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Book Review

Exploring the Immunomodulatory Effects of Vitamin D3 in Sars-Cov2 Management

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ABSTRACT

Severe SARS-CoV2, the latest pandemic infectious diseases, is a serious threat to human health. SARS-CoV2 infection causes immune activation and systemic hyper-inflammation which can lead to respiratory distress syndrome (ARDS). ARDS victims are linked to sustained IL-6 and IL-1 increase. Macrophage activation associated with the "cytokine storm" promote the dysregulation of the innate immunity. So far without vaccines or specific therapy, all efforts to design drugs or clinical trials are deserving. Vitamin D and its receptor vitamin D receptor (VDR) exert an important role in infections for their remarkable impact on both innate and adaptive immune responses and on the suppression of inflammatory process. The protective effects of vitamin D supplementation have been proved by several observational studies and by meta-analysis of clinical trials for prevention of viral acute respiratory infection. In this review we compare the mechanisms of host immune response to SARS-CoV2 infection and the immunomodulatory actions that vitamin D exert in order to explore the preventive effect of vitamin D supplementation on SARS-CoV2 viral infection.

Keywords: Vitamin D; SARS-CoV2 Immunopathology; Immunomodulation; Prevention

INTRODUCTION

The new corona virus SARS-CoV2 has now become a major global concern. To date, have been typified 30 CoVs that can infect humans, mammals and birds. The corona virus family have been divided into four genera, the corona virus α, β, ɣ and δ. Human CoV infections are caused by α and β CoVs. The new SARS-CoV2 may have developed from the recombination of RNA between existing corona virus [1]. Human CoV infections mostly cause respiratory, gastrointestinal, hepatic and central nervous system diseases. The clinical symptoms such as dry cough, fever, dyspnea, expectoration, fatigue, and myalgia of SARS-CoV2 infection are similar to those of SARS-CoV and MERS-CoV [2]. SARS-CoV2 clinical manifestations vary considerably throughout the population. A large portion of subjects with SARS-CoV2 may remain asymptomatic [3]. Several others patients present renal insufficiency, severe pneumonia, bilateral ground-glass opacities on chest CT scans [4]. About 20-25% of SARS-CoV2 patients are affected by intestinal symptoms such as those observed in MERS-CoV or SARS-CoV [3]. In severe SARS-CoV2 infection subjects, the symptoms may include acute respiratory distress syndrome (ARDS), septic shock, multiple organ failure, metabolic acidosis, and bleeding and coagulation dysfunction and even death [5].The mortality rate is variable in the different population and currently cannot be quantified. SARS-CoV2 is transmitted through droplets, close contact, aerosol and even fecal-oral transmission can be a possible route of infection [6]. Asymptomatic subjects and patients in the incubation period can infect other persons, leading to exceptionally arduous procedures for detecting and isolating patients to contain epidemic spread [7]. Based on genome similarity with SARS, analysis of nucleic acid sequence within the spike protein receptor-binding domain (RBD) has been shown that SARS-CoV2 use angiotensin converting enzyme (ACE)-2 and dipeptidyl peptidase (DPP)-4 as a cell receptor [8]. Like SARS-CoV and MERS-CoV, SARS-CoV2 infection leads to inflammation and immune activation and the release of pro-inflammatory cytokines, including interleukin 1 beta (IL-1 β)

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and IL-6 [9]. Therefore, innate and adaptive immune response exert crucial role in protective or destructive responses. In the last decades many experimental evidences indicate that 1 α , 25(OH)2D3 influences the innate and adaptive immune response [10]. It regulates the immune response through VDR, which is expressed in immune cells, including naïve or activated CD4+ and CD8+ T cells, B cells, neutrophils monocytes, macrophages and dendritic cells [10]. Patients with Vitamin D deficiency have been found to have increased IL-6 and tumor necrosis-alpha (TNF- α) levels as well as activated monocyte phenotypes [11]. Therefore, a successful immune response is significantly influenced by the vitamin D endocrine system which balances inflammation versus anti-inflammation. In this review we compare the mechanisms between the host immune response to the infection and the immunomodulatory actions of Vitamin D in order to evaluate whether the causative agent SARS-CoV2 is an infection that takes advantage of Vitamin D deficient patients thereby affecting the course of the disease and if vitamin D supplementation may be useful to prevent this disease.

VITAMIN D3

The vitamin D3 [1,25(OH)2D3] is a fat-soluble steroid with endocrine function. In addition to its conventional role in calcium homeostasis exerts others biological effects, such as regulation of cell proliferation, differentiation and apoptosis, on numerous cell types and tissues. The two major forms of Vitamin D, D2 (ergocalciferol) and D3 (cholecalciferol) are obtained in the skin when exposed to sunlight or in lesser extent through diet and supplementation (Figure 1).

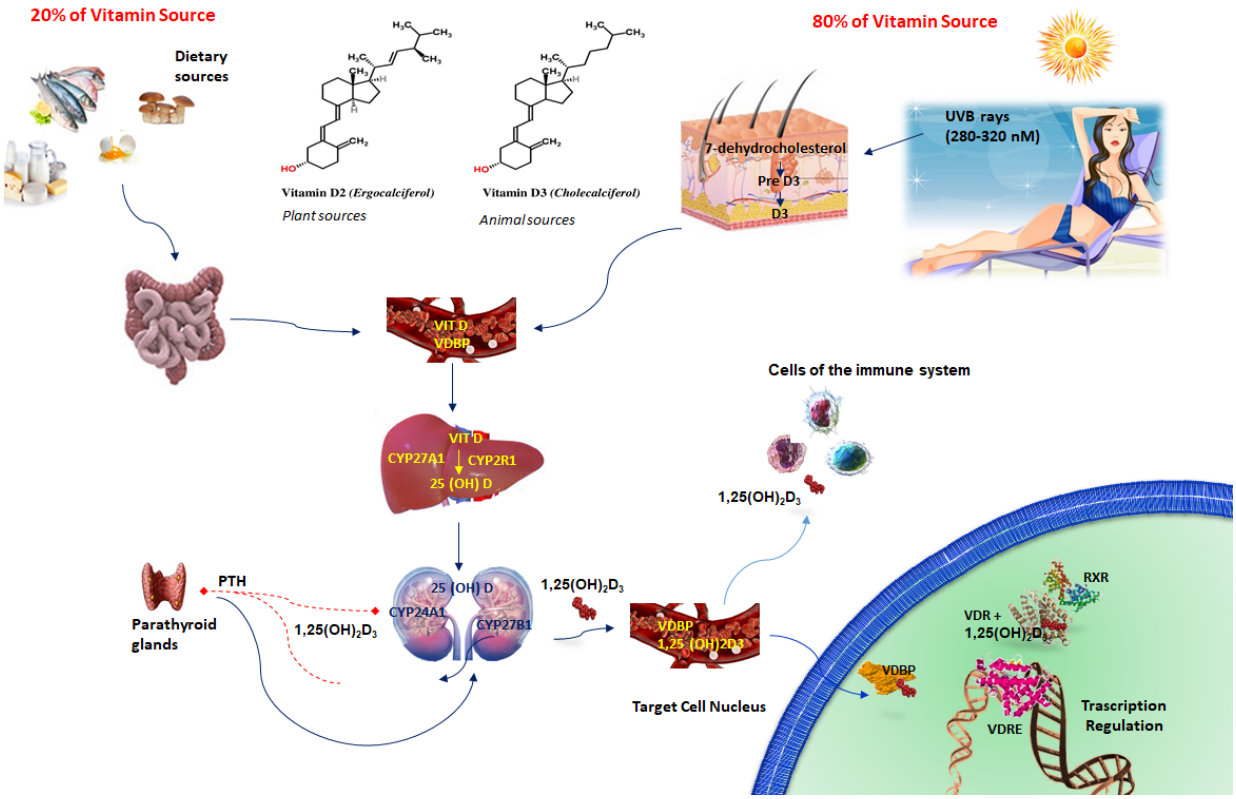

Figure 1: Vitamin D Synthesis and Mechanism of action: 1,25(OH)2D3 is biologically inactive and requires two enzymatic steps to become biologically active. Cutaneous 7-dihydrocholesterol is converted into preVitD3 after irradiation by ultraviolet light (UVB) from the sun. The first step occurs principally in the liver, where cholecalciferol is hydroxylated to 25-hydroxy-VitD [25(OH)D] or calcidiol, by the cytochrome P450 hydroxylase enzymes CYP27A1 and CYP2R1.25(OH)D is further converted, through a second hydroxylation in the kidneys by the mitochondrial cytochrome P450 enzyme (CYP27B1), into the active 1,25-dihydroxyvitamin D3[1 α ,25(OH)2D3] or calcitriol. The activity of 1 α ,25(OH)2D3 is regulated by 24-hydroxylation (carried out by CYP24A1), which inactivates the hormone. The Vitamin D activation takes place not only in the kidneys but also in other but it is also activated locally by CYP27B1 in many tissues including the cells of the immune system, where vitamin D is able to influence a variety of cellular functions. Active 1 α ,25(OH)2D3binds the vitamin D receptor (VDR) and afterward interacts with vitamin D response elements (VDRE) to modulate gene transcription.

Vitamin D deficiency is a public health problem around the world in all age groups, even in countries nearer to the equator with sufficient UV radiation and in developed countries where 1 α ,25(OH)2D3is commonly supplemented [12]. Vitamin D deficiency (VDD) may be due to advanced age, limited sunlight exposure, skin pigmentation, black ethnicity, low levels of dietary 1 α ,25(OH)2D3intake, gastrointestinal absorption disorders, liver and kidney diseases, obesity, diabetes mellitus, and alcohol ingestion [10]. Usually, vitamin D status is evaluated by serum 25(OH)D levels (Figure 2).

25(OH) D status

25(OH) D Concentration	
<10 ng/mL	Severe deficiency
10–20 ng/mL	Deficiency
20–30 ng/mL	Insufficiency
≥30 ng/mL	Optimal Level
≥100 ng/mL	Toxicity

Risk factors

Latitude
Skin color
Aging
Obesity
Sunscreen
Malabsorption syndrome
Kidney diseases
Inflammatory bowel disease
Occupational activities
Drugs (anti-epileptic, antiretroviral, etc)

Daily intake

Age	Recommended Intake (IU/day)	Upper Limit (IU/day)
National Institute of Medicine		
Children (0-18 years)	400-600	2500 (1-3 years) 3000 (4-8 years) 4000 (13-18 years)
Adults (19-70)	600	4000
Elders (>70)	800	4000
Pregnancy/Lactation	600	4000
Endocrine Society		
Children (0-18 years)	400-1000	2500-4000
Adults (19-70	1500-2000	10,000
Elders (>70)	1500-2000	10,000
Pregnancy/Lactation	600-1000 (14-18 years) 1500-2000 (19-50 years)	10,000

Figure 2: Vitamin D deficiency, daily Intake and risk factors: the international guidelines focused on pleiotropic effects of vitamin recommend a target 25(OH)D concentration of 30 ng/mL. Vitamin D doses recommended based on age, limited sunlight exposure, ethnicity, skin pigmentation, gastrointestinal absorption disorders, obesity, diabetes mellitus, liver and kidney disease, alcohol intake, range between 400 and 2000 IU/day.

IMMUNOPATHOLOGY OF SARS-COV-2

The initial infection site of SARS-Cov-2 has not yet been fully identified and its pathogenesis is still under investigation. However, it is established that the SARS-Cov-2 infection activates the innate and adaptive immune responses that cause a tremendous inflammatory scenario resulting in tissue damage both local and systemic. Patients with severe SARS-Cov-2 show significantly reduced numbers of CD4+ T cells, CD8+ T cells, B cells and natural killer (NK) cells [3,5] and a reduced percentage of monocytes, eosinophils and basophils[13]. An increase in neutrophil count and in the neutrophil-to-lymphocyte (NLR) ratio indicates higher disease severity and poor clinical outcome [14]. Exhaustion markers, such as NKG2A, on cytotoxic lymphocytes such as NK cells and CD8+ T cells, are increased in patients with SARS-Cov-2. In convalescent patients CD4+ T cells, CD8+ T cells, B cells and NK cells and the markers of exhaustion on cytotoxic lymphocytes get back to normal [15]. Patients with severe COVID-19 show elevated serum levels of C-reactive protein and D-dimer and of pro-inflammatory cytokines including IL-6, IL-1 β , TNF- α , IL-2, IL-8, IL-17, G-CSF, GM-CSF, IP10, MCP1, MIP1 α (or CCL3) named as cytokine storm[3,5,13]. The cytokine storm generate shock and tissue damage in the heart, liver, kidney and multiple organ failure. In addition, it has been observed spleen atrophy and lymph node necrosis, indicative of immune-mediated damage in deceased patients. Cytokine storm facilitate a severe pulmonary pathology, leading to massive infiltration of neutrophils and macrophages, diffuse alveolar injury with the formation of hyaline membranes and a diffuse thickening of the alveolar wall [5]. In particular, over-production of IL-6 is a valuable indicator of poor outcome in SARS-CoV2 patients with ARDS.

ACE2 RECEPTOR AND HOST–VIRUS INTERACTION

Renin angiotensin system (RAS) is an endocrine system playing physiological roles in electrolyte homeostasis, body fluid volume regulation and cardiovascular control in peripheral circulation. Renin, an enzyme produced from the kidney, acts on angiotensinogen (AGT), a liver-precursor, to release an inactive decapeptide, angiotensin I (Ang I) ACE2 catabolizes Ang II into Ang (1–7), which binding to mitochondrial assembly receptor (MasR) deactivates the deleterious actions of Ang II the effector peptide of RAS [16]. Upon binding to the Ang II type 1 receptor (AT1R), Ang II enables vasoconstriction, inflammation, cell proliferation, fibrosis, increased renal sodium absorption, aldosterone and arginine vasopressin (AVP) release. Ang I and Ang II can also bind with angiotensin type 2 receptor (AT2). AT2 counteracting AT1 overstimulation offsets the effects of the AT1 receptor inducing anti-inflammatory actions, vasodilation, cell apoptosis, and natriuresis [17]. SARS-CoV2 uses a spike protein S1 that facilitates the binding of the virion to the cell membrane by interacting with host ACE2 receptor [18] (Figure 3).

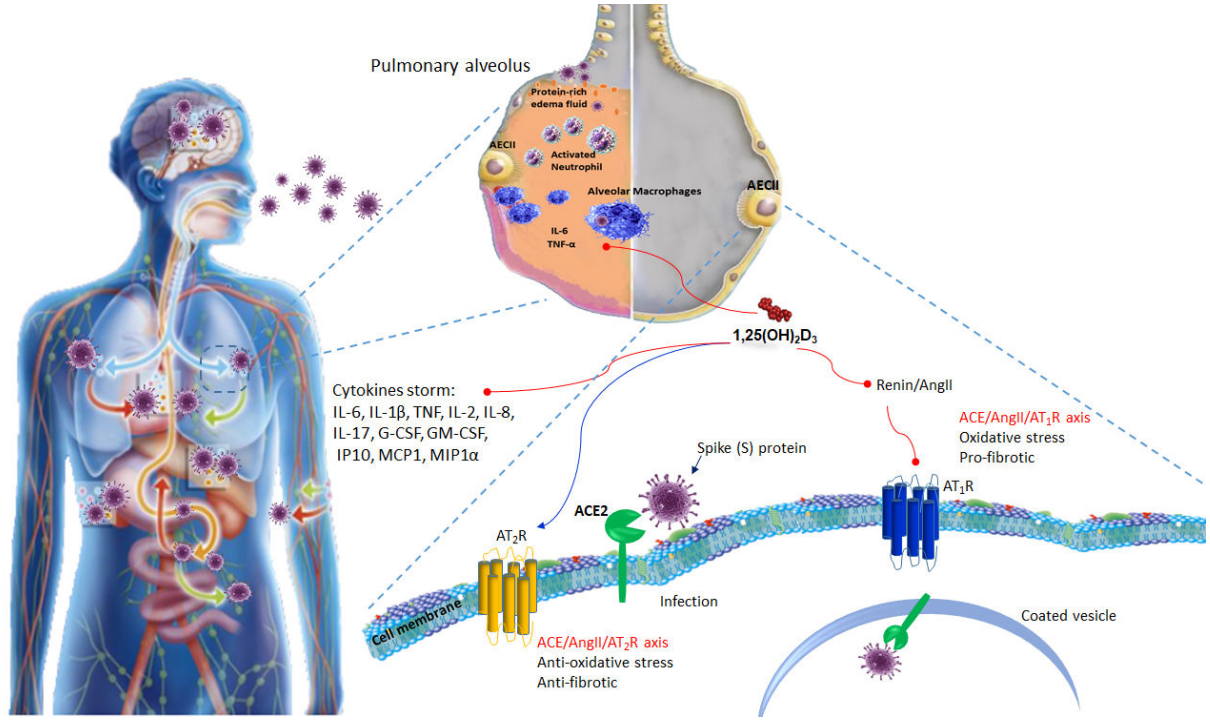

Figure 3: Host-SARS-CoV2 interaction and Vitamin D action: SARS-CoV2 interacts with host ACE2 receptor using a spike protein S1 that facilitates the binding of the virion to the cell. Infection generates Cytokines storm. ACE2-expressing Alveolar epithelial type II cells (AECII) AECII helps corona viral replication. 1 α ,25(OH)2D3inhibits renin, ACE and Ang II expression, and induced ACE2. 1 α ,25(OH)2D3 induces ACE2/Ang-(1-7) axis and inhibits renin and the ACE/Ang II/AT1R cascade and thus prevents vasoconstriction, inflammation, cell proliferation, fibrosis, oxidative stress, activates autophagy.

It has been demonstrated that the ACE2 binding affinity of the SARS-CoV2 spike protein ectodomain is 10–20 fold higher than that of the SARS-CoV spike protein, that may explain the higher binding affinity of the SARS-CoV2 spike protein to the human receptor. ACE2 receptor is expressed in numerous tissues including lung, heart, luminal surface of intestinal epithelial cells, kidney, and endothelium [18]. Hence, the clinical symptoms of respiratory injury, hepatic failure, acute kidney injury or diarrhea are, at least in part, associated with the pervasive ACE2 expressing cells in these tissues. Additionally, n-CoV targeting ACE2-expressing cells affects various immune cells in different tissues especially macrophages in lung, liver and stomach. Macrophages, recruited by nCoV-targeted cells through CD74-MIF interaction and other signaling pathways during infection, may play both defensive and destructive roles. ACE2 is modestly expressed in the lung, nevertheless it is the most susceptible target organ. About 83% of ACE2-expressing cells are alveolar epithelial type II cells (AECII). Therefore, these cells can act as a storage for viral invasion [19]. Gene ontology enrichment studies proved that the ACE2-expressing AECII possesses high levels of multiple viral process-related genes, comprising regulatory genes for viral processes, viral life cycle and viral assembly [20], indicating that the ACE2-expressing AECII helps corona viral replication. In addition, human coronaviruses use other peptidases as their receptors. Alanyl aminopeptidase (ANPEP), Glutamyl Aminopeptidase (ENPEP) and DPP4 are the top three genes correlated with ACE2 [21].

ACE2 RECEPTOR AND VITAMIN D

ARDS is one of the main cause of morbidity and mortality in severely SARS-CoV-2 patients, and is characterized by endothelial impairment and increased barrier permeability. In a murine model of ARDS, ACE2 knockout mice developed a severe lung disease caused by increased vascular permeability and pulmonary edema compared to wild mice [22]. Inhibition of the prorenin receptor decreases interstitial edema, hemorrhage, neutrophil count and the amount of non-proteolytically activated prorenin in the lung tissues of rats [23]. Therefore, since ACE2 can protect against acute lung injuries its inhibition by SARS-CoV-2 could be closely associated with ARDS. Systemic infusion of Ang II promotes ALI [24].Moreover, dysregulation of local and circulating RAS, with enhanced ACE/Ang II expression levels and reduced ACE2/Ang-(1 – 7) expression, promote ischemia-reperfusion-induced acute lung injury (ALI) in mice [25]. ACE2 overexpression reduces LPS-induced ARDS via the Ang-(1 – 7)/-MasR pathway by inhibiting extracellular signal-regulated kinase/nuclear factor-κ B (NF- κ B) activation [26]. Several findings indicate that 1 α , 25(OH)2D3regulates RAS inhibiting renin biosynthesis [27]. Vitamin D-mediated pathway may avoid acute lung injury. 1 α , 25 (OH)2D3inhibits renin, ACE and Ang II expression, and induced ACE2 levels in LPS-induced ALI. Therefore, vitamin D attenuate LPS-Induced ALI by, at least partially, inducing ACE2/Ang-(1-7) axis activity and inhibiting renin and the ACE/Ang II/AT1R cascade. VDR is highly expressed in the lung. It has been demonstrated that VDR inactivation results in

deregulated stimulation of RAS. VDR and 1,25 (OH)2D counteract fibrosis in lung, liver and kidney [28-30] through a negative regulation of the RAS and the inhibition of nuclear factor kappa B (NF-κB) and wnt/β-catenin [26,31]. In addition, VDR mediating this activity protects against sepsis-induced lung injury by inhibiting the angiopoietin-2-TEK receptor tyrosine kinase-myosin light-chain kinase pathway [31]. Vitamin deficiency (VDD) is common in people who develop ARDS [32].VDD may promotes lung fibrosis by activating RAS [33]. Consequently, RAS inducing fibrosis decreases lung function and compliance [34]. It has been observed that VDR-knockout mice suffer higher severity ALI induced by LPS, which occur for the collapse of the alveolar epithelial tight junctions via reduced occludin and zonula occludens-1 expression [35]. Vitamin D pretreatment decreases seawater aspiration-induced ALI by inhibiting inflammatory responses, reducing lung epithelial-endothelial barrier permeability and modulating the RAS cascade. Paricalcitol, a vitamin D analog, protecting alveolar barrier function alleviated LPS-induced ALI. These extraordinary effects occur by inhibition of NF-κB and ofras the homolog family member A/Rho kinase signaling pathways [36]. Inflammation may be responsible for impaired 1α-hydroxylase activity in the kidneys, resulting in reduced production of 1α,25(OH)2D3by blocking the PTH-stimulated conversion of 25(OH)D to 1α,25(OH)2D3 [37]. Interestingly, 1α-hydroxylase-deficient mice display increased activity of the intra-renal RAS that decreased following 1α, 25(OH)2D3administration [38]. In various pathogenic conditions, VDRA/VDR complex could alleviate RAS activity, inhibit epithelial-to-mesenchymal transition, prevent apoptosis, decrease inflammation, activate autophagy, regulate mitochondrial function and induce immune tolerance through various signaling pathways. Experimental and clinical evidence showed that pharmacological activation of VDR may offer an attractive target for various inflammatory diseases [10].

THE HOST IMMUNE RESPONSE COVID19 INFECTION

Observing the remarkable similarities in genomic structure, in clinical and experimental data between the recent SARS-Cov-2 and previous SARS-COV and MERS-COV viruses, it is possible hypothesize, step by step, how the host immune system acts with SARS-Cov-2 virus. The innate immune system is a conserved defense apparatus essential for the early recognition and restraining of pathogens and the consequent activation of the adaptive immune response. Once a corona virus is inhaled commonly it binds to non-specific receptors on the respiratory epithelium such as adhesion molecule CEACAM1 [39]. Follows an endocytosis process which internalizes the virus and allows successive replication, transcription and translation of new viruses that are then released to infect new cells. The infected cells activate the innate immunity employing various pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and RIG-I-like receptors (RLRs) and nucleotide binding-oligomerisation domain (NOD)-like receptors (NLRs) that recognize pathogen-associated molecular patterns (PAMPs). Despite differences in each viral genome, replication strategies, and the types of PRRs activated, common signaling pathways are utilized.

TOLL-LIKE RECEPTORS

Airway epithelial cells express TLRs and RLRs which perceive viruses. Ligands for TLRs and RLRs activate epithelial cells triggering a rapid immune response against viral invasion [40]. Besides to the early infection of the epithelial cells, tissue-resident macrophages, dendritic intraepithelial cells (DCs), which reside just under the respiratory epithelium, internalize particles in the lumen of the airways by phagocytosis and macropinocytosis, thus stimulating PRR and starting an immune response [41]. The TLRs belong to single-pass transmembrane receptors expressed on innate immune cells. They are important sensors for a wide range of pathogen-derived molecules and influence host-pathogen interactions. The recognition of PAMPs by TLRs take place in cell membranes, endosomes, lysosomes, and endocytolysosomes and other sites of the cells [42]. TLRs identifies proteins, lipids, lipoproteins, and nucleic acids of the bacterial, viral, parasite, and fungal origins [42].TLRs are either expressed on the cell surface (e.g., TLR2 and 4) or intracellularly (e.g., TLRs 3, 7, 8 and 9) mainly located on the endoplasmic reticulum (ER) membrane [43]. TLRs identify ss-RNA (TLR 7/8) or unmethylated CpG double-stranded DNA motifs (TLR 9) of the viral genome, or the intermediary double-stranded RNA (TLR 3) formed during viral replication [44]. Further, TLR4 and TLR2 receptor complexes play a role in viral recognition carrying them to the endolysosome [45]. In particular, TLR7 which is one of PRRs recognizing ssRNA viruses via the recognition of the viral RNA genome [46] is able to detect SARS-CoV. TLR7 induces pro-inflammatory cytokines such as TNF-α, IL-6 and IL-12, recruits immune responder cells into the lungs, promotes ARDS and even lung fibrosis in the late phase [47]. Usually, Coronaviruses affect the signaling downstream of IFNAR and inhibit type I IFN production, which are linked with the disease severity [48]. Once type I IFN is induced, SARS-CoV prevents by ubiquitination and degradation RNA sensor adaptor molecules MAVS and TRAF3/6 inhibiting IRF3nuclear translocation [49]. MERS-CoV also utilizes these strategies but also suppresses histone modification [49]. Hence, coronaviruses implement mechanism to inhibit IFN signaling decreasing STAT1 phosphorylation [50].The viral proteins modulating this host type I IFN response are both structural proteins, such as M, N, and non-structural proteins such as ORF proteins, which are encoding polyprotein replicases. It has been shown that papain-like protease (PLPro), encoded by ORF1, is involved in SARS-CoV replication exhibiting an antagonistic activity against type I IFN. It directly interacts with IFN regulatory factor 3 (IRF-3) to block IRF-3 phosphorylation and nuclear translocation [51].Alternatively, PLPro disrupts the stimulator of interferon gene (STING)-mediated signaling and then negatively regulates type I IFN induction [51]. PLPro interacting with the STING-TRAF3-TBK1 complex, reduces the ubiquitinated forms of STING, RIG-I, TRAF3, TBK1 and IRF-3. PLPro cooperates with these regulators of TLR signal pathways which characterizes the antagonistic mechanisms of TLR signal pathways. In SARS-

Cov-2 infection, a similar scenario with a varying degree of immune interference may occur.

VITAMIN D AND TOLL LIKE RECEPTORS

An important anti-viral pathway in monocytes and macrophages take account of the TLRs activation [52]. Vitamin D metabolism in macrophages is associated to pathogen recognition [52]. In macrophages TLR induces up-regulation of the VDR and CYP27B1 gene expression [53]. Initiating a signaling cascade essential to upregulate VDR and CYP27B1, which in turn, induce the conversion of 25(OH)D to 1,25(OH)2D. This conversion could represent a key step to trigger the TLR pathway. It is well-known that the binding of 1,25(OH)2D to the VDR allows the expression of multitarget genes, which modulate the function of monocytes/macrophages during infection. Macrophages internalize serum VDBP-bound 25(OH)2D3 from the extracellular fluid by endocytosis and use 25(OH)2D3 as substrate to upregulated CYP27B1. Since VDR is efficient when exogenous 1,25(OH)2D3 is added, it can facilitate the ligation of the TLR2/1 heterodimer in macrophages and up-regulate CYP27B1[10, 54]. Therefore, extra-renal 1,25(OH)2D synthesis is regulated by TLR ligation and cytokine secretion, utilizing an intricate cross-talk between various signaling pathways [54]. It is also possible that the expression of TLRs in multiple cell types, and the ability to respond to a variety of pathogens promotes other TLRs or alternate PRRs to induce extra-renal expression of CYP27B1, allowing that locally generated 1,25(OH)2D performs widespread effects on the immune response. The mechanism by which TLR ligation improves CYP27B1 expression involves NF-κB, JAK-STAT, and p38 MAPK pathways, and phosphorylation of the transcription factor C/EBPβ by p38 MAPK [55]. Cytokines, such as IL-1, IL-2, IL-4, IL-13, IFNγ and TNF-α, regulate CYP27B1 expression and vitamin D metabolism [55,56]. It is possible that the pathways involved in cytokine-regulated transcription of CYP27B1 are the same as those used by TLR ligation-induced regulation [56]. The TLR8 ligands CL097 and ssRNA40 have both been demonstrated to induce a dose-dependent increase in CYP27B1 mRNA and protein expression in macrophages [57]. 1α,25(OH)2D3promotes TLR-2 and TLR-4, the receptor of interferon gamma (IFN-γ) or CD40 expression in monocytes[58] supporting a state of hypo-responsiveness to PAMPs [10,59]. In response to TLR-2/1 activation in human macrophages, 1α,25(OH)2D3 triggers innate immunity inducing the synthesis of antimicrobial peptides [10]. Another interesting finding is that 1α, 25(OH)2D3modifies the TLR9-dependent production of IL-6 and increases NE/PAD4/COX-3/GAPDH, TLR7 and type I interferon (IFN) mRNA levels (Figure 4) [60].

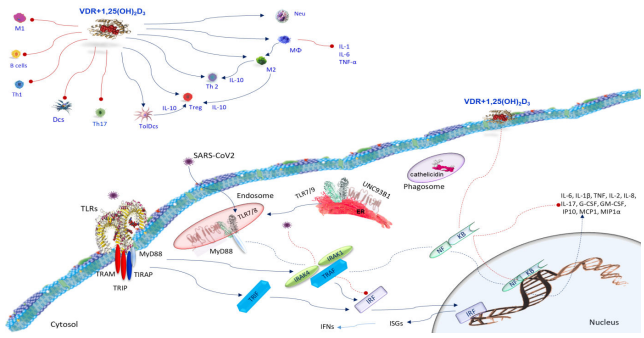

Figure 4: Host Immune Response COVID19 infection and Vitamin D function:TLRs play a role in viral recognition carrying them to the endosome. Different TLRs can induce different biological responses via subsequent activation of varied adapter proteins, such as MyD88, TIRAP, TRIP, and TRAM. MyD88 activates the nuclear factor kappa B (NF κ B) pathways to induce inflammatory cytokines production. UNC93B1 is essential for signalling of TLR3, TLR7 and TLR9. It interacts TLRs in the endoplasmic reticulum (ER) following viral infection. TLRs activate different adapter proteins, such as MyD88, TIRAP, TRIP, and TRAM. After a TLR is activated by the corresponding PAMP, MyD88 recruits IL-1 receptor-associated kinase (IRAK)-4, then leads IRAK4 to activate other members of the IRAK, like IRAK1 and IRAK2. IRAK4 plays an important role in activating NF with TNF receptor-associated factor (TRAF) 6 and activate NFTRIF is an adapter protein of TLR3 and TLR4. TRIFdent pathways activate NF receptors(IRFs). They induce expression of a variety of interferon-stimulated genes (ISGs), which reduce infection by their antiviral and immunomodulatory actions. SARS-CoV recognizing TLR7 and induces the pro-inflammatory cytokines such as TNF-α, IL-6 and IL-12. Coronaviruses inhibit type I IFN production, prevents the signaling downstream inhibiting IRF3 nuclear translocation. 1α,25(OH)2D3promotes TLR-2 and TLR-4. In response to TLR-2/1 activation in human macrophages, 1α,25(OH)2D3 triggers innate immunity inducing the synthesis of Cathelicidin.

It is possible that in individuals with adequate circulating levels of 25(OH)D, infection by SARS-CoV2 viruses following recognition by TLRs and cytokine production is able to intensify the levels of 1,25(OH)2D, which modulate the immune response to counteract the infections.

SARS-COV2 AND IMMUNE CELLS

Severe subjects with SARS-CoV2 show altered leukocytes count and NLR [13].The infiltrated immune cells in alveoli are mainly monocytes and macrophages [61]. Both helper T cells and suppressor T cells in patients with SARS-CoV2 are lower than the normal levels whereas the percentage of naïve helper T cells increases [13]. In addition, SARS-CoV2 severe patients have lower level of regulatory T cells (Tregs) [62]. Severe cases showed elevated levels of infection-related biomarkers and inflammatory cytokines [62]. Likewise, to SARS-CoV or MERS-CoV infection, delayed type I IFN production compromises the early viral control, leading to influx of hyper-inflammatory neutrophils and monocytes-macrophages [63]. Infiltrated myeloid cells are the main cause of lung dysfunction. The increases in these innate immune cells yields deteriorating consequences to

infected host that negatively impact the outcome of the infection.

VITAMIN D AND NEUTROPHILS

Often SARS-CoV2 patients show altered percentage of neutrophils [13]. Neutrophils are phagocytic cells that are specialized in early defense against invading pathogens [64]. They participate to innate immune responses controlling infectious diseases through microbicidal mechanisms such as phagocytosis, degranulation and the release of neutrophil extracellular traps (NETs). NETs are DNA structures released through the decondensation and spreading of chromatin and the adherence of various proteins, including neutrophil elastase (NE), myeloperoxidase (MPO) and peptidyl arginine deiminase 4 (PDA4). NETs are crucial for the host's defenses and inflammation, they enhance IL-1β and IFN-α of activated neutrophils. In several SARS-CoV2 patients high levels of NETs have been found [65]. 25(OH)2D3 plays an important role in modulating neutrophils activation and in preventing infections. It has been reported that 1,25(OH)2D3, is able to restrict the spread of pathogens, such as virus, by inducing NETs[66] and the expression of TLR7 and of IFN-α [60]. Nevertheless, excessive NET formation contributes to cytokine release and initiates a cascade of inflammatory reactions that facilitates micro-thrombosis generating a long-lasting organ damage of the pulmonary, cardiovascular, and renal systems [67]. Remarkably, these are three frequently affected organs in severe SARS-CoV2 [68]. These observations suggest that in severe SARS-CoV2, the administration of Vitamin D3 could be ineffective or even deleterious.

VITAMIN D AND MONOCYTES/ MACROPHAGES

Macrophages are phenotypically and functionally heterogeneous cells. They exert a multitude of biological activities conditioned both by tissue microenvironment stimulation and cytokines signals [69]. This happens because macrophage polarization, that generates classically activated (M1), following IFN-γ and LPS induction and alternatively activated macrophages (M2) following IL-4 and IL-13 or indirectly through Th2 cells induction, modifies their immune asset [70]. M1 and M2 exert opposite activities anti-versus pro-inflammatory response, immunogenic versus tolerogenic activities, and tissue-repair versus tissue destruction [70]. M1 macrophages stimulate inflammation and type 1/Th1/Th17 immune responses and produce a plethora of proinflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α, and various cytotoxic molecules that stimulate the acquired immune response and promote the clearance of invading pathogens [71]. Vitamin D influences macrophage polarization towards M2 phenotype [72] (Figure 3). M2 macrophages are the greatest producers of IL-10 which avoid the differentiation of M1 macrophages [73]. 1α, 25(OH)2D3modifies macrophage phenotype through the inhibition of IFN-γ, and through IL-10 prevents M1 macrophage differentiation[74]. M2 macrophages inhibit inflammation eliciting type 2/Th2 immune responses [75]. When macrophages have impaired functions, airway occlusion can occur following SARS-CoV2 infection due to the accumulation of inflammatory cells, virus-infected cells, apoptotic debris and serum proteins, resulting in severe and potentially fatal ARDS [76]. In this context the ability of M2 macrophages to phagocytose infected cells and apoptotic cellular debris, improved by the action of 1α,25(OH)2D3, could help the achieve the clearance of infection and the resolution of inflammation

VITAMIN D AND AMPS INDUCTION

The 1,25/VDR/RXR complex enhances in the macrophages chemotactic and phagocytic capabilities and meanwhile activates the transcription of anti-microbial peptides (AMPs) in several cell types(Figure 3). In macrophages TLRs activation elicits cathelicidin antimicrobial peptide (CAMP) expression via a vitamin D-dependent pathway [78]. 1,25(OH)2D-VDR complex binds VDRE in the promoter of the cathelicidin gene enhancing hCAP-18 production [79]. The induction of CAMP vitamin D-mediated intensifies antimicrobial activity against pathogens specifically by direct killing cathelicidin leucin-leucin-37 (LL-37) and increasing phagosome formation [78].This induction can be one of the mechanism by which vitamin D enhance innate immunity to respiratory infections. LL-37 is present in epithelial cells, monocytes, neutrophils and macrophages, NK cells, B-cells and γδ T-cells [80], and can be secreted by respiratory epithelial cells onto the airway surface to form a first line of defense against invading pathogens [81]. LL-37 modifies cytokine production enhances TLR3 signaling. Moreover, repressing directly viral particles, it reduces spread of infection and of infected epithelial cells and exerts an anti-viral activity of against both enveloped and non-enveloped viruses [82-84]. In monocytes TLR activation generates expression of the defensin beta 4 gene (DEFB4) through the synergistic action of IL-1β and VDR pathways [85]. The proximal promoter region of DEFB4 gene contains a VDRE, allowing 1,25(OH)2D to upregulate expression of β-defensin 2 [79]. This is another antimicrobial peptide, which, similarly to LL-37, is able to induce chemotaxis of immune cells and to inhibit viral infection [86].

VITAMIN D AND ROS AND iNOS GENERATION

1α,25(OH)2D3modulates the innate immune system in a variety of ways. One of the signaling pathways regulated by vitamin D is the class III phosphatidylinositol 3-kinase complex (PI3KC3), with PI3K signaling associated with monocyte and macrophage generation of ROS and iNOS [87]. The oxidative burst has beneficial antiviral effects [88], nevertheless aberrant induction is associated with pathophysiology and tissue damage [89]. 1α,25(OH)2D3plays an important role in redox homeostasis in both pro-oxidative induction of ROS and iNOS to boost the antiviral response [90], and ant oxidative inhibition of Inos and induction of ROS scavenging pathways to prevent immunopathology [91].

VITAMIN D AND AUTOPHAGY

Autophagy acts as part of the immune system to remove damaged proteins and organelles, and is an important host defense tool against viral infections [92]. In human monocytes, 1α,25(OH)2D3 elicits the maturation of auto phagosomes and

auto phagolysosomes by means a hCAP18/LL-37-mediated pathway [93].1 α ,25(OH)2D3 induces autophagy by regulating multiple associated pathways, such as Bcl-2, mammalian target of rapamycin (mTOR), class III phosphatidylinositol 3-kinase complex, and cathelicidin production, thus potentially enhancing clearance of viruses and viral components [94]. Vitamin D triggering autophagy in macrophages could inhibit replication SARS-CoV2. VDR and adequate levels of Vitamin D could be associated with a natural resistance SARS-CoV2. This may stem from the upregulation of anti-inflammatory IL-10 and induction of defensin in mucosa of SARS-CoV2-exposed individuals.

ADAPTIVE IMMUNE RESPONSE

Humoral immune response producing neutralizing antibody, plays a protective role by limiting infection at later phase and prevents re-infection. In SARS-CoV, both T and B cell epitopes were widely mapped for the structural proteins, S, N, M and E protein [95].The Th1 type immune response plays a dominant role in an adaptive immunity to viral infections. Cytokines, generated by antigen presenting cells, directs T cell responses. T helper cells coordinate the global adaptive response, while cytotoxic T cells are indispensable in killing viral infected cells. In MERS-CoV infection, early rise of CD8+ T cells correlates with disease severity. In the convalescent phase, dominant Th1 cells have been observed [96]. In a murine model, airway memory CD4+ T cells specific for conserved epitope are protective against fatal challenge and can cross-react with SARS-CoV and MERS-CoV [97]. CD8+ T cell responses is more pronounced than CD4+ T cell responses. Th1 type response is crucial for an efficient control of SARS-CoV and MERS-CoV. This could be possible for SARS-CoV-2 as well. However, CD8+ T cell response needs to be well controlled to not cause lung pathology.

VITAMIN D AND THE ADAPTIVE IMMUNE RESPONSE TO RESPIRATORY PATHOGENS

Vitamin D modulates the adaptive immune response, and since it influences the antigen presentation acts as a bridge between innate and adaptive immunity. 1 α ,25(OH)2D3 inhibits proliferation and differentiation of B lymphocytes and IgG production, proliferation of Th1 cells and their cytokines production, whereas increases the Th2 pathway [10]. Low vitamin D levels impair normal Th1 and Th2 cytokine balance, resulting in a higher Th1 cytokine expression. 1 α ,25(OH)2D3 affects indirectly T-cell responses influencing the dendritic cells (DCs) phenotype[98]. DCs are effective antigen-presenting cells affecting lymphocyte activation and inducing the adaptive immune response. DCs express VDR, CYP27A1 and CYP27B1, thereby generating locally bioactive 1 α ,25(OH)2D3[99]. Nonetheless, human monocyte-derived DCs convert 25(OH)D to 1 α ,25(OH)2D3 in a reduced quantity compared to macrophages, probably because DCs express a truncated CYP27B1 transcript, which may result in a deficiency in Vitamin D activation[100]. Addition of 1 α ,25(OH)2D3 inhibits DC differentiation, maturation and antigen presentation, decreases the co-stimulatory molecules CD40, CD80 and CD86[101], reduces markers such as CD1a, MHC class II and abolishes the chemotactic response to CCL4 and CCL19[102]. The main function of DCs is to initiate T-cell responses and, thus, the effect of 1 α ,25(OH)2D3 on DCs has a major impact on T-cells. In vitro1 α ,25(OH)2D3 induces a stable maturation-resistant tolerogenic phenotype and increases interleukin 10 (IL-10)/IL-12p70 ratios. Introduction of an antigen along with 1,25(OH)2D3 can induce antigen-specific tolerogenic DCs (tolDCs) [103] with the capacity to induce infectious tolerance, changing the behavior of other pro-inflammatory mature DCs through the induction of antigen-specific regulatory T cells (Tregs), and causing the perpetuation of the tolerogenic response[104]. Even when cultured with committed T-cells, these tolDCs cause hypo responsiveness, inhibit T-cell proliferation and reduced IFN- γ secretion [105]. The increased IL-10 production induces Tregsthat, in turn, secrete more IL-10, TGF- β , and CCL22 [59].The proliferation and cytokine profiles of T-cells are also directly altered by 1 α , 25(OH)2D3. Production of IL-2, IL-17 IL-21, IFN- γ and TNF- α , are all inhibited [106]. The IFN- γ further prevents macrophage activation, thus attenuating antigen presentation and the recruitment of other T-cells [107].This direct inhibition of Th1-priming cytokines further directs T-cell differentiation towards a Th2 phenotype. 1 α ,25(OH)2D3 is also able to upregulate the Th2-specific transcription factors GATA-3 and c-maf, resulting in increased production of IL-4, IL-5 and IL-10 [108]. Th17 cells and IL-17, have also been shown to be decreased, with 1 α ,25(OH)2D3 reducing IL-17 production. Th17 cells, by releasing IL-17, initiate an inflammatory response dominated by neutrophils. Th17 cells are a CD4+ T subset, their growth is influenced by signals mediated by IL-6 and TGF β , IL-21, and IL-23 and by stimulation of the lineage-specifying transcription factor, retinoic acid-related orphan nuclear receptor (ROR γ T). However, with conflicting cellular studies on the effects of vitamin D on Th2 cells [109] regarding both enhancement [108] and inhibition of IL-4 synthesis the mechanisms behind any potential beneficial role of vitamin D are unclear [110]. In addition, 1 α ,25(OH)2D3 down-regulates DC-derived Ox40L, which is required for Th2 priming, thus, resulting in a reduced Th2 cytokine response in CD4+ T-cells [111], thus contradicting evidence that vitamin D leads the T-cell phenotype towards a Th2 one. The decrease in Th1 immunity, which has been observed [10], would suggest a diminished immune response to pathogens, contrasting to the evidence that suggested an improved response to respiratory tract infections after vitamin D supplementation. Finally, while studies have demonstrated direct effects of 1 α ,25(OH)2D3 administration on lymphocytes, others have shown that in DCs to exert its immunomodulatory effects is required to convert the inactive metabolite 25(OH)D to the active 1 α ,25(OH)2D3 [112]. This indicates that administration of different vitamin D metabolites may result in different responses. Therefore, while vitamin D evidently acts as an immunomodulatory molecule with a wide range of effects, the specific mechanisms are at this time vague. Besides the conflicting results reported adds uncertainties to his action B-cells are also affected by vitamin D, with modulation of T-cell responses altering the B-cell compartment, as well as having direct effects on B-cells themselves [113]. Human B-cells also express VDR and CYP27B1, which are upregulated upon activation, suggesting that B-cells may also be susceptible to 1 α ,

25(OH)2D3 stimulation. It is able to inhibit proliferation, plasma-cell differentiation, immunoglobulin secretion and memory B-cell generation, even though inducing B-cell apoptosis [114] (Figure 5).

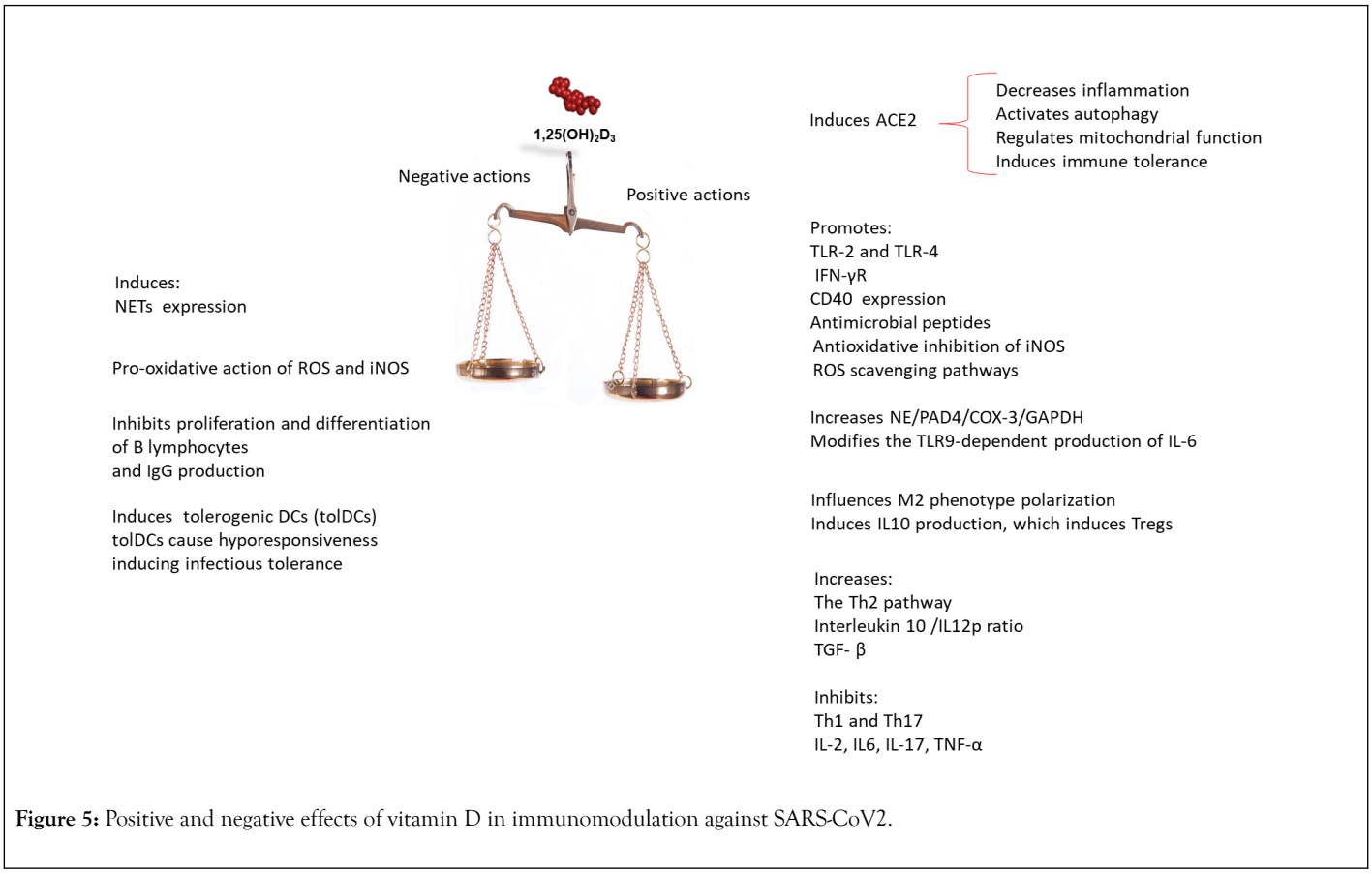

Figure 5: Positive and negative effects of vitamin D in immunomodulation against SARS-CoV2.

Emerging data reveal the promising role of Vitamin D in preventing cytokine and consequently determining outcomes of SARS-Cov2 [115-124]. It has been shown latitude-related vitamin D insufficiency, obesity, diabetes, hypertension, ethnicity, sex are conditions associated with the increased risk of severe SARS-Cov2 and mortality. Even it has been suggested that the different susceptibility gender-related, involve testosterone levels associated with vitamin deficiency in men [119].

CONCLUSION

In this review we analyzed the immune response of the host to the infection with SARS-Cov2, also referring to the knowledge of the immune response against MERS-CoV and SARS-CoV given the high homology that these viruses show. In parallel we evaluated in which effector arms of the immune response Vitamin D could intervene to modulate the inflammatory response. This examination shows that vitamin D exerts contrasting effects in modulating the immune response. Therefore, we can deduce that the supplementation of Vitamin D could be effective to prevent infection or in the early stages of the disease, instead the administration in serious SARS-CoV2 could be deleterious because it could stimulate or inhibit some cellular function that would amplify further the inflammatory response. Since Vitamin D has multiple cellular and intracellular targets, additional studies are needed to determine the consequences of the interaction of Vitamin D in the immune-response against SARS-CoV2 in order to harvest a significant vision into its prophylactic and therapeutic strategy for the prevention of this viral infection.

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