BRIEF COMMUNICATION

Vitamin D can reduce severity in COVID-19 through regulation of PD-L1

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Abstract

COVID-19 is a highly contagious viral infection that has killed millions of people around the world. The most important diagnostic feature of COVID-19 is lymphocyte depletion, particularly the depletion of T cells. In COVID-19 infections, there is a link between destruction of T cells and increased expression of inhibitory immune checkpoint molecules (PD-1/PD-L1) on T cell surfaces. It was shown that PD-1/PD-L1 levels increase in severely COVID-19 infected individuals. Higher proinflammatory cytokine levels cause increased PD-1/PD-L1 expression. In severe COVID-19, higher proinflammatory cytokine levels may increase PD-1/PD-L1. Vitamin-D is an important immune regulator. It is known that the numbers of $CD4^+$ and $CD8^+$ T lymphocytes decrease in vitamin D deficiency while vitamin D supplementation increases CD + 4 lymphocytes. Vitamin D can increase regulatory T cell (Treg) activity. Vitamin D also has a diminishing effect on proinflammatory cytokines. In severe COVID-19 cases, vitamin D supplementation may inhibit the increase of PD-L1 expression through reducing proinflammatory cytokine levels. Thus, vitamin D supplementation could eliminate the suppressive effect of PD-L1 on $CD4^+$ and $CD8^+$ T cells, preventing lymphopenia and reducing disease severity and mortality in patients infected with COVID-19. Besides, vitamin D supplementation can reduce inflammation by increasing Treg activity. The aim of this letter is to discuss the functions of inhibitory immune checkpoint molecules and their effects on dysfunction and depletion of T-cells as well as to explain the possible modulatory effect of vitamin D on these checkpoints and T cells.

Keywords $CD4^+$ · $CD8^+$ · Regulatory T cell · COVID-19 · PD-1/PD-L1 · T lymphocyte · Vitamin D

To the Editor,

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has left the health systems of all countries in strain and killed millions of people. COVID-19 presents with a range of signs and symptoms varying from mild illness to acute pneumonia. Acute respiratory distress syndrome (ARDS) is the most common complication of COVID-19. Although numerous vaccines have been developed to provide protection against COVID-19 and many antiviral drugs have been used for the treatment of COVID-19 infected patients, the pandemic still prevails across the world.

Recently, there have been many studies indicating that vitamin D can reduce the severity of COVID-19 and deaths caused by COVID-19. It was shown in the present study that vitamin D can reduce the severity of COVID-19 via the PD-1/PD-L1 signaling pathway. Our hypothesis on the involved mechanism is explained under the following headings.

COVID-19 and T cells (CD4 + T, CD8 + T, and regulatory T [Treg])

Understanding the link between the immune system of patients infected with COVID-19 and disease severity could play a crucial role in the fight against this pandemic. The adaptive immune system, governed by T and B lymphocytes, has fundamental functions in alleviating the severity of COVID-19 (Qin et al. 2020a, b). $CD4^+$ T cells facilitate virus-specific antibody production through T-dependent activation of B cells (Xu and Gao 2004). $CD4^+$ T cells regulate the suppression and proliferation of immune cells, particularly $CD8^+$ T cells, in regulating immune responses

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(Xu and Gao 2004). CD8$^+$ cytotoxic T cells, on the other hand, play a vital role in viral clearance by secreting a series of molecules such as granzymes (A, B), perforin, and IFN-y to destroy viruses from the host (Mescher et al. 2006). In addition, another important component of the adaptive immune system is regulatory T cells (Treg) (Sakaguchi et al. 2010). In general, Tregs suppress CD4 + and CD8 + T-cell responses and reduce the infiltration of natural killer (NK) cells, eosinophils and neutrophils, providing the first line of defense against uncontrolled inflammation and viral infections (Littwitz-Salomon et al. 2018; Quillien et al. 2019; Weir et al. 2020). Tregs play a critical role in maintaining immune homeostasis and limiting immunopathology (Wang et al. 2021).

In more severe cases, COVID-19 is characterized by lymphopenia, which usually results from increased neutrophil cell and decreased T lymphocyte counts (Diao et al. 2020; Tan et al. 2020a, b; Chen et al. 2020a, b; Xiong et al. 2020; Ricci et al. 2021). Compared to milder ones, severe cases were reported to have advanced lymphopenia more frequently in T cells along with higher C-reactive protein (CRP) and markedly elevated levels of interleukin (IL)-2R, IL-6, IL-10, and TNF-α levels (Chen et al. 2020a, b). It was reported that the numbers of T lymphocytes, CD4$^+$ T cells, and CD8$^+$ T cells decrease in almost all patients, and these decreases were reported to be more prominent in severe cases compared to moderate ones (Chen et al. 2020a, b). Lymphocytopenia in COVID-19 patients is mainly manifested by reductions in CD4$^+$ T lymphocyte count and CD4$^+$/ CD8$^+$ ratio. It was reported that decreased CD4$^+$ T lymphocyte count and elevated TNF-α and IL-6 levels were correlated with the severity of COVID-19 (Sun et al. 2020). It was observed that both CD4$^+$ T-helper and CD8$^+$ T-cytotoxic lymphocytes could be affected and that the decreases in the number of CD4$^+$ T cells were more prominent when the severity of the disease was higher (Ricci et al. 2021). Thus, decreases in the number of CD4$^+$ and CD8$^+$ T lymphocytes could be used to determine the severity of the disease. In addition, Tregs may play an important role in regulating immune responses to COVID-19. It was revealed that Treg levels were considerably low in COVID-19 patients, especially in severe cases, compared to normal (Qin et al. 2020a, b; Wang et al. 2020). In COVID-19 patients with acute respiratory distress syndrome (ARDS), prolonged hospitalization characterized by excessive systemic inflammation (cytokine storm) and delayed lung repair due to partially reduced or defective Tregs could take place (Blanco-Melo et al. 2020). Chen et al. (2020a, b) demonstrated that CD45RA + Treg expression levels were decreased in both moderate and severe COVID-19 patients. The CD45RA + Treg level was found to be significantly lower in severe cases than in moderate ones (0.5 vs. 1.1%, respectively) (Chen et al. 2020a, b). In an interesting study, on the other hand, it was observed that IL-6, IL-8,

IL-12, TNF-α, and IFNγ levels decreased after allogeneic Treg infusion in two critically ill COVID-19 patients (Gladstone et al. 2020).

However, contrasting findings regarding the changes in Treg levels were also reported in COVID-19 patients. Neumann et al. (2020) found that Treg levels increased in moderate to severe COVID-19 patients and that IL-10-producing Treg levels were particularly high in severe COVID-19 patients. Tan et al. reported that Treg levels increased considerably in mild cases but moderately in severe cases, indicating immunosuppression in COVID-19 patients (Tan et al. 2020a, b). Another study reported that aTreg levels were 4.4 times higher than that of healthy controls on day 7, peaking on day 22 and decreasing on day 28 (Yang et al. 2020). Studies on whether increasing or decreasing Treg levels increase the severity of COVID-19 is controversial.

PD-1/PD-L1 signaling and immune cell tolerance

Inhibitory immune checkpoint molecules expressed on the surface of T cells, B cells, dendritic cells, and natural killer T cells [programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1)] play important roles in innate and, especially, adaptive immune response (Chen et al. 2016). PD-1 was identified as a negative immune regulator that limits T and B cell function (Fife and Bluestone 2008). Under normal conditions, PD-1 expression creates an activation threshold that must be exceeded before an immune response can be initiated (Fife and Bluestone 2008; Riley 2009). Therefore, PD-1 is considered to be a potent modulator of the immune system. PD-L1 maintains immune cell tolerance through binding to PD-1 receptor (Boussiotis 2016). High expression of PD-L1 prevents proliferation and differentiation of T cells whereas knockdown of PD-L1 leads to autoimmunity (Nishimura et al. 2000; Probst et al. 2005). PD-1 deficiency leads to species-specific autoimmunity that occurs much later in life (Okazaki and Honjo 2006). Disruption of the gene encoding PD-1 in BALB/c mice results in autoimmune cardiomyopathy, while C57BL/6 PD-1-knockout (KO) mice develop progressive arthritis and a lupus-like glomerulonephritis, and NOD-PD-1-KO mice develop autoimmune diabetes (Nishimura et al. 1999; Nishimura et al. 2001; Wang et al. 2005). Thus, PD-1 plays a vital role in maintaining immune homeostasis and limiting immune responses. Therefore, a balanced PD-1/PD-L1 pathway is crucial (Fig. 1).

PD-1/PD-L1 signaling and COVID-19

Binding of PD-1 to its ligand PD-L1 transmits a signal that inhibits T cell proliferation, cytokine production, and cytolytic function. The balance between positive
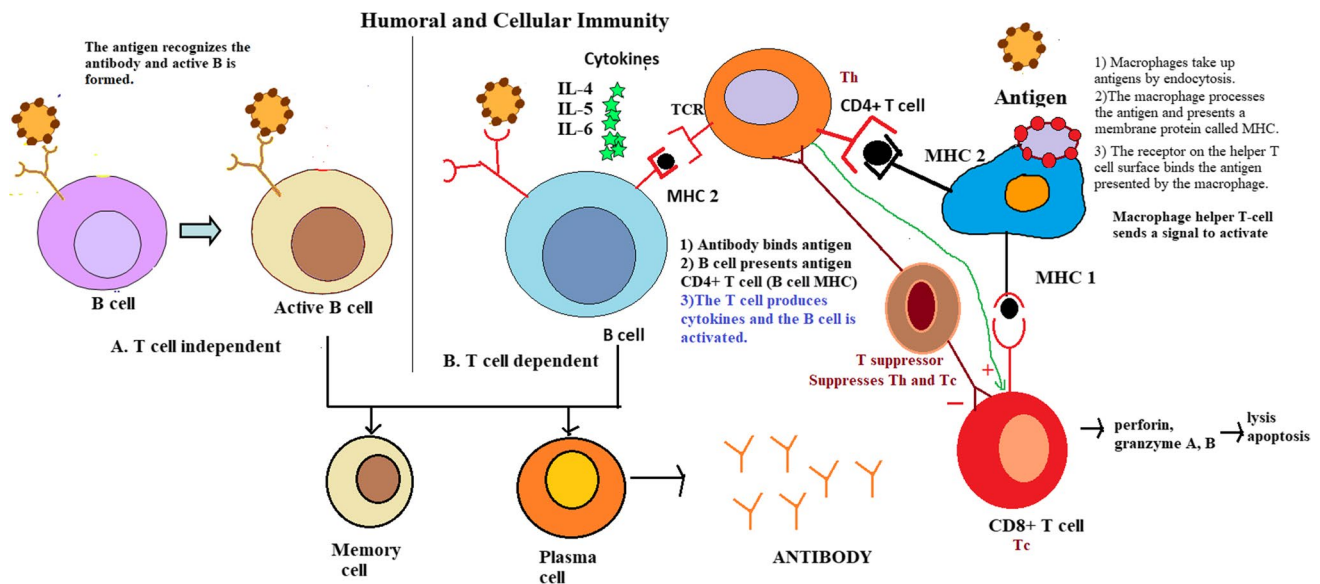
Fig. 1 Humoral and cellular immunity

and negative signals transmitted to T cells by PD-1 and PD-L1 is critical for cellular immune responses (Riley 2009). It was reported in many studies that in severe COVID-19 cases PD-1/PD-L1 pathway is disrupted and the expression of these inhibitory immune checkpoint molecules increases (Zheng et al. 2020a, b; Diao et al. 2020; Sabbatino et al. 2021). Increased PD-1 expressions were reported to be directly related to severity of the disease (Diao et al. 2020). Since PD-1/PD-L1 blockade provides immunological control of viral infections, it can theoretically heal COVID-19 patients (Barber et al. 2006; Koralnik 2019; Zheng et al. 2020a, b). On the other hand, PD-1/PD-L1 blockade could theoretically increase the hyperactive immune phase of COVID-19 and worsen the outcomes (Moore and June 2020). Recently, PD-L1 inhibitors have been shown to be promising therapeutic agents in the treatment of a wide variety of cancer types. PD-L1 antibody induces tumor regression by reducing immunosuppressive PD-1/PD-L1 signaling to achieve maximum immune activation (Luo et al. 2020a). However, it was observed that PD-1/PD-L1 blockade treatment did not have an extra positive effect on recovery in cancer patients infected with COVID-19 (Damato et al. 2020). Another similar study showed that PD-1 blockade did not affect the severity of COVID-19 in patients with lung cancer (Luo et al. 2020b). In the same study, after adjusting for smoking status, PD-1 blockade reduced hospitalization, intensive care unit (ICU)/intubation, and death rates. These findings may be promising for the safety of use of PD-1 blocking therapy during the COVID-19 pandemic. In addition, it can be concluded

that PD-1 blockade does not worsen but may improve, or do not change, the condition of patients with COVID-19.

PD-1/PD-L1 signaling, T cell, and COVID-19

A study by Diao et al. on COVID-19 patients reported that PD-1 levels in T cells were considerably higher; that the total number of T cells, CD4^{+} and CD8^{+} T cells was particularly low in patients requiring intensive care; and that serum IL-6, IL-10, and TNF-a concentrations were higher. On the other hand, number of T cells increased and IL-6, IL-10, and TNF-a concentrations decreased in recovering patients (Diao et al. 2020). In a very recent study, high PD-L1 levels have been found to be associated with lymphopenia, elevated CRP levels, and increased mortality in COVID-19 patients (Sabbatino et al. 2021). Potential importance of PD-L1 as a biomarker for the prognosis of COVID-19 was reported (Sabbatino et al. 2021). It was also shown that PD-L1 expression increased in both monocytes and dendritic cells of patients with more severe condition (Parackova et al. 2020). PD-1 is more commonly observed on the surface of CD8^{+} and CD4^{+} T cells. PD-L1 inhibits functioning of T cells, and this inhibitory function is achieved by binding of PD-1 to its ligands (PD-L1) expressed on the surface of peripheral tissues (Keir et al. 2008). In a recent study, it has been reported that the binding of PD-L1 to PD-1 expressed on the surface of CD8^{+} T-cells may inhibit the antiviral activities of CD8^{+} cells and may ultimately lead to disease progression (Aghbash et al. 2021). While the theory put forward by that study

seem plausible for severe COVID-19 cases, it may not be viable for cases with mild disease severity because in mild cases the proportions of CD8^{+} T cells were reported to be high. However, during acute infections, PD-1 molecules were shown to be expressed on the surface of CD8^{+} T cells but did not lead to loss of function (Peng et al. 2020; Westmeier et al. 2020). Elevated proinflammatory cytokines during COVID-19 infection increase the expression of PD-1 on the surface of T cells, resulting in a decrease in CD4^{+} lymphocytes.

After COVID-19 infection, the negative regulatory mechanism mediated by Tregs is activated. However, in critically ill patients, excessive and prolonged inflammatory responses eventually lead to lymphocyte apoptosis and even lymphocyte failure in the late stage of infection (Tan et al. 2020a, b). During COVID-19 infection, there is a positive correlation between destruction of T cells and increased expression of inhibitory immune checkpoint molecules on their surface (Zheng, et al. 2020a, b). Programmed death 1 (PD-1) is selectively expressed on the surface of Tregs. During chronic viral infection, PD-1 expression is upregulated on the surface of Treg cells (Park et al. 2015). The upregulation of PD-1 on Treg cells and its interaction with the PD-1 ligand (PD-L1) on effector T cells results in potent T cell suppression and depletion. For this reason, agents that keep the PD-1, PD-L1 pathway in balance may play an important role in coping with COVID-19 infection.

COVID-19 and vitamin D

Recently, a new era has started for the immunomodulatory role of vitamin D in autoimmunity. Vitamin D plays an important role in both adaptive and innate immunity. Vitamin D is also known to increase the expression of two antimicrobial peptides called cathelicidin and β-defensins and to play a key role in innate immunity (Aygun 2020a). Vitamin D exerts its effect through the vitamin D receptor. This receptor is widely found in the body and is abundantly expressed in the immune system. The vitamin D receptor acts as a modulator of innate and adaptive immunity. Several recent studies reported that patients with low vitamin D levels are at high risk of contracting COVID-19 infection, that they are more likely to be PCR positive, and that they have poor prognosis for the disease and have higher mortality rates (Carpagnano et al. 2021; Demir et al. 2021). On the other hand, patients who received vitamin D supplementation and whose serum 25(OH)D levels reached ≥ 30 ng/mL were reported to have a lower risk of COVID-19 infection, COVID-19 severity and mortality compared to 25(OH)D-deficient patients who did not receive vitamin D supplementation (Oristrell et al. 2021). Carpagnano et al. (2021) reported that while the probability of death was 50%

in COVID patients with severe vitamin D deficiency, this probability falls to 5% in patients with vitamin D levels of ≥ 10 ng/mL. In general, the vitamin D deficiency status appears to be associated with an increased risk of COVID-19. Vitamin D deficiency is more common in type 2 diabetes patients, obese individuals, people with hypertension, cancer patients, individuals with cardiovascular disease, and the elderly. All of these are demographic parameters associated with increased risk of serious COVID-19 and deaths from COVID-19 (Rhodes et al. 2021; Aygun 2020b). However, it should be kept in mind that healthier people spend more time outdoors compared to less healthy individuals and can benefit more from ultraviolet light (UVB) rays and eat healthier, so they may experience relatively less vitamin D deficiency (Bergman 2021). When mortality per million is plotted against the latitude, it could be noticed that all countries located below the 35° north latitude have a relatively low mortality rate (Rhodes et al. 2021). After adjusting for age, Rhodes et al. found a 4.4% increase in COVID-19 mortality for each degree of latitude north of 28°, which was indirectly associated with a person's vitamin D status as a result of UVB exposure (Rhodes et al. 2020). To understand the potential impact of seasonal UV and temperature levels on COVID-19 cases, a large-scale study analyzing meteorological data and daily COVID-19 cases per million in a population of 26 European countries was carried out (Mukherjee et al. 2022). The study found that low UV exposure can affect the body's production of essential vitamin D, which is linked with the transmission dynamics and severity of COVID-19. Although Scandinavian countries are located in northern latitudes, vitamin D deficiency is rare among their citizens possibly due to the widespread use of supplements, and COVID-19 related mortality is relatively low (Ips et al. 2019; Rhodes et al. 2021). This suggests that vitamin D deficiency has a potential role in COVID-19 infection.

COVID-19, PD-1/PD-L1 signaling, and vitamin D

There are several pathophysiological mechanisms that could explain the benefits of vitamin D against COVID-19 or against its adverse outcomes. The most important of these mechanisms is the modulatory effect exerted by vitamin D especially on the immune system. Vitamin D modulates the adaptive immune response via T lymphocytes. Several studies reported association of vitamin D deficiency with low CD4^{+} T cell counts. Coelho et al. (2015) showed that 88% of individuals with vitamin D deficiency had low CD4^{+} count and that calcidiol (25(OH) D) supplementation increased number of CD4^{+} cells. Similarly, another study reported a significant increase in CD4^{+} count and a decrease in viral load after vitamin D supplementation (Prietl et al. 2013;

Stallings et al. 2015; Dougherty et al. 2014). A recent study reported that patients with very low plasma vitamin D levels had a low CD4$^+$/CD8$^+$ ratio, decreased CD8$^+$ T lymphocytes, high thoracic CT scan involvement, and that these patients had higher risk (Ricci et al. 2021). It is still unclear why CD4$^+$, CD8$^+$ T lymphocytes decrease in vitamin D deficiency and how these lymphocytes increase with vitamin D supplementation. However, in a recent study, Morita et al. (2021) reported that vitamin D supplementation may have a bimodal function of increasing serum PD-L1 when serum PD-L1 levels are very low and decreasing serum PD-L1 levels when serum PD-L1 levels are very high. Presumably, vitamin D could increase the number of T lymphocytes by eliminating the suppressive effect of the PD-1 PD-L1 pathway on T lymphocytes. In severe COVID-19 patients, vitamin D supplementation may help to achieve adequate immune response reducing the elevated PD-L1 level and removing the suppression of PD-L1 on CD4$^+$ and CD8$^+$ T cells, thereby reducing the severity of the disease and reducing mortality (Fig. 2).

It is well known that the expressions of PD-L1 and PD-1 are regulated by various signaling pathways activated by pro-inflammatory cytokines (Chen et al. 2016). It was reported that PD-L1 expression increases especially in response to proinflammatory cytokines such as IFNy and IL-4 (Sharpe et al. 2007). In patients infected with COVID-19, increases were reported in interleukin (IL)-1, IL-1B, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, interferon-γ inducible protein 10 (IP-10), granulocyte colony stimulating factor (GCSF), tumor necrosis factor-a (TNF-a), macrophage inflammatory protein 1-a (MIP1a), monocyte chemoattractant protein 1 (MCP-1), and IFN-y concentration (Liu et al. 2020; Huang et al. 2020; Aygun 2020a). Many studies indicated that vitamin D reduces inflammation by reducing proinflammatory cytokines such as IL-6, IL-8, IL-12 and IL 17, nuclear factor-kB (NF-kB), TNFα gamma interferon (IFN-y), and IL-2, thereby lowering oxidative stress and eliminating cell damage in COVID-19 infected patients. Vitamin D supplementation may decrease the PD-L1 expression by lowering proinflammatory cytokine levels. In this case, thanks to its stabilizing effect on PD-L1, vitamin D can

reduce the severity of the disease by preventing the suppressive effect of PD-L1 on the immune system.

It is known that especially elderly patients are more affected by COVID-19 (Mueller et al. 2020; Leung 2020). Comorbidities such as diabetes, cardiovascular disease, and obesity are considered as the primary cause of more severe course in COVID-19, but these comorbidities alone do not explain why age is such a strong risk factor. Aging could be mediated by the changes in the immune system that particularly affect the three pillars of adaptive immunity, namely, CD4$^+$ T cells, CD8$^+$ T, and B cells (Sette and Crotty 2021). PD-L1 levels were reported to increase in an age-related manner (Chen et al. 2011; Cubillos-Zapata et al. 2019). Increased serum PD-L1 and decreased T lymphocyte count caused by COVID-19 infection could explain why elderly patients are more affected by COVID-19. Vitamin D supplementation can prevent the increase in PD-L1 expression especially in the elderly. As a result, the suppressive effect of PD-L1 in T lymphocytes could be eliminated and appropriate immune response may develop. Thus, the mortality and hospitalization rates and severity of the disease may decrease in elderly patients infected with COVID-19.

In some studies, it was reported that Treg expression is decreased in COVID-19 patients (Qin et al. 2020a, b; Wang et al. 2020; Chen et al. 2020a, b; Blanco-Melo et al. 2020). Thanks to its immunomodulatory properties, vitamin D has the capacity to specifically inhibit effector T cell responses and to stimulate the responses of Tregs (Chambers and Hawrylowicz 2011). Besides, vitamin D synthesizes anti-inflammatory mediators such as FoxP3 + and IL-10 + Tregs and transforming growth factor (TGF)-β, and exerts inflammatory effect through stimulating FoxP3 (Unger et al. 2009; Urry et al. 2009; Chambers and Hawrylowicz 2011). In addition, vitamin D supplementation can induce the proliferation of Tregs, thereby reducing inflammation and controlling ARDS associated with SARS-CoV-2.

At the same time, the stimulation of Tregs by vitamin D supplementation may increase PD-1 expression on the Treg cell surface (Park et al. 2015) and cause T cell depletion when PD-1 binds to its ligand in the effector cell (PD-L1). However, in this case, the severity of COVID-19
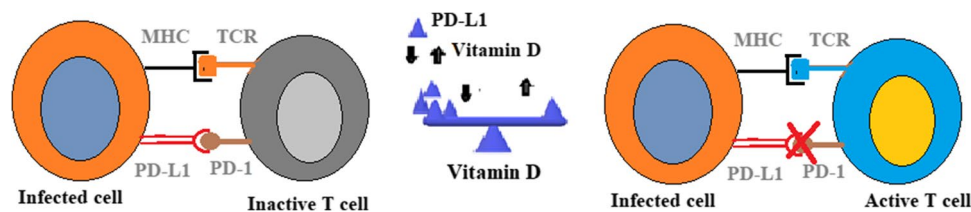

Fig. 2 Upon binding of PD-1 PD-L1, T cells become inactive and undergo apoptosis over time, and then immunosuppression develops. Vitamin D keeps PD-L1 levels in balance. Thus, the suppressive effect of PD-L1 on the immune system disappears and it shows an immune modulatory effect

infection could increase and the prognosis of the disease could worsen. Nevertheless, in many studies conducted so far, it has been reported that vitamin D supplementation reduces the death rate in patients with COVID-19, shortens the length of hospital stay, and suppresses inflammation (Murai et al. 2021; Carpagnano et al. 2021). In addition, a PUBMED survey revealed no studies indicating that the prognosis of COVID-19 worsens after vitamin D supplementation. It is possible that vitamin D could result in inhibition of inflammatory responses and reduction of COVID-19 severity through the induction of Tregs.

In some studies, it was reported that the expression of Tregs is increased in severe COVID-19 patients (Neumann et al. 2020; Tan et al. 2020a, b; Yang et al. 2020). It is known that activation of the PD-1/PD-L1 pathway is increased in patients with severe COVID-19 (Zheng et al. 2020a, b; Diao et al. 2020; Sabbatino et al. 2021). Activation of the PD-1/PD-L1 pathway induces T-cell depletion and enhances the function of Tregs. Therefore, expression of Tregs may be increased in patients infected with COVID-19. Vitamin D supplementation can reduce the severity of the disease by inhibiting the excessive PD-1/PD-L1 activation in patients with COVID-19. However, in order to clarify this controversial situation, in vivo and in vitro studies investigating the effect of vitamin D on Treg and PD-1/PD-L1 pathways in the immunopathogenesis of COVID-19 are needed.

In the fight against COVID-19 infection, vitamin D supplementation can prevent the increase of PD-L1 through reducing proinflammatory cytokines, and the suppressive effect of inhibitory immune checkpoint molecules on T lymphocytes is eliminated. Thus, in severe COVID-19 patients, T lymphocytes could provide appropriate immune response, and the severity and mortality rate of the disease may decrease.

Author contribution HA: research concept and design, preparation of article and revisions. All data were generated in-house and that no paper mill was used.

Data availability Data available on request from the author.

Declarations

Ethics approval Not require ethics approval.

Consent to participate Not require the informed consent to participate in the study.

Consent for publication The author affirms the informed consent for publication of the images in figures.

Conflict of interest The author declares no competing interests.

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