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REVIEW

Role of vitamin D in regulating COVID-19 severity—An immunological perspective

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Abstract

Vitamin D, a key nutrient/prohormone classically associated with skeletal health, is also an important immunomodulator, with pleotropic effects on innate and adaptive immune cells. Outcomes of several chronic, autoimmune, and infectious diseases are linked to vitamin D. Emergent correlations of vitamin D insufficiency with coronavirus-induced disease 2019 (COVID-19) severity, alongside empirical and clinical evidence of immunoregulation by vitamin D in other pulmonary diseases, have prompted proposals of vitamin D supplementation to curb the COVID-19 public health toll. In this review paper, we engage an immunological lens to discuss potential mechanisms by which vitamin D signals might regulate respiratory disease severity in severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infections, *vis a vis* other pulmonary infections. It is proposed that vitamin D signals temper lung inflammatory cascades during SARS-CoV2 infection, and insufficiency of vitamin D causes increased inflammatory cytokine storm, thus leading to exacerbated respiratory disease. Additionally, analogous to studies of reduced cancer incidence, the dosage of vitamin D compounds administered to patients near the upper limit of safety may serve to maximize immune health benefits and mitigate inflammation and disease severity in SARS-CoV2 infections. We further deliberate on the importance of statistically powered clinical correlative and interventional studies, and the need for in-depth basic research into vitamin D-dependent host determinants of respiratory disease severity.

KEYWORDS
immunoregulation, vitamin D

1 | INTRODUCTION

Respiratory viruses such as coronavirus and influenza pose a major public health threat due to their fast-spreading nature, epidemic/pandemic potential, and high morbidity/mortality through rapid induction of respiratory disease. In this regard, the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), has proven particularly challenging, with relatively short latency and prolonged infectious periods,[1] case fatality rates ranging from 2 to 10% in the most affected countries (see mortality analysis at https://coronavirus.jhu.edu/data/mortality), and confoundingly disproportionate effects on certain vulnerable populations such as the elderly, the overweight, African Americans, and patients with preexisting chronic disease conditions.[2–4] Given the scale of the SARS-CoV2-induced coronavirus disease 2019 (COVID-19) pandemic—with over 59,100,000 confirmed cases and over 1,400,000 deaths in 216 countries worldwide (as of November 24, 2020; World Health Organization)—there is an urgent need to define the host determinants of disease susceptibility. In this review paper, we delve into the clinical and empirical evidence supporting vitamin D regulation of host immunity in the context of COVID-19 and other diseases, and speculate on possible immunologic mechanisms by which vitamin D might dictate COVID-19 patient outcomes. It is proposed that vitamin D signals temper lung inflammatory cascades during SARS-CoV2 infection, and insufficiency of vitamin D causes increased inflammatory cytokine storm, thus leading to exacerbated respiratory disease.

Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxy vitamin D; 25OHD, 25-hydroxy vitamin D; ARDS, acute respiratory distress syndrome; CD, cluster of differentiation; COVID-19, coronavirus-induced disease 2019; CYP, cytochrome P450 mixed-function oxidase; DC, dendritic cells; LCMV, lymphocytic choriomeningitis virus; RSV, respiratory syncytial virus ; IBD, inflammatory bowel disease ; MS, multiple sclerosis; SARS-CoV2, severe acute respiratory syndrome coronavirus 2; TB, tuberculosis; Treg, regulatory T cells; VDR, vitamin D receptor.

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2 | VITAMIN D METABOLISM IN MAMMALS

Vitamin D_3 (cholecalciferol) is produced in the skin from 7-dehydrocholesterol through a 2-step process by UVB radiation from the sun, to first form pre-D_3, which then isomerizes to vitamin D_3 in a nonenzymatic process. The rate of vitamin D_3 production in the skin is regulated by multiple factors, such as melanin pigmentation level and UVB intensity (as determined by seasonal angle of the sun, latitude, clothing, and sunscreen usage). Vitamin D may also be procured from dietary sources in the form of vitamin D_3 or cholecalciferol (from animal sources such as fatty fish), and in the form of vitamin D_2 or ergocalciferol (from plant sources such as mushrooms). The cytochrome P450 mixed-function oxidase (CYP) family of enzymes in the endoplasmic reticulum and mitochondria, next catalyze the conversion of vitamin D_3 into 25-hydroxy vitamin D (25OHD, also known as calcidiol), largely in the liver. The CYP27B1 hydroxylase next converts 25OHD into the bioactive form 1,25-dihydroxy vitamin D [1,25$(OH)_2$D, also known as calcitriol], mainly in the kidneys. 1,25$(OH)_2$D is implicated in a variety of physiologic effects such as bone and calcium homeostasis, as well as transcriptional regulation. Gene regulatory effects of 1,25$(OH)_2$D are largely mediated through the vitamin D receptor (VDR) transcription factor of the steroid hormone nuclear receptor family, by binding to the vitamin D response elements in transcriptional regulatory genomic regions. The genomic effects of VDR are exerted in the context of a heterodimeric partner, RXR, and may vary from one cell-type to another depending on distinct binding partners (see previous review papers on the topic).[5–8] In this review paper, we use vitamin D to broadly include the bioactive 1,25$(OH)_2$D and the 25OHD precursor forms, both of which together typically define the vitamin D status of an individual.

Based on the 2020 Consensus statement from the 2nd International Conference on Controversies in Vitamin D 25(OH)D, the serum concentrations between 50 nmol/L (20 ng/ml) and 125 nmol/L (50 ng/ml) appear to be safe and sufficient in the general population for skeletal health.[9] However, it has been shown in numerous studies that higher 25-hydroxyvitamin D serum concentrations are associated with a reduction of diseases other than rickets and osteomalacia, for instance, a lower cancer incidence and cancer mortality.[10] The dose of vitamin D required to increase serum 25OHD in people to a minimum serum 25OHD of 20 ng/ml (50 nmol/L) is approximately 800 IU daily, whereas increasing to a minimum level of 30 ng/ml (75 nmol/L) requires approximately 4,000 IU daily.[9] Potential risk of vitamin D toxicity is typically defined as a 25(OH)D level > 100 ng/ml (>250 nmol/ml) in adults, thus offering a substantial range of vitamin D dose for supplementation efforts. Of note, in various disease states, Endocrine Society recommended doses of 10,000 IU/day (250 μg/day), and in some studies even higher doses have been used.[11,12] Hence, short-term treatment with high doses of vitamin D can be safely used in emergency situations, such as COVID-19 patients requiring supplemental oxygenation. In the future, to maximize the beneficial outcomes of supplementation in the context of immune health, an in-depth, systematic dose-dependent risk versus benefit analyses of vitamin D supplementation in its relatively newer role of immune regulation and disease mitigation beyond skeletal health are critically needed.

3 | VITAMIN D AND COVID-19

In general, age, certain genetic or ethnic backgrounds, and preexistent chronic disease conditions are linked to disproportionately exacerbated respiratory disease severity in COVID-19.[13–16] Intriguingly, vitamin D insufficiency is also pronounced in the elderly and African Americans owing to inefficiencies in vitamin D uptake, metabolism, and/or signaling.[5,17–20] Likewise, chronic disease conditions such as heart disease and diabetes are also linked to inadequate vitamin D levels, thus supporting a possible indirect link between vitamin D insufficiency and COVID-19 severity.[8] Promisingly, preliminary retrospective analyses of serum vitamin D levels in South Asian and Swiss COVID-19 patients reveal a strong correlation between vitamin D insufficiency and respiratory disease severity.[21,22] A similar indirect correlation between vitamin D and COVID-19 severity is implicated by recent observations of increased mortality in the higher latitude northern US states that receive lower UVB dose compared with the southern states.[15,16] Likewise, cursory cross-sectional global analyses have uncovered higher COVID-19-related mortality in countries with lower average vitamin D status.[14,23]

Linkage between vitamin D insufficiency and COVID-19 severity is consistent with several historic, anecdotal, and clinical studies linking inadequate vitamin D levels to a variety of infectious, chronic, and autoimmune diseases such as respiratory syncytial virus (RSV), tuberculosis (TB), HIV, HBV, HSV, Dengue virus, malaria, leprosy, cancer, multiple sclerosis (MS) and inflammatory bowel disease (IBD).[5,6,24–40] Likewise, strong association between vitamin D status and seasonal influenza infections incidence is well known.[41–44] Hepatitis C virus infection also shows an inverse relationship between vitamin D levels and viral load, liver fibrosis, and treatment outcomes.[45] Direct studies using mouse models of mycobacterial and listeria infections demonstrate that disease progression is severely aggravated when mice are rendered vitamin D deficient.[46–49] Recent data from our studies demonstrate that vitamin D deficiency also underlies increased susceptibility to chronic lymphocytic choriomeningitis virus (LCMV) infection in mice (unpublished observations). Collectively, the above-referenced clinical correlations and meta-level pathogen clearance/disease outcome analyses linking vitamin D and disease severity underscore the importance of investigations into vitamin D-dependent immunological mechanisms of disease control or susceptibility.

4 | IMMUNE REGULATION BY VITAMIN D

Cells of both innate and adaptive immune systems work in concert to clear pathogens. In addition to mediating direct antipathogen functions, innate immune responses also serve as beacons and catalysts for adaptive immune responses to effectively cull the pathogen.

Notably, vitamin D influences cells of the both innate and adaptive immune systems.[17,50,51] Macrophages,[37,46,47] dendritic cells (DCs),[52] neutrophils,[53] NK cells, NK-T cells, effector cluster of differentiation (CD)4 and CD8 T cells,[54] regulatory T cells (Tregs),[55–60] and B cells,[60,61] all express the VDR (a zinc-finger nuclear transcription factor that exerts direct gene regulation and cell signaling effects) or CYP27B1 enzyme (required for formation of bioactive vitamin D for intracrine action), or both. The expression of VDR changes in a cell-type specific manner with their developmental state.[62,63] For example, macrophages and DCs down-regulate VDR following maturation, whereas T cells tend to up-regulate VDR expression following activation. Types of infections also differentially regulate VDR expression through distinct mechanisms. For instance macrophages/monocytes up-regulate the expression of VDR and CYP27B1 during mycobacterial infections through TLR-triggering, thus enabling increased responsiveness to paracrine and intracrine vitamin D signals.[64] Other innate cells such as neutrophils are also functionally activated by vitamin D signals.[65] In fact, immunologic role of vitamin D was first identified in mycobacterial infections, where it promotes host defense by augmenting chemotaxis, phagocytosis, and production of antimicrobial peptides by macrophages and monocytes.[46,66–68]

Mouse and human CD8 and CD4 T cells express VDR and the CYP27B1 enzyme (required for formation of bioactive vitamin D), and up-regulate VDR expression upon polyclonal *in vitro* stimulation.[17,37,54,69,70] We have recently shown that antigen-specific CD8 T cells also up-regulate VDR following *in vivo* activation during viral infection.[71] Intriguingly, contrary to immunostimulatory effects of vitamin D on monocytes/macrophages, vitamin D signals are largely inhibitory in adaptive immune cells such as T cells and B cells.[5,65] Exposure of CD4, CD8 T cells, and B cells to calcitriol *in vitro* suppresses proliferation and effector differentiation.[17,72] Our data show reduced expression of effector molecule, granzyme B, in VDR-deficient LCMV-specific effector CD8 T cells.[71] Likewise, B cells produce reduced IgM and IgG effector antibodies in the absence of vitamin D signals.[72] Differentiation of T_{H1} and T_{H17} effector subsets is also suppressed by vitamin D, while the effects of vitamin D on T_{H2} cells are seemingly stimulatory, albeit this needs to be further clarified due to conflicting reports.[73–75] Development and function of iNKT cells is also regulated by vitamin D.[76–79] In contrast, vitamin D promotes the development and the suppressive function of Tregs—the prototypic suppressive cells of the adaptive immune system, critical for inhibition of innate and effector responses.[80–91] Treg-enhancing effects of vitamin D are mediated either through direct signaling in Treg cells, or indirectly through induction of tolerogenic DCs.[52,92–94]

As a critical link between innate and adaptive immune systems, DCs serve as an important target for vitamin D-mediated immunoregulation.[52,93,94] Vitamin D signals lead to impaired expression of key functional markers in DCs including: (i) MHC-II and costimulatory molecules (such as CD80. CD86, CD40), (ii) inflammatory cytokines and chemokines (such as IL-12 and IL-23, which drive inflammatory T_{H1} and T_{H17} responses), and (iii) also promote the expression of immunosuppressive cytokines and chemokines (such as IL-10 and CCL-22, which support immunosuppressive Treg cell function and migration).[52,94–98] The migratory properties and tissue tropism of T cells are also modulated by DCs in a vitamin D-dependent manner through regulation of chemokine receptor expression. For example, in the *in vitro* studies, vitamin D promotes CCR10 expression and skin homing of T cells in response to CCL27, but suppresses the expression of gut-homing receptors CCR9 and $\alpha 4\beta 7$.[55] Similar to DCs,[99] vitamin D also suppresses the ability of macrophages to activate T cells by reducing expression of MHC-II, costimulatory molecules, and inflammatory cytokines, despite activating their phagocytic and chemotactic activity.[100–104] Vitamin D also suppresses the production of other inflammatory or prodifferentiation cytokines such as IFN-γ, IL-2, IL-17, and IL-21 by CD4 T cells.[87] The antipathogen effects of vitamin D possibly extend to nonimmune cells as well, as indicated by increased intestinal epithelial barrier function in case of autoimmune intestinal disorders.[105–107] Therefore, vitamin D regulates several immune cells including macrophages, DCs, NK cells, B cells, and T cells by largely inhibiting inflammation and proliferation while also enhancing antipathogen innate immunity at the same time.[52,108–110]

5 | DISEASE CONTEXT-DEPENDENT REGULATION OF HOST IMMUNITY BY VITAMIN D

Given the multitude of immune cells regulated by the bioactive form of vitamin D [1,25$(OH)_2$D], it is proposed that protective effects of vitamin D are mediated by unique immunoregulatory mechanisms in a disease-specific manner. Although the precise vitamin D-regulated immune mediators may vary in each disease, in general vitamin D promotes immunoprotection by maintaining a healthy balance of inflammation and effector responses.[111] For instance, in mycobacterial infection, vitamin D inhibits pathologic inflammatory mediators and matrix degrading enzymes, while enhancing the IFN-γ-induced antimicrobial peptides to block mycobacterial replication.[26] In the mouse model of MS, vitamin D signaling in T_{H1} cells regulates their migration in response to inflammatory CXCR3 ligands,[112] and also suppresses pathogenic B cell responses.[60,61,72] In the case of HSV-induced Behcet's inflammatory disease, vitamin D mediates protective, anti-inflammatory effects through down-regulation of TLRs.[113,114] Anti-inflammatory effects of vitamin D are also implicated in successful organ transplantation.[73,115–118] In endotoxin-induced acute respiratory distress syndrome (ARDS), inadequate vitamin D level is associated with increased levels of inflammatory cytokines such as IL-1β and TNF-α.[119,120] In contrast to largely beneficial effects of vitamin D, certain infections (e.g., citrobacter and leishmania) were confusingly enhanced or unaffected by vitamin D. Subsequent categoric immunologic studies revealed inhibitory effects of vitamin D on inflammatory T_{H1} effector subset necessary for pathogen control.[38,121,122] These instances of disease context-dependent immune modulation by vitamin D underscore the importance of studying vitamin D regulation of SARS-CoV2 immune

responses for the design of novel immunotherapies and exploring timely vitamin D supplementation in vulnerable populations.

6 | VITAMIN D AND RESPIRATORY DISEASE SEVERITY

Strong host inflammation is a lead cause of exacerbated lung disease in TB and influenza.[123,124] Although rapid induction of proinflammatory cytokines and chemokines is a key part of early immune anti-viral defense, and is also critical for recruitment of additional innate cells (such as monocytes and NK cells) as well as adaptive immune cells to sites of viral replication in the lung, overexuberant and/or prolonged inflammation in the lung causes significant lung damage, ARDS and mortality.[111] In the highly pathogenic 1918 Influenza A, pulmonary pathology and fatal outcome were associated with accelerated activation of proinflammatory genes that remained unabated until death.[125–128] Hence, therapies aimed at reducing the cytokine storm are proposed to be beneficial for severe cases of influenza.[129]

As in pathogenic influenza, SARS-CoV2 infection in humans is associated with replication of the virus in the lower respiratory tract, which is accompanied by severe inflammation owing to increased localization of inflammatory macrophages to lung airways.[13,123,125,130–135] Profound impact of vitamin D on immunity to a variety of respiratory/mucosal diseases characterized by inflammation (influenza, TB, MS)[13,42,44,125] supports vitamin D-dependent regulation of innate and adaptive inflammatory mediators/regulators of pulmonary immunopathology and ARDS during COVID-19 as well (Fig. 1). Increased inflammation is implicated as the prime cause for morbidity/mortality in vulnerable patients, with more severe COVID-19 cases associated with greater inflammatory cytokine induction and vitamin D insufficiency.[14–16,125,130,134–138] Atypical innate immune responses comprising reduced type-I IFN production and increased inflammatory cytokines such as IL-6 have been recently identified in COVID-19[132,139–145] (Fig. 1). 1,25$(OH)_2$D exerts suppressive effects on the production of IL-6 by innate monocytes,[146] albeit its effect on type-I IFNs in the context of viral infections remains largely unexplored. Additional potential vitamin D-regulated innate immune cell targets include neutrophils, inflammatory M1 macrophages and plasmacytoid DCs. Other immune cells implicated in protecting against severe lung injury and pulmonary fibrosis in respiratory infections such as influenza, SARS, MERS, RSV include innate lymphoid cells 2 ILC2, invariant NKT iNKT cells, M2 macrophages, myeloid-derived suppressor cells MDSC, and $\gamma\delta$T cells.[123] Hence, the role of these cell-types in regulating COVID-19 lung immunopathology in a vitamin D-dependent manner needs to be rigorously evaluated in the future.

The adaptive immune components, CD8 and CD4 T cells might also serve to exacerbate inflammation at lung sites as "latecomer" inflammatory mediators attracted by the initial innate chemoattractants at lung sites (Fig. 1). Indeed, vitamin D-dependent regulation of chemokine receptor expression on effector T cells and Tregs, the anti-proliferative effects of vitamin D on effector T cells, and the anti-inflammatory effects of vitamin D likely modulate the overall expansion, migration, differentiation status, and function of T cells in lung sites of SARS-CoV2 infection. In addition to immune mediators of inflammation, it is important to consider epithelial/endothelial cells as candidate vitamin D-regulated mediators of lung damage and ARDS in COVID-19[110,129] (Fig. 1). Airway epithelial and endothelial cells secrete a variety of immune mediators such as antimicrobial peptides, cytokines, and chemokines, such as IFN-I, IL-6, G-CSF, and GM-CSF. Potential vitamin D-dependent innate and adaptive immune regulators of lung inflammation and COVID-19 severity are summarized in Fig. 1.

7 | VITAMIN D SUPPLEMENTATION IN SARS-COV2 INFECTIONS

According to the National Health and Nutrition Examination Survey (NHANES), while massive public health efforts of milk fortification have effectively controlled severe rachitic deficiency, vitamin D insufficiency is widespread in the US as well as Europe.[147–151] Notable vitamin D insufficiency in the elderly, African Americans, and patients with chronic disease conditions (diabetes and heart disease) and their increased susceptibility to COVID-19 has prompted proposals for vitamin D supplementation.

Potential beneficial effects of vitamin D supplementation are supported by vitamin D restoration of antimycobacterial immune defects in sera from TB-susceptible African Americans with vitamin D insufficiency[46,67,68] (also see selected studies highlighted in Table 1). Furthermore, Dr. Niels Finsen's Nobel Prize winning cure of epidermal TB through sunlight lends historic support.[152] Metadata analysis of studies involving a large cohort of participants (>11,000) also promisingly found a protective effect of higher vitamin D levels in acute respiratory infections[153] (Table 1). Even in bacterial sepsis, AIDS, and parasitic infections, meta data analysis of controlled human trials have shown improved clinical outcomes following supplementation (Table 1).[29,31,154] These findings have reinvigorated vitamin D supplementation trials in influenza vaccines after unclear results in the past due to confounding factors such as lack of pre- and posttreatment vitamin D measurements, high baseline vitamin D, and use of low vitamin D doses.[44,155] Random controlled trials showing effective augmentation of bioavailable vitamin D and modulation of CD4 T cells, inflammation, and Treg cells support the proposal that protective effects of vitamin D might be mediated through immune-regulation.[156,157] Likewise, induction of Treg cells and concomitant suppression of inflammation in organ transplant recipients through vitamin D supplementation further bolsters the inflammation regulatory role of vitamin D.[83] Moreover, recent amelioration of MS, diabetes and cardiac disease (with inflammation as a key underlying factor) through vitamin D supplementation bolsters the proposal for vitamin D supplementation in mitigating respiratory disease in SARS-CoV2 infections as well[28,30,58,112] (Table 1). It is predicted that augmenting the overall vitamin D levels in the population to "sufficient" levels will mitigate disease severity in the general population, and will be especially

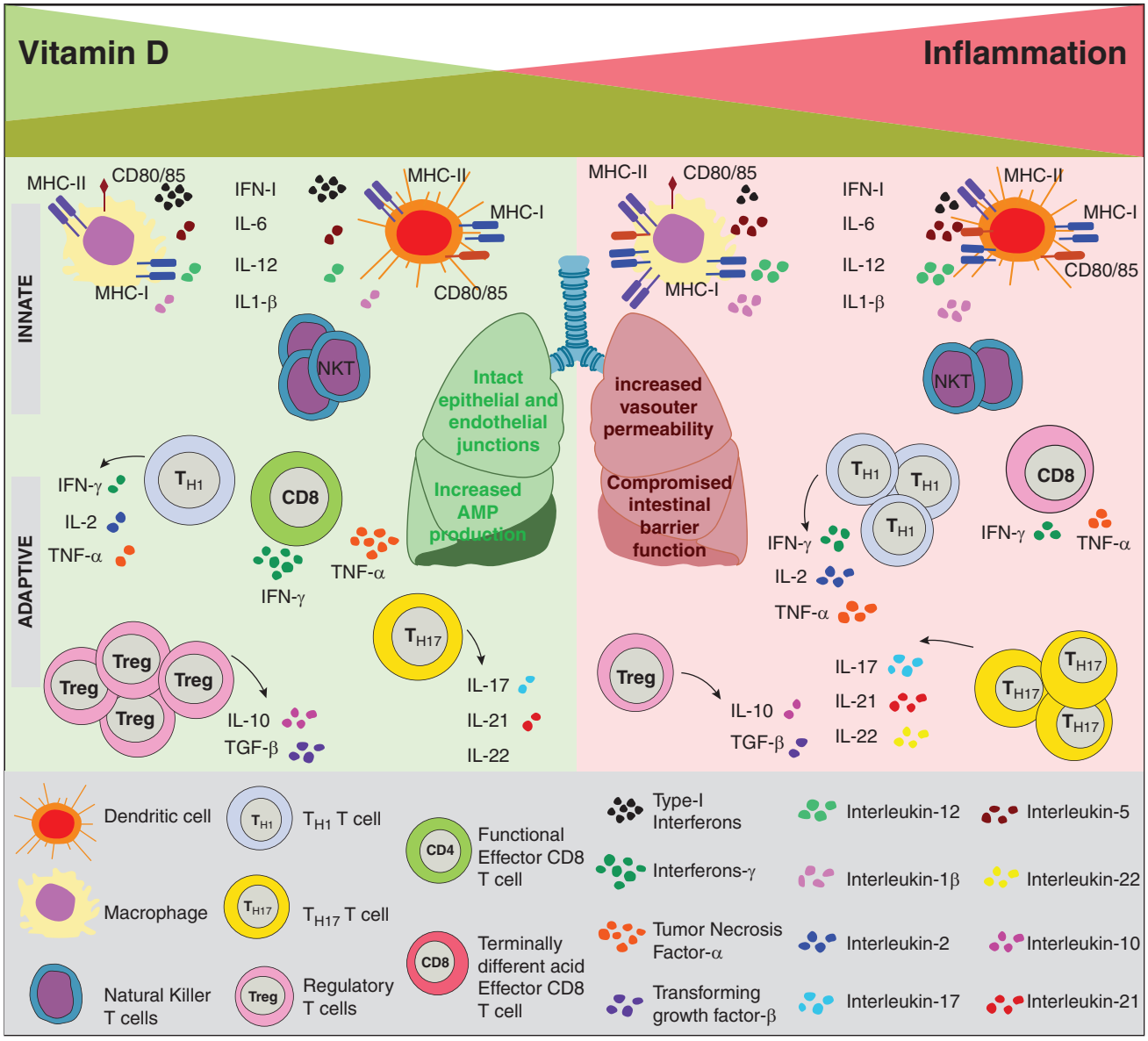

FIGURE 1 **Model of immunomodulation by vitamin D in COVID-19.** The decreasing green shaded triangle indicates decreasing vitamin D status. Increasing intensity of red shade indicates increasing inflammation with decreasing vitamin D levels. Infection in vitamin D sufficient hosts (green half of the figure, corresponding to serum levels of 25(OH)D > 25–30 ng/ml, defined as sufficient) is expected to induce optimal activation of innate immune cells such as macrophages (with robust antimicrobial peptide, AMP, production) and DCs with robust up-regulation of MHC and costimulatory molecules, and regulated production of proinflammatory cytokines. Balanced differentiation of effector CD8 and CD4 T cell subsets under conditions of vitamin D sufficiency is also expected to promote robust antiviral responses, with regulated production of inflammatory cytokines. Vitamin D sufficiency is also predicted to promote the development of immunoprotective NK-T cells, and maintain epithelial junctional integrity and endothelial vascular permeability, thus minimizing pulmonary damage. Contrarily, host vitamin D insufficiency or deficiency (red half of the figure, corresponding to serum levels of 25(OH)D $\leq$ 10 ng/ml (25 nM) defined as deficient, and 10–20 ng/ml defined as insufficient) is expected to lead to aberrant activation of innate inflammatory mediators such as macrophages and DCs, leading to exacerbated inflammation, more pronounced expansion and terminal differentiation of effector CD8 and inflammatory CD4 T cell subsets, and diminished Treg induction and NK-T cell development. Likewise, the junctional integrity of lung epithelial and vascular endothelial cells is also predicted to be impaired, thus leading to pulmonary edema, lung injury and functional impairment and ARDS. Conditions of vitamin D hyper-supplementation are not depicted in this model. A better understanding of disease-context-dependent immunomodulatory effects of vitamin D in SARS-CoV2 infections at pulmonary sites of viral growth and secondary lymphoid sites of immune activation will illuminate potential beneficial effects of normalizing vitamin D levels to sufficient or hyper-supplemented levels

TABLE 1 Clinical studies showing beneficial effects of vitamin D supplementation

Disease	References
Tuberculosis	Martineau et al.[153] Nursyam et al.[175] Morcos et al.[176] Wejse et al.[177]
Influenza	Aloia and Li-Ng,[43]
AIDS	Arpadi et al.[178] Reviewed in Alvarez et al., 2019 and Teixeira et al.[29,31]
Schistosomiasis	Snyman et al.[179]
Sepsis	Amrein et al.[180] Leaf et al.[181] Quraishi et al.[182] Miroliaee et al.[183,184] Ginde et al.[151]
Coronary disease	Sokol et al.[185] Farrokhian et al.[186] Bahrami et al.[187,188] Manson et al.[28]
Diabetes	Pittas et al.[30]
Multiple sclerosis	Kouchaki et al.[34] Berezowska et al.[35]
Dengue	Ahmed et al.[32] Martinez-Moreno et al.[33]

Selected clinical studies of vitamin D supplementation and meta-analysis of multiple disease-relevant clinical trials showing infection or disease protective effects are presented.

beneficial in curbing the disproportionate COVID-19 morbidity and mortality in vulnerable subsets through direct regulation of immune cell activation, proliferation, and inflammatory responses. Several vitamin D-regulated immune target genes (such as MHC-I CCR10, FBP, CAMP, RANKL, IL-6, IL-1β, NFKBIA, CCL2, TNF-α, and cell cycle progression genes ATM p53, CDK6, CDKN2A) have been identified in primary white blood cells as well as in the monocytic cell line THP-1.[158–164] Previous studies supporting beneficial effects of vitamin D supplementation in a variety of infectious diseases and other chronic diseases with underlying inflammatory issues are summarized in Table 1. Rigorous correlations of host vitamin D status with molecular and cellular mediators of inflammation and respiratory disease severity in COVID-19 in large-sized diverse patient cohorts, as well as in preclinical murine models of SARS-CoV2 infection will help advance vitamin D supplementation efforts on a solid footing. Currently there are over 16 clinical trials listed on clinicaltrials.gov aimed at correlating patient vitamin D status with disease severity and evaluating the dose-dependent beneficial effects of vitamin D supplementation.

8 | VALUE OF PRECLINICAL STUDIES

Given that inflammatory responses are (i) rapidly induced after infection, (ii) undergo dynamic changes with disease progression, and (iii) likely show tissue-specific dichotomy in local lung tissues of viral replication compared with the secondary lymphoid sites of immune activation, murine SARS-CoV2 infection model is indispensable for better understanding of when and how vitamin D regulates COVID-19. The hACE2-Tg mouse model of SARS-CoV2, expressing the human ACE2 receptor under the control of the human cytokeratin 18 promoter in airway and gut epithelial cells, closely simulates the human viral replication patterns and COVID-19 disease pathology—with interstitial pneumonia, lymphocyte, and monocyte infiltration in alveolar interstitium and macrophage infiltration in alveolar cavities—thus, offering high translational value for SARS-CoV2 pathogenesis and immunologic studies.[165–167] Importantly, the full range of vitamin D disparity and supplementation modalities observed in the clinic are easily recapitulated in mouse models through controlled diets with well-defined levels of bioactive vitamin D, thus resulting in serum calcidiol levels that aptly mimic clinically defined states of vitamin D deficiency, insufficiency, sufficiency, and supplementation. Likewise, ready use in the clinic of low calcemic vitamin D analogs such as paricalcitol offers rapid translational potential for the preclinical supplementation studies, where equivalency of dose based on body weight and route of administration are easily achieved due to similarities in vitamin D source, absorption, metabolism, and signaling.[170,172]

Powerful genetic models of vitamin D deficiency ($VDR^{-/-}$), supplementation ($CYP27B1^{-/-}$), and conditional ablation ($VDR^{fl/fl}$) exist to query systemic and cell-type specific vitamin D function. Mice harboring VDR deficiency[168] are unable to transmit vitamin D signals and exhibit clinical manifestations of severe vitamin D deficiency (i.e., rickets, osteomalacia, hypocalcemia, and hyperparathyroidism). The $CYP27B1^{-/-}$ mice are unable to convert inactive calcidiol into its bioactive calcitriol form, and develop rickets, osteomalacia, and so on. This is a powerful reagent to tightly control the dose and duration of vitamin D signals by rapidly elevating serum levels via administration of bioactive calcitriol (half-life < 12 h), but not calcidiol.[169,170] Additionally, conditional VDR knockout mice ($VDR^{fl/fl}$) have been developed for controlling vitamin D signals in specildiscfic cell types using the classic immunology tool of bone marrow chimerism.[171] Thus, the field is uniquely poised to conduct rigorous and systematic inquisitions into when and how vitamin D signals regulate COVID-19, as a springboard for further clinical studies and future supplementation trials, to commandeer immune responses based on clinical needs.

9 | SUMMARY

Several respiratory diseases are strongly linked to vitamin D inadequacy, and potential beneficial effects of vitamin D supplementation are indicated in multiple disease conditions with underlying immune alterations. Similar albeit limited preliminary indications of association between vitamin D insufficiency and COVID-19 severity exist. Given the magnitude of the pandemic, larger-scale clinical studies will provide more robust correlations between vitamin D status, inflammation, respiratory disease severity, and mortality from COVID-19. In addition, immune system-independent effects of vitamin D on SARS-COV2 replication and disease progression also need to be considered through possible modulation of ACE2 and the renin–angiotesin

system. Similarly, indirect regulation of immune responses to SARS-CoV2 by vitamin D-dependent regulation of mucosal microbiota is also plausible, as shown in the case of cystic fibrosis.[173,174] Promisingly, the ready availability of highly translational murine models of vitamin D deficiency, insufficiency, and supplementation are expected to catalyze the critical cause–effect type studies in the tractable murine models of SARS-CoV2 infection. Methodical stepwise evaluation of how vitamin D regulates lung immunopathology during early and late stages of SARS-Cov2 infection will illuminate the immunologic underpinnings of ARDS in COVID-19, and provide mechanistic insights into vitamin D-dependent regulation of inflammation and ARDS. This will also offer evidence-based support for vitamin D supplementation as a safe, inexpensive, and readily available option to improve COVID-19 outcomes in vulnerable people (e.g., elderly and African Americans who generally have lower amounts of vitamin D), and help identify potential new therapeutic targets to temper inflammation and ARDS in SARS-CoV2 and other respiratory infections of future pandemic potential (such as novel coronaviruses and influenza viruses).

AUTHORSHIP

V.K. and S.S. contributed equally to this work. All authors contributed to the manuscript preparation.

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DISCLOSURES

The authors declare no conflicts of interest.

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