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1 **Effects of vitamin D on macrophages and myeloid-derived suppressor cells (MDSCs)**  
2 **hyperinflammatory response in the lungs of COVID-19 patients**

3

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28

29 **Abstract**

30 Vitamin D regulates homeostasis, anti-microbial response, and inflammation. The vitamin D  
31 receptors are expressed in the macrophages and other immune cells, regulating the transcription of  
32 many different genes, including those coding the anti-microbial peptides. One of the most severe  
33 complications of the SARS-CoV-2 infection is the acute respiratory distress syndrome (ARDS)  
34 caused by the hyperinflammatory response (commonly called cytokine storm) of the lung

35 macrophages. Studies showed that Vitamin D deficiency increases the severity of the ARDS in  
36 COVID-19 infection. We discuss here how the vitamin D supplementation may influence  
37 macrophage and myeloid-derived suppressor cells (MDSCs) inflammatory response, subdue the  
38 hyperinflammatory response, and lessen the ARDS in COVID-19 patients.

39  
40 **Key words:** macrophages, myeloid-derived suppressor cells (MDSCs), hyperinflammatory response,  
41 vitamin D, vitamin D receptor, COVID-19, SARS-CoV-2

## 42 43 **Introduction**

44 COVID-19 pandemic has revived and increased interest in vitamin D as a potential modulator of the  
45 immune response in SARS-CoV-2 infection. As the macrophage immune response plays an  
46 important role in the severity of COVID-19, any factor modulating their functions is, currently, of the  
47 high interests. Besides macrophages, the myeloid-derived suppressor cells (MDSCs), which suppress  
48 T cells activity and attenuate the overall immune response, are also the target of Vitamin D. This  
49 indicates that Vitamin D may have therapeutic applications as an additive to conventional anti-viral  
50 therapies.

51 Vitamin D (25 (OH)<sub>2</sub> D) is a fat-soluble secosteroid (steroid with a “broken” ring) hormone that  
52 regulates absorption and homeostasis of magnesium, calcium and phosphate, and various aspects of  
53 human health, including mitochondrial integrity, systemic inflammation, and the anti-microbial  
54 immune response (1, 2). The active metabolite of vitamin D, the 1, 25 dihydroxy vitamin D (1,25  
55 (OH)<sub>2</sub>D<sub>3</sub>) that circulates in the blood, functions through the binding to vitamin D receptor (VDR),  
56 also called the NR1H1 (nuclear receptor subfamily 1, group I, member 1), which is the member of the  
57 nuclear receptor family of transcription factors. The 1,25 (OH)<sub>2</sub> D/VDR complex heterodimerizes  
58 with the retinoic-X receptor (RXR), causing nuclear translocation, and binding to the vitamin D  
59 response elements (VDREs) on DNA. This, in turn, dissociates repressors, recruits the co-factors, and  
60 regulates the transcription of over 900 different genes (Fig. 1); (3, 4). Because the VDR is also  
61 abundantly expressed in the immune cells such as T cells, dendritic cells, and macrophages, many of  
62 these target genes have immune response-related functions (4). For example, such targets are the  
63 cathelicidin, and defensin, genes that encode the anti-microbial peptides that reduce viral replication  
64 rate and promote chemotaxis of macrophages and other immune cells to the inflamed organs (3, 7, 8).

## 65 66 **Macrophages and hyperinflammatory response in the lungs of COVID-19 patients**

67 One of the deadliest effects of SARS-CoV-2 infection is the acute respiratory distress syndrome  
68 (ARDS) caused by the overdrive of the inflammatory response of lung macrophages (Fig. 2); (9-16).

69 There are two different (intrinsic and extrinsic) mechanisms inducing macrophage inflammatory  
70 response. In the intrinsic response, the alveolar macrophages, express the ACE2 (angiotensin-  
71 converting enzyme-2), which acts as the receptor recognizing spike proteins on the surface of SARS-  
72 CoV-2 and similar viruses (SARS-CoV, and NL63), and facilitates virus entry, are infected with the  
73 virus (17,18). This, in turn, switches on a rapid and severe immune response flooding the lungs with  
74 the inflammatory cytokines and factors such as tumor necrosis factors (TNFs), IL-1, IL-6, IL-8, and  
75 IL-12, and many others (16, 19), which affect B cells, neutrophils, basophils, and T cells, sending  
76 additional pro-inflammatory signals to macrophages and amplifying the inflammatory response (19).  
77 In the extrinsic response, the macrophage immune response is induced by the incoming inflammatory  
78 signals from the lung epithelial cells (that also express ACE2 and are infected by the virus),  
79 macrophages in the pulmonary lymph nodes and spleen, or/and immune cells of other infected  
80 organs. The produced cytokines, and chemokines (cytokines which have a chemotactic function) can  
81 also recruit monocytes and additional macrophages to the lungs propagating further inflammatory  
82 response (19). Some studies show that the effectiveness of the currently used anti-inflammatory  
83 therapies for the treatment of various diseases relies not only on the inhibition of cytokine production  
84 but also on the decrease of macrophage infiltration (15).

85

86

### 87 **Molecular mechanisms of Vitamin D effects on the hyperinflammatory response in COVID-19**

88 Recent analyses of COVID-19 patients' data from Germany, UK, US, France, Spain, Italy, China,  
89 and South Korea showed that a severe vitamin D deficiency correlates with a high  
90 (C-Reactive Protein) CRP level in patients with COVID 19 infection (10). As we described in  
91 previous sections, the ARDS is caused by the overdrive of the lung macrophage and other immune  
92 cells (B cells, neutrophils, basophils, and T cells) inflammatory response (19). Here we discuss how  
93 vitamin D may be involved in the modulation/suppression of macrophage response in the COVID-19  
94 patients. Such a suppressing effect of vitamin D on the hyperinflammatory response was already  
95 suggested during the influenza pandemic in 1918-1919 (20).

96

### 97 **Expression and role of vitamin D receptors**

98 The response to and modulation of immune cells activity by vitamin D depends on the vitamin D  
99 receptors expressed by these cells. One of the recently proven functions of VDRs is the prevention of  
100 the immune response of T cells and dendritic cells (4). Mouse studies showed that VDR-KOs have  
101 more pro-inflammatory Th17 effector cells, which produce more IL-17 (4, 21). In contrast, the  
102 upregulation of VDR expression inhibits transcription of the IL-2 gene and prevents the immune

103 system overdrive. Similarly, it has been shown that vitamin D, by promoting the development of  
104 tolerogenic dendritic cells and the suppressive iTregs involved in immune tolerance, prevents  
105 potential over-reaction of the immune system (4, 22). The VDR is also crucial for the integrity of  
106 mitochondria and prevents increased respiratory activity and production of damaging reactive oxygen  
107 species (ROS) that are the important activators of pro-inflammatory signaling in the macrophages  
108 (23-25).

109 Already one hundred years ago, in the pre-antibiotic era, the increase of vitamin D by sun exposure or  
110 fish oil consumptions was used for the treatment of tuberculosis, and the vitamin D supplementation  
111 may still be considered today as a beneficial adjunct to the antibiotic therapy for pulmonary  
112 tuberculosis (26, 27). Recent studies showed that macrophages, including alveolar macrophages that  
113 are crucial for the development of the hyperinflammatory response in the lungs of COVID-19  
114 patients, have an inducible expression of vitamin D 1 $\alpha$ -hydroxylase Cyp27B1 that converts the  
115 inactive form of vitamin D to its active metabolite 1,25 (OH)<sub>2</sub> D that binds macrophage VDRs (28).  
116 Studies also showed that the genetic deletion of macrophage VDRs, which are activated either by the  
117 circulatory or macrophage-produced 1,25 (OH)<sub>2</sub> D, impairs the immune response to cutaneous injury  
118 in mouse wound-healing model (29). Zhang et al. (30) showed that vitamin D treatment increased  
119 the binding of the VDR to the vitamin D response element in the promoter of the mitogen-activated  
120 protein kinase phosphatase-1 (MKP-1) promoter. This caused the upregulation of MKP-1 expression,  
121 and, in turn, inhibited the production of pro-inflammatory IL-6 and TNF- $\alpha$  in the monocytes and  
122 macrophages. It is still unknown if in the response to vitamin D, the VDR receptors, which regulate  
123 gene transcription, become localized in the cell nucleus permanently or if they shuttle between the  
124 nucleus and cytoplasm (31). Studies on the chronic inflammatory lung disease such as cystic fibrosis  
125 showed that vitamin D, acting through its receptors, upregulates transcription of the anti-  
126 inflammatory Dual specificity protein phosphatase 1 (DUSP1) gene, which down-regulates the  
127 expression of inflammatory chemokine IL-8 produced by over-reactive (hyperinflammatory)  
128 macrophages (32). This suggests a therapeutic potential of vitamin D for the treatment of  
129 inflammatory lung diseases. Also, a recent large-scale analysis of COVID-19 patients suggests that  
130 vitamin D activates the innate, and suppresses the adaptive immune response, which, by lowering the  
131 cytokine expression level may downregulate the hyperinflammatory response responsible for  
132 COVID-19 severity and mortality (10, 33-35). In line with these findings, the National Institute of  
133 Health posted on their ClinicalTrials.gov website, several clinical trials, which will assess the  
134 efficacy of vitamin D in the prevention and treatment of COVID-19. A recent studies by Rastogi and  
135 colleagues (36) show that a high dose of vitamin D supplementation by oral administration helped to

136 achieve SARS-CoV-2 RNA negativity along with a significant decrease of the inflammatory markers  
137 (Fig 1).

138

### 139 **Potential functions of myeloid-derived suppressor cells (MDSCs) in COVID-19**

140 The myeloid-derived suppressor cells (MDSCs) exit from the bone marrow as functionally immature  
141 cells. Depending on the signals from the microenvironment they mature into monocytic MDSCs  
142 (mMDSCs) and granulocytic MDSCs (gMDSCs). They suppress the T-cell cycle and immune  
143 checkpoints, downregulate T cell receptors, and recruit Tregs (37). They also suppress the activity of  
144 other immune cells through the production of ROS, RNS, degradation of L-arginine, and the  
145 production of the anti-inflammatory factors, such as (TGF)- $\beta$  and IL-10 (38), (Fig.1).

146 Recent studies indicated that the granulocyte-colony-stimulating factor (G-CSF) granulocyte-  
147 macrophage colony-stimulating factor (GM-CSF), which are the main factors driving recruitment and  
148 differentiation of MDSCs are abundant in the lungs of COVID-19 patients. Recent analyses of  
149 MDSCs in 128 SARS-CoV-2 infected patients, showed a very high frequency of MDSCs, especially  
150 in the intensive care patients. It is very plausible that the immunosuppressive function of MDSCs  
151 prevented virus elimination and increased the severity of the disease (39). This suggests that the  
152 MDSCs may be a valuable target for therapeutic intervention in COVID-19 patients (40). Like other  
153 immune cells, the MDSCs express Vitamin D receptors, and as such can be a target for vitamin D  
154 intervention (41). Studies also show that the level of expression of VDRs correlates with the  
155 immunosuppressive activity of MDSCs and that the active form of vitamin D, 1,25(OH)<sub>2</sub>D, reduces  
156 the suppressive activities of MDSCs by 70%, especially in the early stages of their maturation (41)

157

### 158 **Vitamin D in COVID-19 pediatric patients**

159 It is now well established that children are less frequently infected with SARS-CoV-2 and are more  
160 often either asymptomatic or suffer less severe symptoms than adults (42-49). The immune system of  
161 newborns and very young children is not yet fully developed (50), and their innate immune response  
162 based on monocytes, macrophages, dendritic cells, and neutrophils seem to work differently than in  
163 the adults and is associated with clearly lower cytokine response. For instance, De Wit and coworkers  
164 showed impaired production of IL-12 and IFN- $\alpha$  and an increased synthesis of IL-10 in the neonatal  
165 cord blood after the exposure to TLR-4 and TLR-3 ligands, in comparison to the adult blood, which  
166 may indicate an impaired anti-viral and anti-Gram-negative bacteria response in the neonates (51).

167 This under-responsiveness may protect, in part, SARS-CoV-2 infected children against the  
168 hyperinflammatory response. The immune response of children is also clearly different than in adults  
169 with respect to the production of the antibodies (52). In short, adults produce anti-spike (S) IgG, IgM,



170 and IgA antibodies, and anti-nucleocapsid IgG antibody, while children have much lower levels of  
171 anti-SARS-CoV-2-specific antibodies, and predominantly generate IgG antibodies specific for the S  
172 protein, but not against the nucleocapsid proteins. Moreover, children`s antibodies have much less  
173 pronounced neutralizing activity than the antibodies of adult patients. The authors concluded that  
174 children clear SARS-CoV-2 faster than adults, probably via more efficient and adequate innate  
175 immunological response due to macrophages involvement.

176 Another important point is that in the developed countries the newborns receive vitamin D  
177 supplementation soon after birth, while in the subtropical and tropical countries babies and young  
178 children are exposed to the sunlight, which supplements them with vitamin D naturally. Another  
179 factor may be the presence of other respiratory viruses common in young children, which could  
180 competitively limit the growth of SARS-CoV2 (53). A recent large non-pediatric study reveals the  
181 cross-reactivity between the SARS-CoV-2 antigens and the antibodies presumably originating from  
182 the previous human coronaviruses infections (54). As children get these diseases more often than  
183 adults and possibly had these infections not long before the COVID-19 pandemic, they have  
184 statistically more chances to be protected by this cross-reactivity than adults.

185 In summary, it seems that vitamin D supplementation should have beneficial effects by lessening the  
186 macrophage-dependent hyperinflammatory response in the lungs of COVID-19 patients. This  
187 supplementation is of special interest in the northern hemisphere during the second wave of COVID-  
188 19 pandemic in winter 2020/2021.

189

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195

## 196 **Conflict of interests statement**

197 None of the authors has any conflict of interests

198

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## 374 Figure Legends

### 375 Figure 1. Regulation of transcription and activities of immune cells by vitamin D.

376 A) The hormonal metabolite of vitamin D, the 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D<sub>3</sub>) is the ligand  
377 for the vitamin D receptor (VDR). In the absence of the 1,25(OH)<sub>2</sub>D<sub>3</sub>, the VDR is localized in the  
378 cytoplasm. Interaction of VDR with the 1,25(OH)<sub>2</sub>D<sub>3</sub> causes heterodimerization with the retinoid X  
379 receptor (RXR). This complex translocates to the nucleus where it binds to the vitamin D responsive  
380 element (VDRE) of the vitamin D-responsive genes. Further recruitment of regulatory factors,  
381 dissociation of repressors, histone modification, and chromatin remodeling, induce RNA polymerase  
382 binding and activate transcription of the target gene(s). B) Reciprocal effect between the  
383 macrophages, T cells, and MDSCs. Inflammatory signaling from the macrophages mature and  
384 activate MDSCs, and T cells regulate the activity of MDSCs [55]. The MDSCs may also suppress the  
385 function of T cells and this, in turn, indirectly, may suppresses the activity of other immune cells.  
386 Vitamin D affects transcription and protein expression in macrophages and MDSCs and modulates  
387 the inflammatory response. Although not shown here, the T cells also express VDR, and they are also  
388 directly affected by the vitamin D supplementation.

389

### 390 Figure 2. Hyperinflammatory response induced by alveolar macrophages

391 Arrow 1. The SARS-CoV-2 virus infects, through the ACE2 receptors the alveolar macrophages and  
392 alveolar epithelial cells that induces the production of proinflammatory cytokines by the  
393 macrophages. The infected epithelial cells send the proinflammatory signals to the alveolar  
394 macrophages enhancing macrophage response and sending inflammatory signals to other immune  
395 cells. Arrow 2. The virus also infects the dendritic cells, which also produce proinflammatory  
396 cytokines and chemokines. Arrow 3. All these pro-inflammatory factors recruit monocytes,  
397 granulocytes, and various leukocytes from the circulation. The recruited immune cells produce more  
398 cytokines and chemokines amplifying the proinflammatory response. Arrow 4. Such an overdrive of  
399 the inflammatory response causes the hyperinflammatory response in the lungs and the acute  
400 respiratory distress syndrome (ARDS) in the COVID-19 patients.

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402

## 403 Highlights

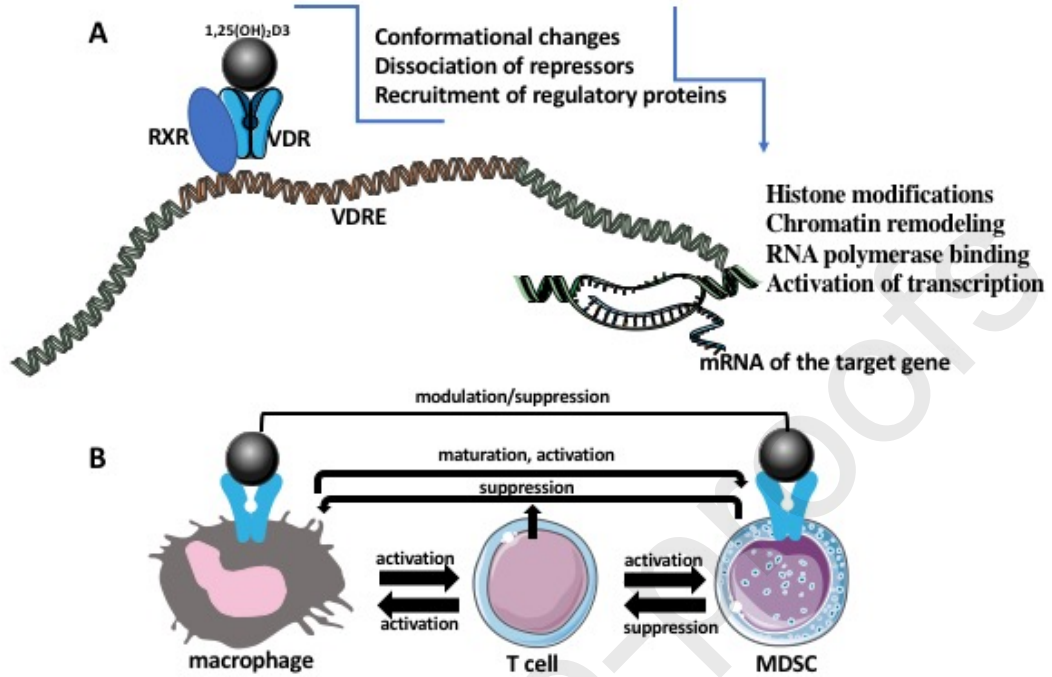
404

405 Vitamin D deficiency increases the severity of the ARDS in COVID-19 infection

406 Vitamin D receptors are expressed in the macrophages and other immune cells

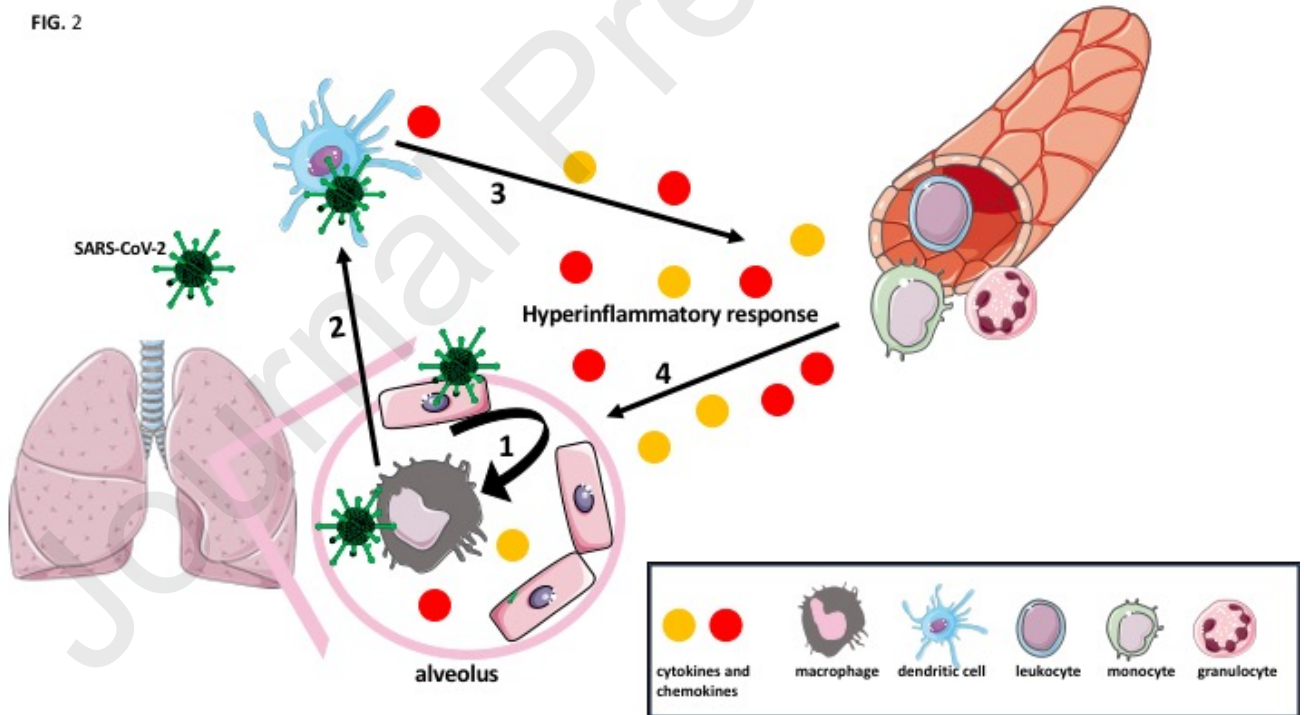
407 Vitamin D supplementation may influence macrophage inflammatory response, subdue the cytokine  
408 storm and lessen the ARDS in COVID-19 patients

FIG. 1



410

FIG. 2



411