Effects of vitamin D deficiency on inflammatory cytokines

VITAMIN D
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Ombretta Viapiana
University of Verona Department of Medicine

Besides playing a key role in maintaining bone health, vitamin D has also been recognised for its antibacterial, antiproliferative, immunomodulatory and anti-inflammatory actions [1,2], whilst its immunomodulatory functions have increasingly become of scientific interest. Indeed, in recent years, both clinical and epidemiological data supporting the link between vitamin D status and the incidence and severity of immunocorrelated conditions, such as multiple sclerosis, psoriasis, diabetes, rheumatoid arthritis, inflammatory bowel disease and infectious diseases, have been published [1,2]. Whereas the association between these pathological events and vitamin D deficiency has been widely demonstrated, the effect of cholecalciferol supplementation on the same phenomena has not. To complicate the picture, published studies are extremely heterogeneous in terms of the populations considered, the basal 25(OH)D levels, the extent of supplementation and the modality applied to administration (daily rather than boluses).

Attention to the effect of cholecalciferol supplementation on immune cells and inflammatory cytokines was certainly rekindled by publication of the VITAL study last year. In this study, 25,571 subjects were enrolled and randomised to take 2,000 IU of cholecalciferol per day (with or without omega-3 supplementation) versus placebo for five years, demonstrating a 22% reduction in the incidence of autoimmune diseases, including rheumatoid arthritis, polymyalgia rheumatica and psoriasis [3].

The regulation of inflammation and cytokine expression is also of crucial importance for the recent "inflammaging" hypothesis, which proposes that with increasing age there appears to be a shift towards a proinflammatory state that tends to create and maintain a chronic low-grade inflammation (only partially detectable by serum biomarkers such as C-reactive protein [CRP]) with a subsequent slow accumulation of damage. This acceleration toward ageing, driven by chronic inflammation, is believed to be the basis for progression to several chronic diseases [4]. This has also been confirmed by a recent Anglo-Saxon biobank study of 397,737 subjects, aged between 37 and 73 years. Vitamin D deficiency was found to be associated with increased mortality from several causes, although not with classical serum inflammatory markers. If this is valid in the general population, however, it may be different in patient populations with high levels of inflammation, such as those with cancer, diabetes mellitus or acute cardiovascular disease, where supplementation in deficient subjects showed a reduction in high-sensitivity PCR [5].

VITAMIN D MECHANISM OF ACTION

Though vitamin D can act through an endocrine mechanism (the typical regulatory action of bone metabolism), it can also act through autocrine-paracrine signalling because of the presence of the enzyme 1 α-hydroxylase capable of producing the active metabolite $1,25(OH)_2D$ within individual cells. Actually, it is the autocrine-paracrine action, which is responsible for the effect on immune system cells and consequently on proinflammatory cytokine production. The action of the active metabolite produced this way is modulated by binding to its receptor (VDR). The VDR found in the nucleus of multiple cell types mediates two types of actions, namely [1-6]:

- The non-genomic pathway: binding of the ligand to VDRs present in the cytosol triggers multiple pathways in intracellular signalling cascades, which lead to immediate responses independent of gene transcription in the cells;
- The genomic pathway: the retinoic acid receptor forms a heterodimer with the VDR bound to $1,25(OH)_2D$. The heterodimer translocates into the cell nucleus and binds to specific vitamin D response elements (VDREs) on target genes, thereby regulating nuclear transcription.

Both VDR and 1-α-hydroxylase are expressed by different types of immune cells, including

Correspondence
Ombretta Viapiana
ombretta.viapiana@univr.it

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macrophages, T-cells, dendritic cells, monocytes and B-cells. Evidence from preclinical studies has shown that vitamin D exerts biological effects on both the innate and adaptive immune systems (Tab. I). Extra-renal 1-α-hydroxylase is not up-regulated by PTH (parathyroid hormone). Therefore, the production of $1,25(OH)_2D_3$ depends on the levels of the $25(OH)D_3$ substrate and can be regulated by inflammatory signals, such as polysaccharide (LPS) and the same cytokines. Vitamin D is believed to have a direct effect on cytokine production, the main mechanisms of which are summarised in Table II [6].

TABLE I.
Main effects of vitamin D on the activity of cells involved in innate and adaptive immunity.

Innate immunity	Adaptive immunity
Increases differentiation of macrophages	Decreases Th1 cytokines
Bactericidal action	Increases Th2 cytokines
Inhibits dendritic cell maturation	Reduces differentiation to Th17
Inhibits antigen presentation	Increases differentiation of T-regs
	Reduces B-cell proliferation
	Induces B-cell apoptosis
	Inhibits plasma cell production
	Inhibits immunoglobulin secretion

Th1: T Helper 1; Th2: T Helper 2; Th17: T Helper 17, T-regs: Regulatory T cells.

TABLE II.
Main mechanisms by which vitamin D exerts its anti-inflammatory effect.

Molecular target	Mechanism	Effect
MAP kinase phosphatase 5	Activates the enzyme which in turn inhibits p38	Blocks amplification of the inflammatory cascade mediated by p38
NF-kB	Inhibits NF-kB transcription factor through VDR binding	Reduces transcription/production of TNF-α, IL-1b and consequently IL-6
Cyclooxygenase 2	Directly inhibits prostaglandin production	Reduces cell proliferation and angiogenesis

TNF-α: tumour necrosis factor-alpha; NF-kB: nuclear factor kappa B; IL-1β: interleukin-1 beta; IL-6: interleukin-6.

VITAMIN D DEFICIENCY AND PRO-INFLAMMATORY CYTOKINES

Vitamin D deficiency, often associated with increased serum levels of pro-inflammatory mediators, including IL-6 and tumour necrosis factor-alpha (TNF-α), are related to both the development and progression of rheumatic and vascular inflammatory diseases [1,2]. Apart from what has by now been considered outdated evidence that observed an association between vitamin D deficiency and pro-inflammatory cytokines in classic inflammatory rheumatological diseases, such as rheumatoid arthritis or connective tissue disorders, a study was recently published that documented a linear correlation between the extent of vitamin D deficiency and increased levels of IL-6 and IL-8 in fibromyalgia. Specifically, reduced vitamin D levels were found to be associated with higher scores for both widespread pain as well as for disease activity [7].

In a similar manner, in another study by the same authors on patients with osteoarthritis of the knee, a correlation was observed between vitamin D deficiency and higher IL-6 levels, whilst the IL-6 levels in turn were found to be associated with the radiographic stage of the disease and with the patient's functionality scale [8].

Finally, in a study on obese patients, it was shown that reduced serum 25(OH)D concentrations were usually correlated with increased levels of other biomarkers of vascular inflammation, such as high-sensitivity PCR and fibrinogen. Similar conclusions were also reached for severely obese children [1].

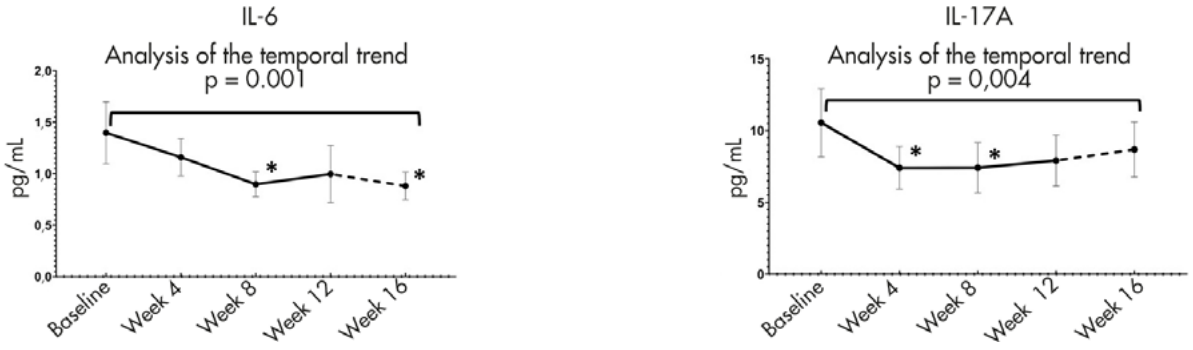
All these studies support the hypothesis that among vitamin D-deficient subjects there is a concomitant rise in pro-inflammatory cytokines regardless of whether the subjects were healthy or were suffering from various rheumatological and non-rheumatological diseases.

EFFECT OF CHOLECALCIFEROL ADMINISTRATION IN CHOLECALCIFEROL-DEFICIENT SUBJECTS

Although a great deal of evidence has been found that supports the association between vitamin D deficiency and increased inflammatory cytokines, few studies have evaluated the effect of cholecalciferol administration on inflammatory status, whereas, often there has been bias that has limited interpretation. In a group of young, healthy subjects, who were however vitamin D-deficient, the effect of cholecalciferol administered over 12 weeks on the production of IL-17A, IL-6, IL-8, IL-10, IL-23 and TNF-α was recently evaluated. Though we observed a progressive reduction in IL-6 and IL-17A levels, no significant differences were found in the serum concentrations of the other cytokines (Fig. 1) [9]. IL-6 and IL-17 are two key cytokines in rheumatoid arthritis and spondyloarthritis, respectively. The reduction in serum levels observed in this study could support a possible role of vitamin D supplementation for patients with rheumatological diseases to optimise their therapeutic response to specific drugs. In support of this option, it was also observed that, depending on serum 25(OH)D levels, vitamin D supplementation would have different (positive) effects on pain and disease activity among patients with rheumatoid arthritis [10].

CONCLUSIONI

Gli studi che hanno valutato l'effetto della supplementazione con vitamina D sulle citochine infiammatorie sono ancora pochi, talvolta con risultati discordanti e spesso non confrontabili tra loro in quanto condotti su popolazioni a volte carenti, a volte no e con comorbilità differenti. Tuttavia negli studi condotti su soggetti giovani, sani e carenti di vitamina D, dove i fattori confondenti sono ridotti, ed è possibile così



IL-6: interleukin-6; IL-17A: interleukin-17°.

FIGURE 1.
Effects of cholecalciferol supplementation on serum levels of IL-6 and IL-17A in young, healthy, vitamin D-deficient subjects [9].

valutare l'effetto "puro" del colecalciferolo, si evidenzia un effetto della supplementazione nel ridurre le citochine pro-infiammatorie. Se questi dati si confermassero, la vitamina D potrebbe diventare un trattamento complementare nella prevenzione e nel trattamento di numerose patologie reumatiche e infiammatorie.

Instead, in another study on healthy but elderly subjects (average age over 70 years), cholecalciferol administration did not change gene expression or serum levels of IL-6, IL-8, IL-10, TNF-α or IFN-γ. Regardless, it should be noted that basal serum 25(OH)D levels were higher than in the previous study and the cholecalciferol dosage varied among treatment groups [11].

The effect on cytokine reduction was also studied in a small group of healthy men undergoing intense endurance exercise. Compared to placebo, the supplemented subjects showed positive effects in terms of increased blood levels of 25(OH)D, of CD4+/CD8+ ratio (immune response) and of aerobic capacity, through the inhibition of inflammatory cytokines (IL-6 and to a lesser extent TNF) and CK(creatine kinase) and LDH (lactate dehydrogenase) (indicators of muscle damage) [12].

Table III summarises the main features of studies that have assessed the effects of cholecalciferol supplementation on serum levels of inflammatory cytokines. The effect has been found to be more controversial in disease conditions. Some years ago, a meta-analysis of over 80 studies on different disease conditions, showed no significant effects of vitamin D supplementation on inflammatory biomarkers, including C-reactive protein, IL-6 and TNF-α. It should be noted that in addition to the heterogeneity of the pathological conditions and their pathogenesis, Il-6 was assayed in only 22 of these studies, whereas TNF-α was measured in only 25 [13].

Assessing some specific clinical conditions, Corrado et al. recently showed that in vitro exposure to increasing doses of 1-25(OH)$_2$D in deficient subjects was associated with a significant reduction in IL-17A and profibrotic cytokines (FGF2, TGF-β, CTGF) whether the patients had systemic sclerosis or were healthy, both with a dose-dependent effect [14].

Instead, in 44 vitamin D deficient multiple sclerosis patients, an increase in serum levels of anti-inflammatory cytokines (IL-10, TGF-β) and the regulatory IFN-γ was observed after 12 months of supplementation with 500-1000 IU/day [depending on basal 25(OH)D levels] of cholecalciferol, while IL-17 (proinflammatory) remained unchanged [15].

Among patients with cardiovascular disease, in deficient subjects, vitamin D supplementation was able to reduce the expression of pro-inflammatory and pro-atherogenic cy-

TABLE III.
Studies that evaluated the effect of cholecalciferol administration on pro-inflammatory cytokines.

Author	No. of patients	Median age (years)	25(OH)D (ng/mL)	Dose administered	Duration	Effect
Fassio et al.	75	34	13.7	• 10,000 IU/day for 8 weeks then 1,000 IU/day for 4 weeks • 50,000 IU/week for 12 weeks • 100,000 IU every other week for 12 weeks	12 weeks	Reduces IL-6 and IL-17a
Berlanga et al.	305	72	20	• 4,000 IU/day • 2,000 IU/day • Placebo	1 year	No significant effect
Liu et al.	18	22	22	• 5,000 IU/day • Placebo	4 weeks	Reduces IL-6

IL-6: interleuchina-6; IL-17A: interleuchina-17A.

tokines such as IL-2 and interferon-γ (IFN-7), which are responsible for T-helper-1 cell activation and vascular inflammation [1].
The condition of obesity deserves a separate discussion. Chronic low-grade inflammation appears to play a crucial role in the development of obesity-associated comorbidities such as insulin resistance, cardiovascular disease and cancer. The systemic inflammatory response brought about by obesity appears to mainly originate from adipose tissue, promoting the infiltration of inflammatory cells (macrophages) and the release of pro-inflammatory mediators, leading to low-grade systemic inflammation. In support of this, previous studies showed some positive correlations between adipose tissue volume and the secretion of pro-inflammatory cytokines [4]. One recent study evaluated the effect of supplementation with probiotics (strains of lactobacilli and bifidobacteria), omega-3 and omega-6 and vitamin D on low-grade inflammation among overweight and obese individuals. The study showed no differences on the primary outcome, which was hs-CRP (high-sensitivity C-reactive protein) levels. However, among the subjects treated, serum levels of IL-6 decreased after administration indicating an albeit modest effect on inflammation [16].
The main limitations of this study were, apart from the limited case series, the simultaneous administration of omega-3-6 probiotics and cholecalciferol, which do not allow the effect of the individual elements to be distinguished, and the low dose of vitamin D administered (200 IU/day, well below the doses that have so far demonstrated extra-skeletal effects).
Although the rationale is very strong, only one study documented a reduction in serum IL-6 concentration after cholecalciferol administration alone among obese subjects [17].

CONCLUSIONS

Though there are few studies that have evaluated the effect of vitamin D supplementation on inflammatory cytokines, they sometimes had discordant results, were often not comparable with one another because they were conducted on populations that were sometimes deficient, sometimes not and often the subjects had different comorbidities. However, in studies conducted on young, healthy, vitamin D-deficient subjects, where confounding factors were reduced, and the "pure" effect of cholecalciferol could thus be assessed, it was shown that one effect was that supplementation reduced pro-inflammatory cytokines. If these data were to be confirmed, vitamin D could then become a complementary therapy in the prevention and treatment of numerous rheumatic and inflammatory diseases.

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