glucocorticoids. Both vitamin D

and glucocorticoids have potent anti-inflammatory actions, and glucocorticoids such as dexamethasone were shown to decrease mortality in hospitalized COVID-19 patients [112]. However, despite these positive effects, the use of glucocorticoids to treat viral infections such as COVID-19 is complicated by data showing that glucocorticoid therapy during early infection is actually associated with impaired viral clearance [114], possibly as a consequence of inhibition of type 1 interferon antiviral immunity [115]. Thus, it has been proposed that the use of glucocorticoids for viral infections is restricted to later stages of disease to prevent pathological damage in tissues such as the lungs [114]. Similar to glucocorticoids, vitamin D is a potent anti-inflammatory steroid hormone but it also promotes antiviral innate immunity. Thus, therapeutic use of vitamin D for infectious diseases is less constrained by the time-frame of disease than for glucocorticoids such as Dexamethasone.

5. Future studies

In the last five years our understanding of the interaction between vitamin D and the immune system has undergone a dramatic transformation. The COVID-19 pandemic brought many aspects of vitamin D and immunity into sharper focus but also expanded the potential impact of vitamin D. We now have a much better understanding of the way in which the vitamin D system interfaces with viral infection and the possible benefits of enhanced vitamin D status for both innate and adaptive immune responses. There is still considerable debate about the epidemiology of vitamin D in relation to patient outcomes for acute respiratory diseases such as COVID-19 [116]. The ongoing discussion as to whether vitamin D supplementation has benefits in either the prevention or treatment of immune disease has still to be resolved. However, the mechanistic observations presented in this review – particularly the powerful data on dysregulation of vitamin D-repressed T cell gene sets in COVID-19 patients [101] – support a role for vitamin D as a regulator of inflammation and the tissue consequences of inflammation in respiratory disease. This, coupled with promising data for the use of Calcifediol as an anti-inflammatory for hospitalized critical care patients [113], suggests that we are now seeing a shift in vitamin D immunity away from the well-characterized effects on innate antibacterial immunity to new facets of adaptive immunity. This includes key areas that are currently poorly understood such as the role of immune responses to vitamin D and vaccination. Previous studies have assessed the effects of vitamin D on immunization against different strains of influenza [117], and there have been some studies reporting effects of vitamin D with respect to COVID-19 vaccination programmes [116]. However, this is a facet of vitamin D immunology that is still poorly understood at a cellular/molecular level and therefore this should be a key focus for future studies. Finally, one of the key mechanistic observations underpinning the link between vitamin D and the immune system has been the extra-renal synthesis of $1,25(OH)_2D$ by APC such as macrophages and DC. The broad assumption has been that capacity for extra-renal $1,25(OH)_2D$ production changes with vitamin D (serum 25 (OH)D) status, but to date this has only been demonstrated *ex vivo* or *in vitro*. However, the advent of new mass spectrometry imaging technologies has enabled tissue mapping of vitamin D metabolites that reflect synthesis in vivo [34,35]. Although initial studies have focused on imaging of 25(OH)D and $1,25(OH)_2D$ in mouse tissues, it is to be hoped that in future similar studies will be carried out for available human tissue, particularly in the setting of variable vitamin D status (serum 25 (OH)D levels) and human disease.

CRediT authorship contribution statement

Martin Hewison: Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of Competing Interest

I have no conflicts of interest to declare.

Acknowledgements

This manuscript was not supported by any specific funding.

Data Availability

No data was used for the research described in the article.

References

[1] R. Bouillon, C. Marcocci, G. Carmeliet, et al., Skeletal and extraskeletal actions of vitamin D: current evidence and outstanding questions, Endocr. Rev. 40 (4) (2019) 1109–1151.
[2] E. Bishop, A. Ismailova, S.K. Dimeloe, M. Hewison, J.H. White, Vitamin D and immune regulation: antibacterial, antiviral, anti-inflammatory, JBMR (2020).
[3] M. Green, Cod liver oil and tuberculosis, Bmj 343 (2011) d7505.
[4] K.I. Moller, B. Kongshoj, P.A. Philipsen, V.O. Thomsen, H.C. Wulf, How Finsen's light cured lupus vulgaris, Photo Photo Photo 21 (3) (2005) 118–124.
[5] I. Greenhalgh, A.R. Butler, Sanatoria revisited: sunlight and health, J. R. Coll. Physicians Edinb. 47 (3) (2017) 276–280.
[6] H.J. Wallace, Tuberculous glands and sinuses treated with calciferol, Lancet 2 (6412) (1946) 88.
[7] G. Herrera, Vitamin D in massive doses as an adjuvant to the sulfones in the treatment of tuberculoid leprosy, Int J. Lepr. 17 (1-2) (1949) 35–42.
[8] N.H. Bell, P.H. Stern, E. Pantzer, T.K. Sinha, H.F. DeLuca, Evidence that increased circulating 1 alpha, 25-dihydroxyvitamin D is the probable cause for abnormal calcium metabolism in sarcoidosis, J. Clin. Investig. 64 (1) (1979) 218–225.
[9] S.E. Papapoulos, T.L. Clemens, L.J. Fraher, I.G. Lewin, L.M. Sandler, J. L. O'Riordan, 1, 25-dihydroxycholecalciferol in the pathogenesis of the hypercalcaemia of sarcoidosis, Lancet 1 (8117) (1979) 627–630.
[10] J.S. Adams, O.P. Sharma, M.A. Gacad, F.R. Singer, Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis, J. Clin. Investig. 72 (5) (1983) 1856–1860.
[11] J.S. Adams, F.R. Singer, M.A. Gacad, et al., Isolation and structural identification of 1,25-dihydroxyvitamin D3 produced by cultured alveolar macrophages in sarcoidosis, J. Clin. Endocrinol. Metab. 60 (5) (1985) 960–966.
[12] A.R. Baker, D.P. McDonnell, M. Hughes, et al., Cloning and expression of full-length cDNA encoding human vitamin D receptor, Proc. Natl. Acad. Sci. USA 85 (10) (1988) 3294–3298.
[13] D.J. Mangelsdorf, H.P. Koeffler, C.A. Donaldson, J.W. Pike, M.R. Haussler, 1,25-Dihydroxyvitamin D3-induced differentiation in a human promyelocytic leukemia cell line (HL-60): receptor-mediated maturation to macrophage-like cells, J. Cell Biol. 98 (2) (1984) 391–398.
[14] H. Tanaka, E. Abe, C. Miyaura, Y. Shiina, T. Suda, 1 alpha,25-dihydroxyvitamin D3 induces differentiation of human promyelocytic leukemia cells (HL-60) into monocyte-macrophages, but not into granulocytes, Biochem. Biophys. Res. Commun. 117 (1) (1983) 86–92.
[15] A.K. Bhalla, E.P. Amento, T.L. Clemens, M.F. Holick, S.M. Krane, Specific high-affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation, J. Clin. Endocrinol. Metab. 57 (6) (1983) 1308–1310.
[16] S.C. Manolagas, D.A. Werntz, C.D. Tsoukas, D.M. Provvedini, J.H. Vaughan, 1,25-Dihydroxyvitamin D3 receptors in lymphocytes from patients with rheumatoid arthritis, J. Lab. Clin. Med. 108 (6) (1986) 596–600.
[17] D.M. Provvedini, C.D. Tsoukas, L.J. Deftos, S.C. Manolagas, 1 alpha,25-Dihydroxyvitamin D3-binding macromolecules in human B lymphocytes: effects on immunoglobulin production, J. Immunol. 136 (8) (1986) 2734–2740.
[18] M. Hewison, Vitamin D and the immune system, J. Endocrinol. 132 (2) (1992) 173–175.
[19] E.P. Amento, A.K. Bhalla, J.T. Kurnick, et al., 1 alpha,25-dihydroxyvitamin D3 induces maturation of the human monocyte cell line U937, and, in association with a factor from human T lymphocytes, augments production of the monokine, mononuclear cell factor, J. Clin. Investig. 73 (3) (1984) 731–739.
[20] Z. Bar-Shavit, D. Noff, S. Edelstein, M. Meyer, S. Shibolet, R. Goldman, 1,25-dihydroxyvitamin D3 and the regulation of macrophage function, Calcif. Tissue Int. 33 (6) (1981) 673–676.
[21] J.M. Lemire, J.S. Adams, R. Sakai, S.C. Jordan, 1 alpha,25-dihydroxyvitamin D3 suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells, J. Clin. Investig. 74 (2) (1984) 657–661.
[22] W.F. Rigby, R.J. Noelle, K. Krause, M.W. Fanger, The effects of 1,25-dihydroxyvitamin D3 on human T lymphocyte activation and proliferation: a cell cycle analysis, J. Immunol. 135 (4) (1985) 2279–2286.
[23] J.D. Nunn, D.R. Katz, S. Barker, et al., Regulation of human tonsillar T-cell proliferation by the active metabolite of vitamin D3, Immunology 59 (4) (1986) 479–484.
[24] L. Binderup, Immunological properties of vitamin D analogues and metabolites, Biochem Pharm. 43 (9) (1992) 1885–1892.

[25] E. Van Etten, B. Decallonne, L. Verlinden, A. Verstuyf, R. Bouillon, C. Mathieu, Analogs of 1alpha,25-dihydroxyvitamin D3 as pluripotent immunomodulators, J. Cell. Biochem. 88 (2) (2003) 223–226.
[26] R. Bouillon, K. Allewaert, J.P. van Leeuwen, et al., Structure function analysis of vitamin D analogs with C-ring modifications, J. Biol. Chem. 267 (5) (1992) 3044–3051.
[27] Q.T. Luong, H.P. Koeffler, Vitamin D compounds in leukemia, J. Steroid Biochem. Mol. Biol. 97 (1-2) (2005) 195–202.
[28] O.P. Sharma, Hypercalcemia in granulomatous disorders: a clinical review, Curr. Opin. Pulm. Med 6 (5) (2000) 442–447.
[29] A.J. Brown, Therapeutic uses of vitamin D analogues, Am. J. Kidney Dis. 38 (5 5) (2001) S3–S19.
[30] M. Hewison, Vitamin D and the immune system: new perspectives on an old theme, Endocrinol. Metab. Clin. North Am. 39 (2) (2010) 365–379.
[31] M. Hewison, Vitamin D and innate immunity, Curr. Opin. Invest. Drugs 9 (5) (2008) 485–490.
[32] M. Hewison, Vitamin D and the intracrinology of innate immunity, Mol. Cell. Endocrinol. 321 (2) (2010) 103–111.
[33] M. Hewison, Vitamin D and immune function: autocrine, paracrine or endocrine? Scand. J. Clin. Lab Invest Suppl. 243 (2012) 92–102.
[34] K.W. Smith, B. Flinders, P.D. Thompson, et al., Spatial localization of vitamin D metabolites in mouse kidney by mass spectrometry imaging, ACS Omega 5 (22) (2020) 13430–13437.
[35] M.B. Meyer, S.M. Lee, S.R. Cichanski, D.F. Cobice, J.W. Pike, Spatial detection and consequences of nonrenal calcitriol production as assessed by targeted mass spectrometry imaging, JCI Insight 9 (15) (2024).
[36] A. Jamilian, F. Ghalichi, F. Hamedi Kalajahi, et al., The role of vitamin D in outcomes of critical care in COVID-19 patients: evidence from an umbrella meta-analysis of interventional and observational studies, Public Health Nutr. 27 (1) (2024) e127.
[37] X. Zhang, J. Wu, H. Dong, et al., The impact of supplementing vitamin D through different methods on the prognosis of COVID-19 patients: a systematic review and meta-analysis, Front Nutr. 11 (2024) 1441847.
[38] T.T. Wang, F.P. Nestel, V. Bourdeau, et al., Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression, J. Immunol. 173 (5) (2004) 2909–2912.
[39] A.F. Gombart, N. Borregaard, H.P. Koeffler, Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3, FASEB J.: Off. Publ. Fed. Am. Soc. Exp. Biol. 19 (9) (2005) 1067–1077.
[40] P.T. Liu, S. Stenger, H. Li, et al., Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response, Science 311 (5768) (2006) 1770–1773.
[41] P.T. Liu, S. Stenger, D.H. Tang, R.L. Modlin, Cutting edge: vitamin D-mediated human antimicrobial activity against mycobacterium tuberculosis is dependent on the induction of cathelicidin, J. Immunol. 179 (4) (2007) 2060–2063.
[42] D.A. Youssef, C.W. Miller, A.M. El-Abbassi, et al., Antimicrobial implications of vitamin D, Dermatoendocrinol 3 (4) (2011) 220–229.
[43] P.G. Barlow, E.G. Findlay, S.M. Currie, D.J. Davidson, Antiviral potential of cathelicidins, Future Microbiol 9 (1) (2014) 55–73.
[44] Y.J. Gordon, L.C. Huang, E.G. Romanowski, K.A. Yates, R.J. Proske, A. M. McDermott, Human cathelicidin (LL-37), a multifunctional peptide, is expressed by ocular surface epithelia and has potent antibacterial and antiviral activity, Curr. Eye Res. 30 (5) (2005) 385–394.
[45] C.B. Jackson, M. Farzan, B. Chen, H. Choe, Mechanisms of SARS-CoV-2 entry into cells, Nat. Rev. Mol. Cell Biol. 23 (1) (2022) 3–20.
[46] K. Lundstrom, A. Hromic-Jahjefendic, E. Bilajac, et al., COVID-19 signalome: pathways for SARS-CoV-2 infection and impact on COVID-19 associated comorbidity, Cell Signal 101 (2023) 110495.
[47] E.H.T. Lim, R.B.E. van Amstel, V.V. de Boer, et al., Complement activation in COVID-19 and targeted therapeutic options: a scoping review, Blood Rev. 57 (2023) 100995.
[48] K.B. Lokhande, T. Banerjee, K.V. Swamy, P. Ghosh, M. Deshpande, An in silico scientific basis for LL-37 as a therapeutic for Covid-19, Proteins 90 (5) (2022) 1029–1043.
[49] L. Zhang, S.K. Ghosh, S.C. Basavarajappa, et al., HBD-2 binds SARS-CoV-2 RBD and blocks viral entry: strategy to combat COVID-19, iScience 25 (3) (2022) 103856.
[50] C. Wang, S. Wang, D. Li, et al., Human Cathelicidin Inhibits SARS-CoV-2 Infection: Killing Two Birds with One Stone, ACS Infect. Dis. 7 (6) (2021) 1545–1554.
[51] K.M. Aloul, J.E. Nielsen, E.B. Defensor, et al., Upregulating human cathelicidin antimicrobial peptide LL-37 expression may prevent severe COVID-19 inflammatory responses and reduce microthrombosis, Front. Immunol. 13 (2022) 880961.
[52] M. Paul, A. Poyan Mehr, R. Kreutz, Physiology of local renin-angiotensin systems, Physiol. Rev. 86 (3) (2006) 747–803.
[53] J.R. Cook, J. Ausiello, Functional ACE2 deficiency leading to angiotensin imbalance in the pathophysiology of COVID-19, Rev. Endocr. Metab. Disord. 23 (2) (2022) 151–170.
[54] B. Getachew, Y. Tizabi, Vitamin D and COVID-19: role of ACE2, age, gender, and ethnicity, J. Med. Virol. 93 (9) (2021) 5285–5294.
[55] J. Kong, X. Zhu, Y. Shi, et al., VDR attenuates acute lung injury by blocking Ang-2-Tie-2 pathway and renin-angiotensin system, Mol. Endocrinol. 27 (12) (2013) 2116–2125.
[56] Y.-g Zhang, S. Wu, R. Lu, et al., Tight junction CLDN2 gene is a direct target of the vitamin D receptor, Sci. Rep. 5 (1) (2015) 10642.
[57] J.A. Schrumpf, A.M. van der Does, P.S. Hiemstra, Impact of the local inflammatory environment on mucosal vitamin D metabolism and signaling in chronic inflammatory lung diseases, Front. Immunol. 11 (2020) 1433.
[58] A.L. Blasius, B. Beutler, Intracellular toll-like receptors, Immunity 32 (3) (2010) 305–315.
[59] Y. Lai, S. Adhikarakunnathu, K. Bhardwaj, et al., LL37 and cationic peptides enhance TLR3 signaling by viral double-stranded RNAs, PloS One 6 (10) (2011) e26632.
[60] D. Singh, R. Vaughan, C.C. Kao, LL-37 peptide enhancement of signal transduction by Toll-like receptor 3 is regulated by pH: identification of a peptide antagonist of LL-37, J. Biol. Chem. 289 (40) (2014) 27614–27624.
[61] D. Singh, R. Qi, J.L. Jordan, L. San Mateo, C.C. Kao, The human antimicrobial peptide LL-37, but not the mouse ortholog, mCRAMP, can stimulate signaling by poly(I:C) through a FPRL1-dependent pathway, J. Biol. Chem. 288 (12) (2013) 8258–8268.
[62] T. Takiguchi, S. Morizane, T. Yamamoto, A. Kajita, K. Ikeda, K. Iwatsuki, Cathelicidin antimicrobial peptide LL-37 augments interferon-beta expression and antiviral activity induced by double-stranded RNA in keratinocytes, Br. J. Dermatol. 171 (3) (2014) 492–498.
[63] Wang T.T., Dabbas B., Laperriere D., et al. Direct and indirect induction by 1,25-dihydroxyvitamin D3 of the NOD2/CARD15-defensin beta2 innate immune pathway defective in Crohn disease. The Journal of biological chemistry; **285**(4): 2227-2231.
[64] J. Bacchetta, J.J. Zaritsky, J.L. Sea, et al., Suppression of iron-regulatory hepcidin by vitamin D, J. Am. Soc. Nephrol. 25 (3) (2014) 564–572.
[65] J.C. Barton, R.T. Acton, Hepcidin, iron, and bacterial infection, Vitam. Horm. 110 (2019) 223–242.
[66] T. Ganz, Hepcidin and iron regulation, 10 years later, Blood 117 (17) (2011) 4425–4433.
[67] C.C. Sun, V. Vaja, J.L. Babitt, H.Y. Lin, Targeting the hepcidin-ferroportin axis to develop new treatment strategies for anemia of chronic disease and anemia of inflammation, Am. J. Hematol. 87 (4) (2012) 392–400.
[68] G. Weiss, U.E. Schaible, Macrophage defense mechanisms against intracellular bacteria, Immunol. Rev. 264 (1) (2015) 182–203.
[69] J. Bacchetta, J.J. Zaritsky, J.L. Sea, et al., Suppression of iron-regulatory hepcidin by vitamin D, J. Am. Soc. Nephrol. 25 (3) (2014) 564–572.
[70] J. Bacchetta, R.F. Chun, B. Gales, et al., Antibacterial responses by peritoneal macrophages are enhanced following vitamin D supplementation, PloS One 9 (12) (2014) e116530.
[71] E.M. Smith, V. Tangpricha, Vitamin D and anemia: insights into an emerging association, Curr. Opin. Endocrinol. Diabetes Obes. 22 (6) (2015) 432–438.
[72] R. Mancinelli, L. Rosa, A. Cutone, et al., Viral Hepatitis and iron dysregulation: molecular pathways and the role of lactoferrin, Molecules 25 (8) (2020).
[73] N. Kumari, T. Ammosova, S. Diaz, et al., Increased iron export by ferroportin induces restriction of HIV-1 infection in sickle cell disease, Blood Adv. 1 (3) (2016) 170–183.
[74] X. Chen, R. Kang, G. Kroemer, D. Tang, Ferroptosis in infection, inflammation, and immunity, J. Exp. Med. 218 (6) (2021).
[75] X. Jiang, B.R. Stockwell, M. Conrad, Ferroptosis: mechanisms, biology and role in disease, Nat. Rev. Mol. Cell Biol. 22 (4) (2021) 266–282.
[76] J. Wang, J. Zhu, S. Ren, et al., The role of ferroptosis in virus infections, Front Microbiol 14 (2023) 1279655.
[77] S. Gao, C. Sun, J. Kong, Vitamin D Attenuates Ulcerative Colitis by Inhibiting ACSL4-Mediated Ferroptosis, Nutrients 15 (22) (2023).
[78] J. Li, Y. Cao, J. Xu, et al., Vitamin D improves cognitive impairment and alleviates ferroptosis via the nrf2 signaling pathway in aging mice, Int J. Mol. Sci. 24 (20) (2023).
[79] J. Yuan, Q. Zhang, S. Chen, M. Yan, L. Yue, LC3-associated phagocytosis in bacterial infection, Pathogens 11 (8) (2022).
[80] J.M. Yuk, D.M. Shin, H.M. Lee, et al., Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin, Cell Host Microbe 6 (3) (2009) 231–243.
[81] D.M. Shin, J.M. Yuk, H.M. Lee, et al., Mycobacterial lipoprotein activates autophagy via TLR2/1/CD14 and a functional vitamin D receptor signalling, Cell Microbiol 12 (11) (2010) 1648–1665.
[82] G.R. Campbell, S.A. Spector, Vitamin D inhibits human immunodeficiency virus type 1 and Mycobacterium tuberculosis infection in macrophages through the induction of autophagy, PLoS Pathog. 8 (5) (2012) e1002689.
[83] G.R. Campbell, S.A. Spector, Autophagy induction by vitamin D inhibits both Mycobacterium tuberculosis and human immunodeficiency virus type 1, Autophagy 8 (10) (2012) 1523–1525.
[84] Y.C. Kim, K.L. Guan, mTOR: a pharmacologic target for autophagy regulation, J. Clin. Investig. 125 (1) (2015) 25–32.
[85] T.S. Lisse, M. Hewison, Vitamin D: a new player in the world of mTOR signaling, Cell Cycle 10 (12) (2011) 1888–1889.
[86] T. Chen, S. Tu, L. Ding, M. Jin, H. Chen, H. Zhou, The role of autophagy in viral infections, J. Biomed. Sci. 30 (1) (2023) 5.
[87] Y. Choi, J.W. Bowman, J.U. Jung, Autophagy during viral infection - a double-edged sword, Nat. Rev. Microbiol 16 (6) (2018) 341–354.
[88] T. Ivanova, Y. Mariienko, N. Mehterov, et al., Autophagy and SARS-CoV-2-Old Players in New Games, Int J. Mol. Sci. 24 (9) (2023).
[89] G.B. Ferreira, A.S. Vanherwegen, G. Eelen, et al., Vitamin D3 induces tolerance in human dendritic cells by activation of intracellular metabolic pathways, Cell Rep. (2015).

[90] A. Munoz Garcia, M. Kutmon, L. Eijssen, M. Hewison, C.T. Evelo, S.L. Coort, Pathway analysis of transcriptomic data shows immunometabolic effects of vitamin D, J. Mol. Endocrinol. 60 (2) (2018) 95–108.
[91] A.M. Garcia, E.L. Bishop, D. Li, et al., Tolerogenic effects of 1,25-dihydroxyvitamin D on dendritic cells involve induction of fatty acid synthesis, J. Steroid Biochem. Mol. Biol. 211 (2021) 105891.
[92] E.L. Bishop, N.H. Gudgeon, G.M. Mackie, et al., 1,25-Dihydroxyvitamin D3 suppresses CD4(+) T-cell effector functionality by inhibition of glycolysis, Immunology 166 (3) (2022) 299–309.
[93] S. Liu, S. Liao, L. Liang, J. Deng, Y. Zhou, The relationship between CD4(+) T cell glycolysis and their functions, Trends Endocrinol. Metab. 34 (6) (2023) 345–360.
[94] M. Hewison, L. Freeman, S.V. Hughes, et al., Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells, J. Immunol. 170 (11) (2003) 5382–5390.
[95] D.M. Provvedini, C.D. Tsoukas, L.J. Deftos, S.C. Manolagas, 1,25-dihydroxyvitamin D3 receptors in human leukocytes, Science 221 (4616) (1983) 1181–1183.
[96] L.E. Jeffery, F. Burke, M. Mura, et al., 1,25-Dihydroxyvitamin D(3) and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3, J. Immunol. 183 (9) (2009) 5458–5467.
[97] D.V. Lopez, F.A.H. Al-Jaberi, A. Woetmann, et al., Macrophages control the bioavailability of vitamin D and vitamin D-regulated T cell responses, Front. Immunol. 12 (2021) 722806.
[98] L.E. Jeffery, A.M. Wood, O.S. Qureshi, et al., Availability of 25-Hydroxyvitamin D3 to APCs controls the balance between regulatory and inflammatory T cell responses, J. Immunol. 189 (11) (2012) 5155–5164.
[99] S. Chen, G.P. Sims, X.X. Chen, Y.Y. Gu, S. Chen, P.E. Lipsky, Modulatory effects of 1,25-dihydroxyvitamin d3 on human B cell differentiation, J. Immunol. 179 (3) (2007) 1634–1647.
[100] J. Lindner, S. Rausch, S. Treptow, et al., Endogenous calcitriol synthesis controls the humoral IgE response in mice, J. Immunol. 199 (12) (2017) 3952–3958.
[101] D. Chauss, T. Freiwald, R. McGregor, et al., Autocrine vitamin D signaling switches off pro-inflammatory programs of TH1 cells, Nat. Immunol. 23 (1) (2022) 62–74.
[102] A. Jayaraman, S. Walachowski, M. Bosmann, The complement system: A key player in the host response to infections, Eur. J. Immunol. 54 (11) (2024) e2350814.
[103] A.K. Bhalla, E.P. Amento, B. Serog, L.H. Glimcher, 1,25-Dihydroxyvitamin D3 inhibits antigen-induced T cell activation, J. Immunol. 133 (4) (1984) 1748–1754.
[104] L.E. Jeffery, K. Raza, M. Hewison, Vitamin D in rheumatoid arthritis-towards clinical application, Nat. Rev. Rheuma 12 (4) (2016) 201–210.
[105] L.E. Jeffery, P. Henley, N. Marium, et al., Decreased sensitivity to 1,25-dihydroxyvitamin D3 in T cells from the rheumatoid joint, J. Autoimmun. 88 (2018) 50–60.
[106] S.A. Joosten, T.H. Ottenhoff, Human CD4 and CD8 regulatory T cells in infectious diseases and vaccination, Hum. Immunol. 69 (11) (2008) 760–770.
[107] S. Sarkar, M. Hewison, G.P. Studzinski, Y.C. Li, V. Kalia, Role of vitamin D in cytotoxic T lymphocyte immunity to pathogens and cancer, Crit. Rev. Clin. Lab Sci. 53 (2) (2016) 132–145.
[108] Y. Yuzefpolskiy, F.M. Baumann, L.A. Penny, G.P. Studzinski, V. Kalia, S. Sarkar, Vitamin D receptor signals regulate effector and memory CD8 T cell responses to infections in mice, J. Nutr. 144 (12) (2014) 2073–2082.
[109] E. Giampazolias, M. Pereira da Costa, K.C. Lam, et al., Vitamin D regulates microbiome-dependent cancer immunity, Science 384 (6694) (2024) 428–437.
[110] E.A. Yamamoto, T.N. Jorgensen, Relationships between vitamin D, gut microbiome, and systemic autoimmunity, Front. Immunol. 10 (2019) 3141.
[111] S.R. Harrison, D. Li, L.E. Jeffery, K. Raza, M. Hewison, Vitamin D, autoimmune disease and rheumatoid arthritis, Calcif. Tissue Int. 106 (1) (2020) 58–75.
[112] R.C. Group, P. Horby, W.S. Lim, et al., Dexamethasone in hospitalized patients with Covid-19, N. Engl. J. Med 384 (8) (2021) 693–704.
[113] X. Nogues, D. Ovejero, M. Pineda-Moncusi, et al., Calcifediol treatment and COVID-19-related outcomes, J. Clin. Endocrinol. Metab. 106 (10) (2021) e4017–e4027.
[114] N. Lee, K.C. Allen Chan, D.S. Hui, et al., Effects of early corticosteroid treatment on plasma SARS-associated Coronavirus RNA concentrations in adult patients, J. Clin. Virol. 31 (4) (2004) 304–309.
[115] A. Marcellini, D. Swieboda, A. Guedan, et al., Glucocorticoids impair type I IFN signalling and enhance rhinovirus replication, Eur. J. Pharm. 893 (2021) 173839.
[116] A.R. Martineau, Vitamin D in the prevention or treatment of COVID-19, Proc. Nutr. Soc. 82 (2) (2023) 200–207.
[117] M.D. Lee, C.H. Lin, W.T. Lei, et al., Does vitamin D deficiency affect the immunogenic responses to influenza vaccination? A systematic review and meta-analysis, Nutrients 10 (4) (2018).