VITAMIN D
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Vitamin D, immunity and inflammation: the experience of the SARS-CoV-2 pandemic

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INTRODUCTION

The biological system of vitamin D comprises active metabolites, enzymes and receptors that give rise to genomic and non-genomic effects at a systemic level. In addition to its impact on the health and attributes of the musculoskeletal system, the vitamin D system has been shown to influence numerous physiological functions at a level of the metabolism and the cardiovascular system [1]. For some time now, important effects of vitamin D and its metabolites on the immune system and its dependent inflammatory reactions have also been acknowledged [2]. The recent outbreak of the COVID-19 pandemic has engaged numerous research centres in an attempt to highlight the possible role of vitamin D in relation to susceptibility to infection, clinical expression of the disease and its clinical course. The aim of this brief review is to summarise the state of knowledge on the role of vitamin D in relation to immunity and inflammation, with particular emphasis on what we have learned so far in relation to its impact on the SARS-CoV-2 infection, whereas, due to a lack of space, its impact on autoimmune diseases will not be considered.

VITAMIN D AND NON-SPECIFIC IMMUNE RESPONSE (NATURAL IMMUNITY)

In the course of infection, all cellular elements of innate immunity, primarily macrophages and monocytes, significantly express the CYP27B1 factor, which converts 25(OH)D into $1,25(OH)_2D$: the latter increases the antimicrobial activity of macrophages and monocytes in an autocrine manner through the VDR-RXR signal, which in turn stimulates the production of the antimicrobial agent cathelicidin LL-37. This latter agent acts against invading bacteria and fungi by destabilising their plasma membrane and it exerts direct antiviral activity against many respiratory viruses, destroying their protein coating and altering the viability of target cells (Fig. 1). The macrophage production of cathelicidin LL-37 is such that it can also affect the function of surrounding lymphocytes by leaving the cellular environment [2]. Then, $1,25(OH)_2D$ also modulates the differentiation and function of APCs (*Antigen Presenting Cells*), primarily dendritic cells and macrophages, making them more immature and immune-tolerant, which results in a reduction in antigen presentation and production of the inflammatory interleukin IL-12 and conversely an increase in IL-10 production. Furthermore, $1,25(OH)_2D$ also suppresses the expression of TLRs (*Toll-Like Receptors*) on monocytes and inhibits the production of other inflammatory cytokines, such as IL-2, IL-6 and IL-17. Experimental studies have also suggested that the differentiation of NK (*Natural Killer*) lymphocytes may be modulated by $1,25(OH)_2D$ [3,4].

VITAMIN D AND SPECIFIC IMMUNE RESPONSE (ACQUIRED IMMUNITY)

Once T lymphocytes have been activated, they are also able to express CYP27B1, and therefore the conversion of 25(OH)D into $1,25(OH)_2D$, as well as the vitamin D receptor (VDR). On the other hand, the $1,25(OH)_2D$ produced by monocytes and macrophages is responsible for a clear shift in the system towards a condition of greater immune tolerance, by acting on the proliferation and differentiation of the same T lymphocytes, meaning a reduced formation of T_H1 and T_H17 cells and an increase in the T_H2 cells. This is associated with the reduced expression of pro-inflammatory cytokines and, conversely, with the increased production of antagonistic cytokines[2,5]. Other mechanisms for the modulation of inflammation include inhibition of COX-2 expression and to stimulate the differentiation of regulatory T-cell (*Treg*), either directly or indirectly through interaction with APC cells [6]. In addition, it has been reported that $1,25(OH)_2D$ exerts an anti-oxidative action on monocytes by increasing

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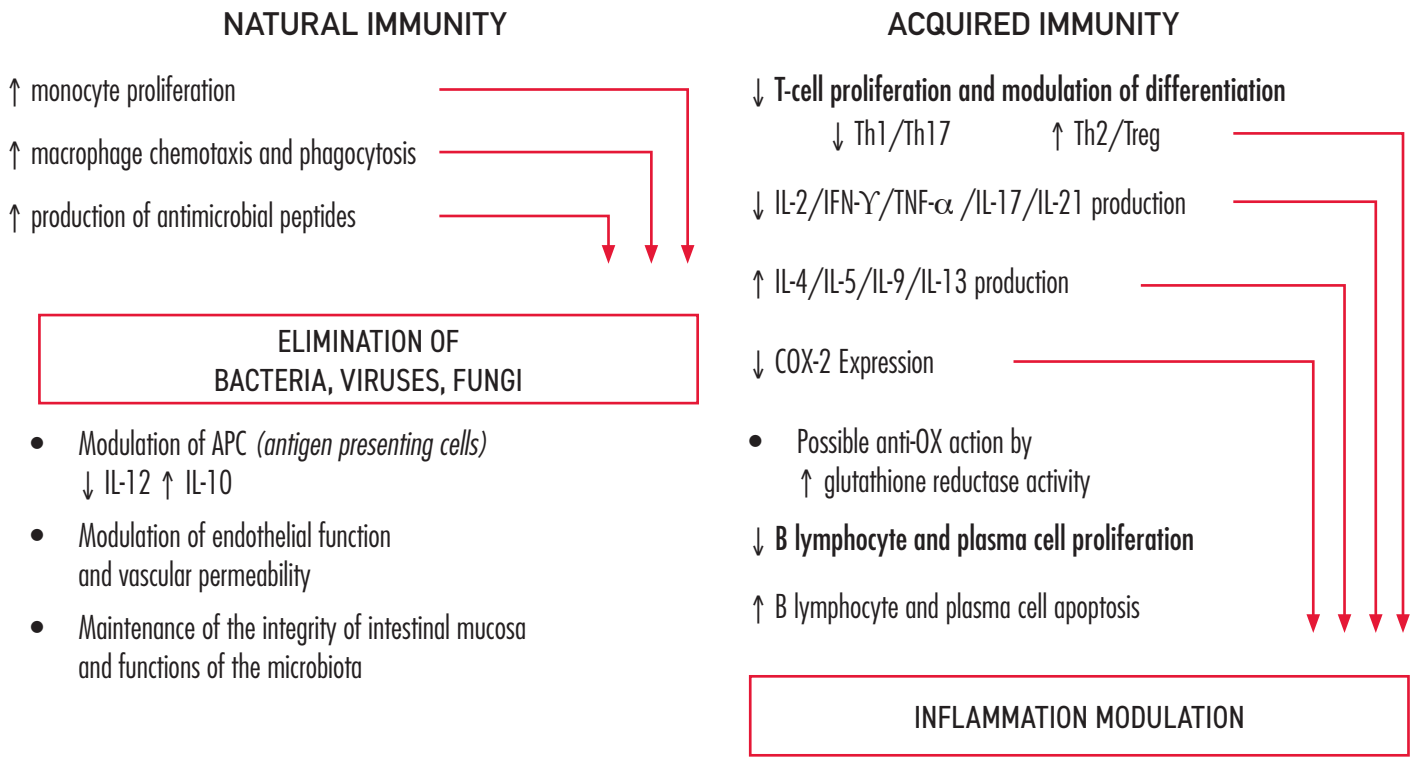

FIGURE 1.
Vitamin D and the immune system. The figure summarises the current state of knowledge regarding the main effects of vitamin D on the immune system.

glutathione reductase activity, resulting in a reduction in the formation of oxygen free radicals [7] (Fig 1).
It has been shown that $1,25(OH)_2D$ modulates the activity of B lymphocytes as well as T lymphocytes. When the system has been activated, $1,25(OH)_2D$ reduces plasma cell formation and induces apoptosis of both activated B lymphocytes and the same plasma cells. Furthermore, it inhibits cytokine-mediated B lymphocyte activation by acting on the T helper lymphocytes, while it directly promotes the production of the anti-inflammatory cytokines IL-10 and CCR10 by B lymphocytes. Finally, $1,25(OH)_2D$ suppresses the differentiation of mature B lymphocytes into plasma cells and immunological memory B cells. It is believed that these actions may reduce the likelihood of autoimmune-type responses often present in conditions of inflammatory response exacerbated by an external agent [8].

VITAMIN D AND INFECTION: CLINICAL FINDINGS

Still common in many developing countries is a latent form of tuberculosis characterised by the formation of a granuloma that confines the mycobacterium in an attempt to control its proliferation. When this containment fails, the patient becomes symptomatic and a diagnosis of active TB can be made [9]. In this condition, vitamin D appears to play an important role in combating the infection by activating macrophages and monocytes as well as by producing cathelicidin. A meta-analysis of seven observational studies showed that the likelihood of contracting TB is significantly higher in the presence of a vitamin D deficiency [10]. Several observational studies have also reported an association between low circulating vitamin D levels and the risk of sepsis, as well as increased morbidity, mortality and ICU stay by septic patients. This relationship could be explained by the modulating effect of $1,25(OH)_2D$ on the over-expression of inflammatory cytokines in the critical patient and also by non-genomic actions on the vascular endothelium oriented toward containing the increase in vascular permeability, an important factor in the pathogenesis of septic shock. On the other hand, a reverse-causality mechanism could also be possible, whereby the low levels of circulating vitamin D in sepsis could instead be explained by extravascular translocation of vitamin D binding protein and an increase in 25(OH)D-24 hydroxylase activity in relation to systemic inflammation. Unfortunately, controlled clinical trials in sepsis have provided mixed results, although most studies have found positive effects on length of stay in the ICU and inpatient mortality [11].
Many studies bear out an independent association between low vitamin D levels and the incidence or severity of respiratory tract infections in children and adults. Respiratory viruses penetrate epithelium of the airways and cause cell and tissue damage, stimulating the immune response resulting in inflammation of the respiratory tract and, in severe cases, even acute respiratory distress syndrome. $1,25(OH)_2D$ exerts antiviral activity, fostering the production of antimicrobial agents such as cathelicidin, modulating the expression of toll-like receptors in lymphocytes, along with NK cell function, and controlling the over-expression of pro-inflammatory cytokines. A recent meta-analysis of 25 randomised controlled trials showed that

vitamin D_2 and D_3 supplementation can provide significant protection against the development of acute respiratory tract infections compared to placebo[12].

VITAMIN D AND SARS-CoV-2 INFECTION

The key element of pulmonary involvement in virus-induced airway disease is intense inflammatory reaction. Although pro-inflammatory cytokine production is an important factor in the response to infection, an intense and prolonged inflam-matory response will cause tissue damage and, in severe cases, may lead to acute respiratory distress syndrome, contributing to fatal outcomes. This sequence of events has been well documented for SARS-CoV-2 infection, leading to the use of suitable drugs to quell the cytokine storm and reduce the level of inflammation in severe cases [13]. The established impact of vitamin D on the immune response in various respiratory tract diseases, such as tuberculosis, influenza and other viral conditions, provides support for the significant role that vitamin D can also play in the immune response to SARS-CoV-2 infection, where an excessive inflammatory response has been deemed responsible for severe and sometimes irreversible lung, heart, kidney and liver damage in the course of the disease [14]. Furthermore, SARS-CoV-2 binds to the ACE2 (angiotensin-converting enzyme 2) receptor on the surface of the epithelial cells of the respiratory mucosa, the alveolar cells of the lung, vascular endothelial cells and macrophages [15]. Coronavirus infection depresses the expression of the ACE2 receptor, thereby inducing a multi-organ accumulation of angiotensin II, which increases the cytokine storm [16]. Vice versa, vitamin D promotes the expression of the ACE2 receptor gene and reduces the expression of the REN gene, thereby modulating, through inhibition, the overall activity of the renin-angiotensin system [17]. Lastly, an additional important pathogenetic element is given by activation of the haemocoagulative cascade with increased levels of D-dimer and fibrinogen in the circulation and diffuse thromboembolic phenomena [18]. A central mechanism of thrombotic events is the generation of thrombin mediated by the massive release of Tissue Factor (TF) secondary to inflammatory vascular damage. In this regard, vitamin D metabolites have been shown to regulate the haemocoagulative cascade by reducing TF expression and activity and, conversely, increasing the expression of thrombomodulin (TM) [19].

Unfortunately, the results of clinical and epidemiological research have unam-biguously supplemented these findings from studies in clinical pathology. One ecological study conducted in 20 European countries showed an inverse correlation between the average level of circulating vitamin D in each country and the respective incidence of COVID-19, as well as between the average level of vitamin D and the number of deaths from COVID-19 [20].

Two systematic reviews and related meta-analyses of observational studies on the relationship between vitamin D and SARS-CoV-2 infection have also been published:

TABLE I. Review of clinical trials on vitamin D and SARS-CoV-2 infection.

Author	Study type	Key Findings
Ilie et al. Aging Clin Exp Res 2020	Ecological	In a comparison among European countries: a) inverse correlation between mean level of circulating vitamin D and number of cases of COVID-19 per million inhabitants (r = -0.44; p = 0.050); b) inverse correlation between mean level of circulating vitamin D and number of deaths from COVID-19 (r = -0.43; p = 0.050)
Butler-Laporte et al. PLoS Med 2021	Mendelian randomisation	In a Mendelian randomisation study based on more than 14,000 cases of COVID-19 and approximately 1,300,000 participants without the disease, genetic predisposition to higher *vs* lower levels of 25(OH)D was not found to be associated with disease risk (OR = 0.95; 95% CI: 0.84, 1.08), hospitalisation (OR = 1.09; CI 95: 0.89, 1.33; p = 0.41) or severe disease (OR = 0.97; 95% CI: 0.77, 1.22; p = 0.77)
Petrelli et al. J Steroid Biochem Mol Biol 2020	Systematic review and meta-analysis of observational studies	Includes 43 cross-sectional, case-control and cohort studies (retrospective or prospective), with over 600,000 patients in total: suggests that vitamin D deficiency is associated with greater severity of COVID-19 disease (OR = 2.6; 95% CI: 1.84-3.67; p < 0.01- and higher mortality (OR = 1.22; 95% CI: 1.04-1.43; p < 0.01) compared to normal levels. Low study quality on average, high heterogeneity and high level of bias with regard to patient selection criteria, threshold levels used and confounding factors
Bassetne et al. Metab Clin Exp 2021	Systematic review and meta-analysis of observational studies	Compared to the other systematic review, only 31 studies were considered because they were published in peer-reviewed journals. They also found a trend towards higher mortality and higher risk of admission to intensive care and need for assisted ventilation for patients with 25(OH)D levels < 20 ng/mL compared to those with higher levels. However, this trend did not reach statistical significance, partly due to the smaller number of studies available for each type of outcome and, moreover, the quality of the studies was generally low and the level of heterogeneity conspicuous with a high risk of bias
Entrenas Castillo et al. J Steroid Biochem Mol Biol 2020	Clinical trial	Among 76 patients hospitalised for COVID-19 (of whom only 50 had received treatment including calcifediol), only 1 calcifediol-treated patient required intensive care compared with half of the untreated patients (p < 0.001)
Rastogi et al. J Postgrad Med 2020	Clinical trial	In a trial of COVID-19 patients with 25(OH)D levels < 20 ng/mL, randomised to treatment with cholecalciferol (n = 16) or placebo (n = 24), after 2 weeks 62.5% of treated patients were SARS-CoV-2 negative *vs* 20.8% of controls. In the first Group there was a reduction in fibrinogen but not in serum CRP, procalcitonin, ferritin or D-dimer
Murai et al. JAMA 2021	Clinical trial	In a trial of 240 COVID-19 patients, half randomised to a single oral dose of 200,000 IU vitamin D_3 and half to placebo, no differences were found in length of hospital stay, use of intensive care and assisted ventilation or mortality

both included cross-sectional observations, case-control comparisons and retrospective or prospective cohort studies. The meta-analysis by Petrelli et al. included 43 studies with a total exceeding 600,000 patients. It suggested that vitamin D deficiency is associated with greater severity of COVID-19 disease (OR = 2.6; 95% CI: 1.84-3.67; $p < 0.01$) and higher mortality (OR = 1.22; 95% CI: 1.04-1.43; $p < 0.01$) compared with normal levels [21]. The second analysis, by Bassatne et al., considered only 31 studies published in peer-reviewed journals. They found a trend towards higher mortality and higher risk of admission to intensive care and need for assisted ventilation for patients with 25(OH)D levels < 20 ng/mL compared to those with higher levels. However, this trend did not reach statistical significance, partly because of the lower number of studies available for each type of outcome: the quality of the studies was generally low whilst the level of heterogeneity in the analysis was very high [22]. Still within the scope of observational studies, added recently was a Mendelian randomisation study, which, by contrasting subjects with a genetic predisposition to lower or higher plasma levels of 25(OH)D, found no differences in susceptibility to infection by SARS-CoV-2, the need for hospitalisation or in disease severity [23]. An important limitation of this research was however, that it did not include subjects with 25(OH)D levels < 20 ng/mL.

At the moment, there are three current clinical trials that have been completed and are available. In the first, conducted on 76 patients hospitalised for COVID-19, of whom 50 were treated with calcifediol and 26 were used as controls, only 1 treated patient out of the 50 required hospitalisation in intensive care compared with 50% of the untreated patients [24]. In the second small trial, in India, on COVID-19 patients with 25(OH)D levels < 20 ng/mL, randomised to treatment with chole-calciferol (n = 16) or placebo (n = 24), after about two weeks, two-thirds of treated participants were SARS-CoV-2 negative *vs* about one-fifth of controls. Furthermore, plasma levels of fibrinogen (but not CRP, procalcitonin, ferritin or D-dimer) were reduced in the treatment Group [25]. In a trial of 240 COVID-19 patients, half randomised to a single oral dose of 200,000 IU vitamin D_3 and half to placebo, no differences were found in the various clinical outcomes analysed [26]. All three of these trials so far published present a risk of significant bias linked mainly to patient selection and randomisation methods.

In conclusion, the findings provided by the experimental studies on the physiological role of vitamin D in regulating the functions of the immune system are extensive and robust. In addition, the benefit of maintaining adequate levels of vitamin D in the prevention of acute respiratory tract infections seems to have been clinically and epidemiologically established. With regards to patients with SARS CoV-2 infections, there are copious indications that vitamin D may exert effective protective action through modulation of the immunological response, attenuation of the cytokine storm and the inflammatory response, preservation of the integrity of the pulmonary epithelial barrier and through its antithrombotic action in turn related to the anti-inflammatory action and modulation of the renin-angiotensin system. Nevertheless, conclusive findings on the effects of supplementation in COVID-19 patients are not yet available. This is because, although they tend to show a favourable effect overall, results from the several observational studies and the few clinical trials available today have not been unambiguous. The discrepancies among the different studies can be explained by the contributions of several factors: the small size of many of the studies conducted, the heterogeneity in patient selection and in disease stage, differences in the cut-off used to define vitamin D deficiency or in the doses and methods of its administration, the possibility that the level of circulating 25(OH)D did not reflect the true bioavailability of the vitamin and its metabolites in the body and, again, the possibility of broad variability among individuals in their response to supplementation due to genetic and/or acquired factors (e.g. presence of obesity).

Hence, clearly, we need to await the results of additional ongoing trials [22], some of which are notably large.

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