Vitamin D and immunity

Robyn M. Lucas[1,2]*, Shelley Gorman[1], Sian Geldenhuys[1], and Prue H. Hart[1]

Addresses: [1]Telethon Kids Institute, University of Western Australia, 100 Roberts Road, Subiaco, Perth, Australia 6008; [2]National Centre for Epidemiology and Population Health, The Australian National University, Canberra, Australia 0200

* Corresponding author: Robyn M. Lucas (robyn.lucas@telethonkids.org.au)

The electronic version of this article is the complete one and can be found at: http://f1000.com/prime/reports/b/6/118

Abstract

Vitamin D deficiency has been linked to an increased risk of a wide range of adverse health outcomes. The active form of vitamin D has an important role in calcium metabolism and in bone mineralisation, but the evidence for other health outcomes is mixed, with the strongest effects seen in the weakest epidemiological study designs. There are plausible pathways whereby vitamin D deficiency can impair immune function, resulting in both overactivity and increased risk of autoimmune disease, as well as immune suppression with poorer resistance to infection. Vitamin D status may influence the bacterial flora that constitute the microbiome and affect immune function through this route. Exposure of the skin to ultraviolet radiation causes the production of a range of chemicals, including vitamin D, and new research is exploring possible vitamin D-independent immunomodulatory pathways.

Introduction

Vitamin D deficiency is reportedly widespread across the world, a consequence of urbanisation and indoor life-styles, migration of dark-skinned populations to low sun environments when their skin type has evolved to be optimal for high sun environments, and possibly sun protection strategies to curb rising skin cancer incidence rates. Over the last 10-15 years, increased risk of a wide range of health outcomes has been linked to vitamin D deficiency, although in many cases the links remain rather weak. After the undisputed importance of vitamin D for bone health, the strongest evidence is probably for an increased risk of immune disorders in association with vitamin D deficiency. Here we briefly review some of that evidence, examine recent literature on possible mechanistic pathways and propose potential explanations for some of the conflicting results in this area.

Clues from epidemiology

Indications that vitamin D may be associated with disorders of human immune function often originate from observations of geographic variation in disease occurrence. In many regions of the world, vitamin D is primarily synthesised in the skin following sun exposure (specifically UV-B irradiation). Levels of UV-B radiation vary strongly according to distance from the equator (latitude) and time of year, with higher levels as the sun is closer to being directly overhead, that is, nearer the equator, in summer, and during the middle of the day. Thus, higher latitude is often taken as a proxy for both lower levels of ultraviolet radiation (UVR) and lower vitamin D status. Latitudinal gradients, where the incidence or prevalence of a disease increases with increasing distance from the equator (lower UV-B radiation), are described for multiple sclerosis [1], type 1 diabetes [2], the autoimmune vasculitides [3], the inflammatory bowel diseases [4], and asthma [5]. Null or inverse associations are also described for other autoimmune disorders [6,7]. Both UVR and vitamin D (in its active form $1,25(OH)_2D$, see Figure 1) have immunomodulatory effects [8] that provide a plausible mechanism whereby higher levels could decrease the risk of autoimmune diseases (see section on mechanisms, below) and, through the same pathways, could impair the immune response to vaccination or infection [9,10]. Accordingly, the effectiveness of BCG vaccination for tuberculosis is reported to increase with increasing latitude (lower UVR) [11].

Figure 1. The synthesis of vitamin D following UVB irradiation of the skin
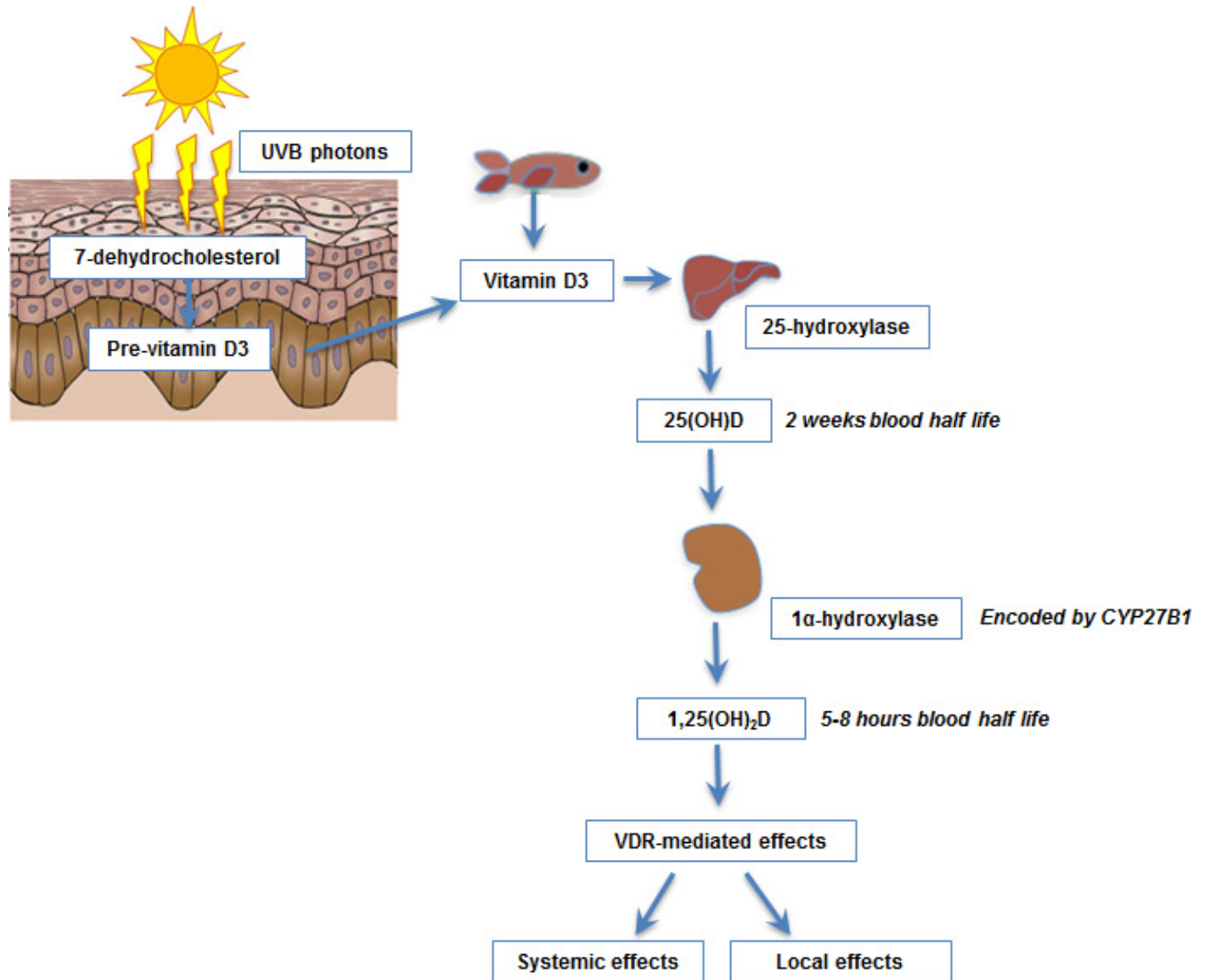
UVB photons are absorbed by 7-dehydrocholestrol in the epidermis and are converted into pre-vitamin D which undergoes a thermal isomerisation to formvitamin D. This then undergoes two hydroxylation reactions, first in the liver to form 25-hydroxyvitamin D (25(OH)D) and then in the kidney to form the active metabolite, 1,25-dihydroxyvitamin D (1,25(OH)2D). Serum 25(OH)D levels are used to determine vitamin D status. VDR, vitamin D receptor.

Observational studies have, in general, supported these ecological patterns. In case-control studies, participants with one of these autoimmune diseases tend to report lower past sun exposure, and/or have lower vitamin D status (measured as the blood concentration of the intermediary metabolite, 25(OH)D, see Figure 1), than healthy controls (see for example recent reviews [12,13]). However, for at least some of the immune-related diseases, it is difficult to determine whether low sun exposure or vitamin D cause, or are caused by, the disease. There is a smaller body of evidence from prospective cohort studies, in particular, because these diseases caused by immune dysfunction are uncommon and require large numbers of participants to be under observation for a sufficient time to achieve required sample sizes. This is a stronger study design because the direction of causality is established, that is, the low vitamin D status precedes the onset of the health outcome. Lower vitamin D status has been linked to increased risk of multiple sclerosis (see case study [14]) and to type 1 diabetes [15], although not all studies show a protective association [16].

Despite these relatively coherent findings from ecologic and observational studies, and plausible biological

mechanisms, trials of vitamin D supplementation for the prevention of immune-related diseases have largely returned null results [17]. It is worth considering the challenges of a true prevention trial for these diseases, they are uncommon, possibly with long subclinical phases, with the optimum time for intervention unknown, amongst other difficulties. Prevention trials to date have focused on high-risk populations where the disease process may already be underway, or on preventing progression in those who have already developed the disease, where different pathological processes may be operating (see comment in [18]). Nevertheless, there is not yet compelling evidence from these studies that vitamin D supplementation ameliorates any immune-related disease.

Mechanisms by which 1,25(OH)2D may regulate immune cell function

It is generally believed that all immune cell types can respond to $1,25(OH)_2D$. This belief is driven by recognition of cellular expression of the vitamin D receptor (VDR), the finding of multiple primary $1,25(OH)_2D$ target genes in immune cells and the discovery that many immune cells (macrophages, dendritic cells, T and B lymphocytes) can convert $25(OH)D$ to $1,25(OH)_2D$ through *CYP27B1* activity and therefore provide significant local levels of $1,25(OH)_2D$ for functional outcomes (for review [8,19]). The amount of $1,25(OH)_2D$ produced may depend on the ability of immune cells to express *CYP27B1* and other enzymatic machinery of the vitamin D pathway, including the *CYP24A1* deactivation enzyme. As an example, *in vitro* stimulated macrophages produced more $1,25(OH)_2D$ than dendritic cells, which expressed truncated *CYP27B1* transcripts resulting in lower CYP27B1 protein levels, and also expressed increased levels of *CYP24A1* mRNA [20]. Despite *in vitro* studies reporting significant biological effects of $1,25(OH)_2D$ on immune cells (frequently under ideal conditions using potentially supra-physiological concentrations of $1,25(OH)_2D$), the question remains as to the real biological effects of $1,25(OH)_2D$ *in vivo*. The issue is further complicated by the finding that human cells are frequently used for *in vitro* investigations, but the majority of studies reporting the biological effects of $1,25(OH)_2D$ *in vivo* have been performed in the experimental mouse. There are also differences between the regulatory potential of supplemented levels versus homeostatic concentrations of $1,25(OH)_2D$. An extreme is seen in mice not expressing the VDR; they have increased sensitivity to autoimmune diseases [21] but this finding contributes little information to the extent by which $1,25(OH)_2D$ may dose-dependently regulate VDR-expressing cells. Further work is required to better understand the regulatory properties of $1,25(OH)_2D$ because, as summarised below, many of the findings *in vitro* have not been replicated

in vivo. A central concept is that $1,25(OH)_2D$ stimulates innate immunity and suppresses adaptive immunity, but the mechanisms by which these outcomes are achieved vary with different experimental models.

Exposure of differentiating dendritic cells to $1,25(OH)_2D$ prevents their full maturation [22]. However, debate remains surrounding the properties of these "tolerogenic" dendritic cells and their ability to interfere with T cell division and promotion of T regulatory cell (T_{Reg}) production and expansion [23]. Some studies suggest that existing CD25+Foxp3+T_{Reg} cells proliferate rather than T_{Reg} cell development occurring *de novo* [24]. Other investigators report that, whilst high non-physiological concentrations of $1,25(OH)_2D$ ($10^{-6}M$) and long culture periods (2 weeks) are required for induction of Foxp3+ T_{Reg} populations, the frequency *in vitro* of Foxp3+ T_{Regs} can be increased by supplementation of cells with TGFβ (2 ng/ml) and lower concentrations of $1,25(OH)_2D$ ($10^{-7}M$) [25]. Upon $1,25(OH)_2D$ administration *in vivo*, the properties of the exposed dendritic cells are varied. In one model, $1,25(OH)_2D$ was applied topically to the skin of mice [26]. After 18 hours, when the dendritic cells would have migrated to the draining nodes, the capacity of these dendritic cells to take up, process and present antigen to co-cultured T cells was not modified. However, upon transfer to new naïve mice, these dendritic cells induced significantly smaller ear-swelling responses, as a measure of contact hypersensitivity. The dendritic cells from the mice treated with topical $1,25(OH)_2D$ expressed increased levels of indoleamine 2,3-dioxygenase which may explain this altered dendritic cell property *in vivo* [26].

T cells may also directly respond to $1,25(OH)_2D$. Naïve T cells express low levels of the VDR that is up-regulated by antigen-specific triggering of T cell receptors and contributes to priming of naïve cells (for review [27]). As VDR expression can also inhibit the transcription of the interleukin (IL)-2 gene, this may represent another point of immune regulation by $1,25(OH)_2D$. Homing receptors on T cells may be altered by $1,25(OH)_2D$ [28] and a recent analysis of the impact of $1,25(OH)_2D$, given by oral gavage to mice immunised with myelin oligodendrocyte glycoprotein to induce experimental autoimmune encephalomyelitis, suggests that this may be one of the most important immunoregulatory roles of $1,25(OH)_2D$ [29]. These authors found that $1,25(OH)_2D$ prevented accumulation of inflammatory cells into the central nervous system, although $1,25(OH)_2D$ did not affect the activation of the pathogenic interferon-γ and IL-17-producing T cells in lymph nodes, spleen or the immunisation site, that is, the dramatic systemic immune reaction to myelin oligodendrocyte glycoprotein was not altered. There was no induction of Foxp3+ T_{Reg} by

$1,25(OH)_2D$. More specifically, $1,25(OH)_2D$ seemed to maintain the activated Th1/Th17 cells in the circulation and prevented them from crossing the blood brain barrier. The mechanism proposed was a significant and reversible reduction in expression of the chemokine receptor, CXCR3 [29]. Modulation of other chemokine receptors on T cells by $1,25(OH)_2D$ has also been reported [30]. Further, $1,25(OH)_2D$ can reduce the migration of macrophages by suppressing the expression of CCR2, the receptor for monocyte chemotactic protein-1, principally by reducing endoplasmic reticulum stress [31].

T_{Regs} may also be direct targets of $1,25(OH)_2D$. Using topical delivery to shaved skin, $1,25(OH)_2D$ enhanced the ability of CD4+CD25+ cells in the skin draining lymph nodes to down-regulate T helper type 2-driven asthmatic responses, upon transfer to allergen-sensitised mice [32]. Further investigations found that $1,25(OH)_2D$, in the presence of IL-2, directly enhanced the regulatory potential of CD4+CD25+ T cells to control immune responses [33].

Circulating levels of $1,25(OH)_2D$ are a log fold lower than those of $25(OH)D$ and are not sufficient for immunoregulation; it is generally proposed that $25(OH)D$ is converted locally to sufficient levels of $1,25(OH)_2D$ to achieve biological activity. Mast cells have recently been identified, with macrophages, dendritic cells and T and B lymphocytes, to express *CYP27B1* for local $1,25(OH)_2D$ production [34]. However, the level of vitamin D binding protein, and its affinity to $25(OH)D$, can also restrict the availability of $25(OH)D$ to dendritic cells and possibly other cells [35]. Cells from individuals with various vitamin D-binding protein (VDBP) variants were studied and for individuals with the strong $25(OH)D$ binding variant, the availability of $25(OH)D$ to dendritic cells was restricted, resulting in less dendritic cell-T cell interaction. In summary, we still have much to learn about the mechanisms by which $1,25(OH)_2D$ may regulate immune cell activity *in vivo*.

Multiple sclerosis: a case study

One of the earliest indications of an association between vitamin D and disorders of human immune function was the geographical variation in prevalence of multiple sclerosis, as first described in 1922 [36]. Additional studies since then have confirmed a latitudinal gradient, with higher incidence or prevalence at locations further from the equator in both the northern and southern hemispheres [1,37,38]. Building on these ecological studies, observational studies confirmed increased risk of multiple sclerosis in association with lower sun exposure [39,40] and with lower $25(OH)D$ levels [14,41]. Yet, despite plausible immunological pathways whereby vitamin D (through stimulation of T_{Regs} and dampening

of T helper type 1 over-reactivity) could diminish the risk of multiple sclerosis or disease activity, vitamin D supplementation trials have shown immunological [42] and radiological [43], but not clinical, benefit for people with multiple sclerosis.

It is challenging to differentiate between vitamin D-dependent and -independent pathways in the effect of sun exposure on disease risk. Limited success in vitamin D supplementation trials suggests that vitamin D-independent effects may contribute more strongly to disease risk. In the mouse, the onset of experimental autoimmune encephalomyelitis, a model for multiple sclerosis, was significantly delayed following chronic irradiation with sub-erythemal doses of UV-B radiation [44]. Serum $25(OH)D$ levels were only slightly elevated and suppression of disease did not occur following oral administration of $25(OH)D$ [44]. However, when $1,25(OH)_2D$ was administered (in food), it suppressed the development of experimental autoimmune encephalomyelitis, but only when given at levels that caused hypercalcaemia; one hypothesis was that it was hypercalcaemia *per se* that was important to disease suppression. T_{Reg}-inducing tolerogenic dendritic cells, induced by UV-B irradiation of the skin, were required for amelioration of experimental autoimmune encephalomyelitis [45]. Serum $25(OH)D$ levels and sun exposure have been shown to be independently associated with the onset of central nervous system demyelination in humans [41] and brain and spinal cord lesions detected by magnetic resonance imaging in people with multiple sclerosis [46]. Additionally, while increased sun exposure during childhood was inversely associated with risk of multiple sclerosis, there was no decreased risk associated with intake of vitamin D supplements during childhood [40].

Sun exposure during childhood may be a more significant factor for disease risk than sun exposure in adulthood. On the basis of migration studies, the risk of developing multiple sclerosis may already have been determined before the age of 15 years [47]. Migrants moving from a country with high prevalence of multiple sclerosis to a country with a lower prevalence retain the high risk of their country of origin if migration occurs after the age of 15, but have the lower risk of their new country if they migrate before 15 years of age [47,48]. However, sun exposure even earlier than childhood may be an important factor in risk of multiple sclerosis. An association between month of birth and risk of multiple sclerosis has been observed in both the northern [49,50] and southern [51] hemispheres, suggesting an important role for sun exposure *in utero* for the risk of developing the disease in later life. In the experimental autoimmune encephalomyelitis animal model, vitamin D supplementation in the postnatal period delays the onset of the disease [52] but there is no evidence that antenatal

vitamin D supplementation has any effect. Although childhood vitamin D levels and sun exposure appear to be important determinants of the risk of disease onset, higher 25(OH)D levels early in the disease course may predict the rate of disease progression in multiple sclerosis [53].

An indirect pathway: vitamin D, immunity and the microbiome

Following interactions with pathogens, immune cells like monocytes can synthesise $1,25(OH)_2D$, possibly through activation of specialised immune receptors like the toll-like receptors [54], or following stimulation with other immune-modulators like TGFβ or interferon-γ [55]. This stimulates autophagy (the degradation of internal cell structures) and the synthesis of antimicrobials, such as cathelicidin, for bacterial killing [54]. Antimicrobial proteins are expressed at common epithelial surfaces, including the gut, skin, urinary tract and lung [55]. Cathelicidins target gram-positive and -negative bacteria, viruses and fungi by membrane disruption, and are expressed by neutrophils, macrophages, natural killer cells, mast cells and epithelial cells [56,57]. They are widely conserved in mammals - the LL-37 peptide in humans and cathelin-related antimicrobial protein in mice. Transcription of the *CAMP*, the gene encoding cathelicidin, is regulated by the vitamin D receptor through $1,25(OH)_2D$ in humans, but not rodents [58]. Induced antimicrobials such as cathelicidin and ß-defensin-4 [54], can also act as immune-modulators [57]. This pathway (Figure 2) may be dependent upon levels of circulating 25(OH)D, the substrate for $1,25(OH)_2D$, or of *CYP27B1*, the enzyme controlling this reaction; however, *in vivo* evidence for this is lacking. Vitamin D supplementation increases serum levels of 25(OH)D and cathelicidin [58–60], suggesting that systemic elimination of pathogenic microbes is possible; but, supplementation trials aiming to control infections with *Mycobacterium tuberculosis* have not been successful [61]. *In vitro* bacterial targets of vitamin D include *M. tuberculosis*, *Pseudomonas aeruginosa* and other species [54], and there are reported benefits of vitamin D for the control of viral or fungal infections, especially those of the lower respiratory tract like influenza A [8].

The microbiome constitutes the commensal bacteria that colonise various parts of the body like the gastrointestinal [62] and respiratory [63] tracts and skin [64]. In murine models, $1,25(OH)_2D$ may be able to suppress tissue inflammation by altering the microbiome [65, 66]. Vitamin D deficiency increased the severity of colitis and bacterial numbers in the colons of mice [66] and, in male mice with allergic airway disease (modelling allergic asthma), vitamin D deficiency increased lung inflammation and bacterial numbers [65]; these effects were reversed by vitamin D supplementation [65]. Other studies have

linked vitamin D with specific changes to the composition of the bacterial flora in the gut microbiome. The absence of the 1α-hydroxylase enzyme in $CYP27B1^{-/-}$ mice with colitis increased the burden of the *Proteobacterium* phylum (including *Helicobaceteraceae* species) in faeces [67]. These observations were linked with diminished numbers of tolerogenic CD103+ dendritic cells in the lamina propria, cells that shape the T_{Reg} cell repertoire of the gut [68]. Treatment of these mice with $1,25(OH)_2D$ (1.25 μg/100 g diet) suppressed colitis severity and *Helicobaceteraceae* numbers. Similar observations have been made in humans, whereby increased dietary vitamin D changed the composition of faecal microbiota [69]. Collectively, these studies suggest that vitamin D is instrumental in regulating the microbiome of the lungs and gut, and is entwined with the maintenance of immune tolerance.

As murine studies are starting to illustrate in the gut and lungs, the interplay between vitamin D, the microbiome and immune tolerance may also curb skin inflammation. Abnormal microbiomes have been detected in diseased skin, including psoriatic plaques and atopic dermatitis [70]. Topical $1,25(OH)_2D$ or related analogues are used to treat psoriasis and may be useful for treating atopic dermatitis and other inflammatory skin diseases [71]. The mechanisms by which $1,25(OH)_2D$ suppresses skin inflammation associated with these diseases may at least partially involve promotion of immune tolerance with the activation of T_{Regs} [72]. An added complexity in psoriasis is the levels of cathelicidins that accumulate in the dermis, which can have pro-inflammatory effects at high concentrations [73]. We anticipate that a deeper understanding of how vitamin D modulates the microbiomes of the skin and other tissues will come through ongoing vitamin D supplementation trials [74].

Other studies suggest that the microbiome may modify the capacity of tissue regulation by vitamin D. In rodent models, probiotics can upregulate expression of the VDR in the colon, reducing inflammation and delaying the transition to dysplasia and cancer [75]. Furthermore, vitamin D supplementation may suppress the development of intestinal tumours in susceptible mice [76]. In a clinical setting, expression of the VDR was decreased in patients with dysplasia and colitis-associated colorectal cancer [77], suggesting that inflammation may also downregulate the VDR. These data suggest that not only can vitamin D modulate tissue inflammation through modifications to the microbiome, but the reverse is also possible—the microbiome and inflammation can change the responsiveness of tissues to vitamin D through regulation of the VDR. $1,25(OH)_2D$ also upregulates the VDR [78], suggesting that this reduced responsiveness may be reversed by supplementation.

Figure 2. A proposed network of interactions between vitamin D and the microbiome that may sway the development of immune tolerance or tissue inflammation
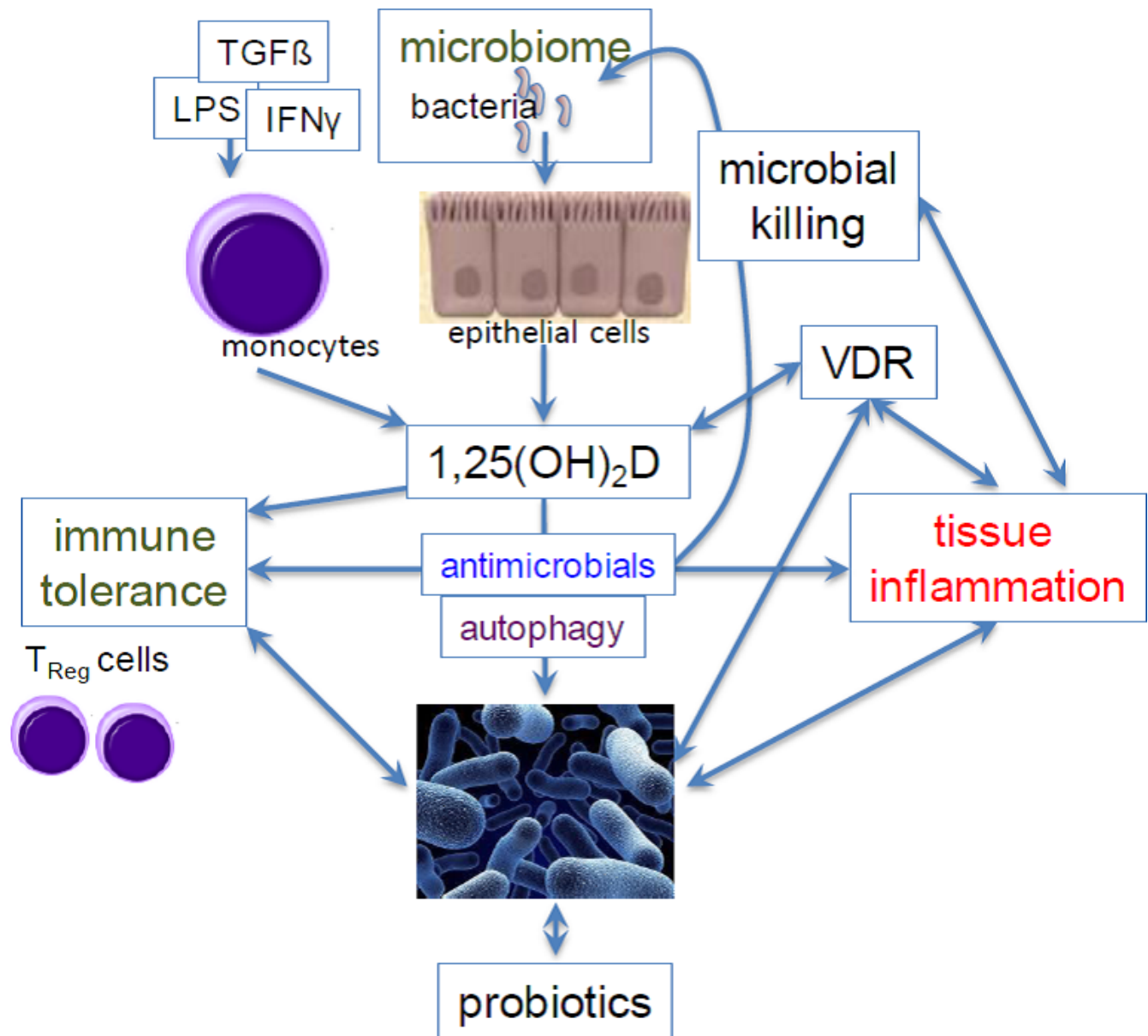
Activation of immune cells like monocytes and epithelial cells by bacterial-derived products (e.g. lipopolysaccharide [LPS], derived from the outer membrane of gram-negative bacteria) and cytokines (such as transforming growth factor-ß [TGFß] and interferon-γ [IFNγ], results in local synthesis of 1,25(OH)$_2$D, and immune tolerance through effects on T$_{Reg}$ cells. 1,25(OH)$_2$D may also have effects on innate pathways including synthesis of antimicrobials and activation of autophagy, which together may modulate the local microbiome. Alternatively, microbiome changes, like those induced following the use of probiotics, may regulate the responsiveness of immune cells to vitamin D by enhancing the expression of the vitamin D receptor (VDR) thus reducing tissue inflammation.

Why doesn't vitamin D supplementation decrease disease risks?

In the face of supportive observational and mechanistic evidence, why doesn't vitamin D supplementation decrease disease risks? A broad range of responses have been proposed to answer this question with a particular focus on reverse causality or confounding. For the former, it has been proposed that inflammation causes depression of 25(OH)D levels [17] and this accounts for the association between low 25(OH)D levels and

increased disease risk. For evidence arising from case-control studies, where disease has already occurred in the cases, this is certainly plausible; however, in the published cohort studies that show a link between low 25(OH)D and development of disease, the latter often occurs many years after the blood sample was taken (e.g. [14]). That asymptomatic disease of sufficient severity to lower 25(OH)D levels was present at this much earlier time seems unlikely. Residual confounding from physical activity or a generally healthier lifestyle may provide an explanation for the significant associations seen in observational studies for some disease outcomes, but neither of these is a known risk factor for autoimmune diseases (and thus could not be a confounder). In addition, with respect to an ability of supplemental vitamin D to alter the course of autoimmune diseases, there is debate about the validity of biochemical or cellular markers from blood as truly representative of disease status.

There are several potential other explanations that need to be considered and each has important implications for research and clinical practice.

Independent beneficial effects of sun exposure

Exposure to UVR and to $1,25(OH)_2D$ both cause immune suppression that is relevant to some (Th-1) autoimmune diseases [8]. Low levels of 25(OH)D seen in observational studies may be a marker for low levels of sun exposure, with the latter also important for disease risk. Vitamin D supplementation studies take account only of vitamin D, without the possibly necessary additional benefits of sun exposure.

Importance of the life stage

There is some evidence to support the importance of early life exposures in the risk of autoimmune diseases. It is challenging to adequately study these exposures. However, several studies suggest that low vitamin D status or low levels of ambient UVR during the *in utero* period [49], or during childhood [40], are associated with increased risk of immune-related disorders. The lack of any protective effect of vitamin D supplementation in adulthood may be because the processes necessary for disease have already been put in place much earlier in life.

Genetic variation in the physiological response to vitamin D supplementation

A high degree of individual variability in the 25(OH)D response to vitamin D supplementation has been described that is the result of demographic, environmental and genetic factors [79]. The effect of these factors on the physiological, or disease-relevant, response to vitamin D supplementation has not been explored.

Corruption of the vitamin D system by the disease

Mismanagement of the vitamin D system may arise during some pathological processes. For example, in leprosy, the *Mycobacterium leprae* may hijack the use of cellular microRNAs to block vitamin D-induced antimicrobial production [80]. In addition, in some granulomatous diseases, such as sarcoidosis, *CYP27B1* is over-expressed in disease-activated macrophages [81] causing high levels of $1,25(OH)_2D$ and increased risk of hypercalcemia.

Free versus total serum 25(OH)D

Vitamin D metabolites in blood are largely tightly bound to VDBP, with a small proportion of the total concentration loosely bound to albumin or unbound (free) [82]. Traditionally the level of total 25(OH)D has been considered the best estimate of vitamin D status, but recent studies have suggested that the "bioavailable" (albumin-bound and free) 25(OH)D concentration may be more relevant to health and disease [83]. The ratio of total to bioavailable 25(OH)D depends on several VDBP-related genes and health states, such as liver disease [84], type 1 diabetes [85], and pregnancy [86].

Vitamin D2 versus vitamin D3 versus active 1,25(OH)2D

There is mixed evidence on the different physiological potency of vitamin D_3 compared to D_2 (for example, [87,88]). In most observational studies that have separately quantified $25(OH)D_2$ and $25(OH)D_3$, levels of the former are generally low or undetectable [89]. Most recent vitamin D supplementation trials use vitamin D_3, suggesting that any differences between D_2 and D_3 do not account for the different findings from observational and vitamin D supplementation trials. The active form, $1,25(OH)_2D$, is seldom used in supplementation trials. It has a short half-life in blood, can cause hypercalcaemia and raising $1,25(OH)_2D$ levels in blood initiates its deactivation by the 24-hydroxlyase enzyme [90,91].

The study participants were not vitamin D deficient at baseline

If there is a causal association between vitamin D and disease risks, increased risk occurs in association with vitamin D deficiency [92]. Vitamin D supplementation trials commonly include participants who do not have low baseline 25(OH)D levels and whose disease risk would thus be unlikely to change much [91]. Future work could focus on supplementation trials including only people with low baseline levels of 25(OH)D.

Risks to supplementing people who are already vitamin D sufficient

Several studies have now reported U-shaped or reverse J-shaped curves for a range of disease outcomes; for example, for all-cause mortality, both high and low 25(OH)D

levels are associated with increased risk [92]. Similarly, in one RCT of high-dose vitamin D supplementation there was an increased risk of falls and fractures [93]. It is likely that there is a loss of study power and a risk of harm if participants in vitamin D supplementation trials already have adequate 25(OH)D levels at baseline [91].

Higher 25(OH)D levels are not maintained over a disease-relevant time period

Many of the diseases for which increased risk has been linked to vitamin D deficiency develop over a long period of time, for example, cancers, and cardiovascular disease. Yet vitamin D supplementation trials are generally relatively short-term, with supplementation for only 1-2 years. This discrepancy in timing may contribute to the failure of vitamin D supplementation trials to decrease the risk of disease onset.

Conclusion

The jury is still out on the importance of vitamin D for immune function: both for vitamin D deficiency increasing the risk of autoimmune diseases and viral infections through loss of regulatory adaptive immune functions, as well as for high vitamin D levels decreasing the risks of tuberculosis and infections with other intracellular pathogens through upregulation of innate immunity. Effects may not be direct, but could work through alterations in the microbiome, or be only partly vitamin D-mediated, with exposure to UVR itself also important. Many of the outstanding questions will be difficult or impossible to resolve in human studies, such as the importance of the timing of any intervention. Pragmatic solutions such as moving whole population distributions of 25(OH)D levels to above a minimum of 40-50 nmol/L may be required, with any beneficial effects only apparent retrospectively.

Abbreviations

1,25(OH)2D, 1,25 dihydroxyvitamin D, the active form of vitamin D; 25(OH)D, 25-hydroxyvitamin D, an intermediate metabolite of vitamin D, the serum concentration is the usual measure of vitamin D status; BCG, Bacillus Calmette-Guérin vaccination for tuberculosis; CCR2, the receptor for monocyte chemotactic protein-1; CD4+CD25+, a type of regulatory T cell; CD25+Foxp3+ TReg, specific type of regulatory T cell; CYP24A1, the gene encoding the 24-hydroxylase enzyme that converts 1,25(OH)2D to 24,25(OH)2D; CYP27B1, the gene encoding for the 1α-hydroxylase enzyme that converts 25(OH)D to 1,25(OH)2D; CXCR3, chemokine receptor 3; DC, dendritic cells; EAE, experimental autoimmune encephalomyelitis, the animal model for MS; IL, interleukin; LL-37, a cathelicidin; LPS, lipopolysaccharide; MS, multiple sclerosis; TGFβ, transforming growth factor beta; Th1 cells, T helper 1 cells; Th17 cells, T helper

17 cells; TReg cell, regulatory T cell; UV-B, shorter wavelength (280-315 nm) ultraviolet radiation; UVR, ultraviolet radiation; VDBP, vitamin D-binding protein; VDR, vitamin D receptor.

Disclosures

The authors declare that they have no disclosures.

References

1. Simpson S, Jr., Blizzard L, Otahal P, Van der Mei I, Taylor B: **Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis.** *J Neurol Neurosurg Psychiatry* 2011, **82**:1132-41.

F1000Prime RECOMMENDED

2. Ball SJ, Haynes A, Jacoby P, Pereira G, Miller LJ, Bower C, Davis EA: **Spatial and temporal variation in type 1 diabetes incidence in Western Australia from 1991 to 2010: increased risk at higher latitudes and over time.** *Health and Place* 2014, **28**:194-204.

F1000Prime RECOMMENDED

3. Gatenby PA, Lucas RM, Engelsen O, Ponsonby AL, Clements M: **Antineutrophil cytoplasmic antibody-associated vasculitides: could geographic patterns be explained by ambient ultraviolet radiation?** *Arthritis Rheum* 2009, **61**:1417-24.

4. Khalili H, Huang ES, Ananthakrishnan AN, Higuchi L, Richter JM, Fuchs CS, Chan AT: **Geographical variation and incidence of inflammatory bowel disease among US women.** *Gut* 2012, **61**:1686-92.

F1000Prime RECOMMENDED

5. Krstic G: **Asthma prevalence associated with geographical latitude and regional insolation in the United States of America and Australia.** *PLoS One* 2011, **6**:e18492.

F1000Prime RECOMMENDED

6. Prieto S, Grau JM: **The geoepidemiology of autoimmune muscle disease.** *Autoimmun Rev* 2010, **9**:A330-34.

7. Staples JA, Ponsonby AL, Lim LL, McMichael AJ: **Ecologic analysis of some immune-related disorders, including type 1 diabetes, in Australia: latitude, regional ultraviolet radiation, and disease prevalence.** *Environ Health Perspect* 2003, **111**:518-23.

8. Hart PH, Gorman S, Finlay-Jones JJ: **Modulation of the immune system by UV radiation: more than just the effects of vitamin D?** *Nat Rev Immunol* 2011, **11**:584-96.

9. Nghiem DX, Kazimi N, Clydesdale G, Ananthaswamy HN, Kripke ML, Ullrich SE: **Ultraviolet a radiation suppresses an established immune response: implications for sunscreen design.** *J Invest Dermatol* 2001, **117**:1193-9.

10. Norval M, Woods GM: **UV-induced immunosuppression and the efficacy of vaccination.** *Photochem Photobiol Sci* 2011, **10**:1267-74.

11. Zodpey SP, Shrikhande SN: **The geographic location (latitude) of studies evaluating protective effect of BCG vaccine and it's efficacy/effectiveness against tuberculosis.** *Indian J Public Health* 2007, **51**:205-10.

12. Hewer S, Lucas R, van der Mei I, Taylor BV: **Vitamin D and multiple sclerosis.** *J Clin Neurosci* 2013, **20**:634-41.

13. Gatenby P, Lucas R, Swaminathan A: **Vitamin D deficiency and risk for rheumatic diseases: an update.** *Curr Opin Rheumatol* 2013, **25**:184-91.

14. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A: **Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis.** *Jama* 2006, **296**:2832-8.

F1000Prime RECOMMENDED

15. Munger KL, Levin LI, Massa J, Horst R, Orban T, Ascherio A: **Preclinical serum 25-hydroxyvitamin D levels and risk of type**

1 diabetes in a cohort of US military personnel. *Am J Epidemiol* 2013, **177**:411-9.

16. Simpson M, Brady H, Yin X, Seifert J, Barriga K, Hoffman M, Bugawan T, Baron AE, Sokol RJ, Eisenbarth G, Erlich H, Rewers M, Norris JM: **No association of vitamin D intake or 25-hydroxyvitamin D levels in childhood with risk of islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young (DAISY).** *Diabetologia* 2011, **54**:2779-88.

17. Autier P, Boniol M, Pizot C, Mullie P: **Vitamin D status and ill health: a systematic review.** *Lancet Diabetes and Endocrinology* 2013, **2**:76-89.

    F1000Prime RECOMMENDED

18. Lucas R, Taylor B: **Challenges in exposure and outcome definition in neuroepidemiology: the case of vitamin D and multiple sclerosis.** *Australasian Epidemiology* 2013, **20**:4-8.

19. Mora JR, Iwata M, von Andrian UH: **Vitamin effects on the immune system: vitamins A and D take centre stage.** *Nature reviews Immunology* 2008, **8**:685-98.

20. Kundu R, Chain BM, Coussens AK, Khoo B, Noursadeghi M: **Regulation of CYP27B1 and CYP24A1 hydroxylases limits cell-autonomous activation of vitamin D in dendritic cells.** *Eur J Immunol* 2014, **44**:1781-90.

21. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, Lieben L, Mathieu C, Demay M: **Vitamin D and human health: lessons from vitamin D receptor null mice.** *Endocr Rev* 2008, **29**:726-76.

22. Penna G, Adorini L: **1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation.** *J Immunol* 2000, **164**:2405-11.

23. Hilkens CM, Isaacs JD, Thomson AW: **Development of dendritic cell-based immunotherapy for autoimmunity.** *Int Rev Immunol* 2010, **29**:156-83.

24. Ferreira GB, Gysemans CA, Demengeot J, da Cunha JP, Vanherwegen AS, Overbergh L, Van Belle TL, Pauwels F, Verstuyf A, Korf H, Mathieu C: **1,25-Dihydroxyvitamin D3 promotes tolerogenic dendritic cells with functional migratory properties in NOD mice.** *J Immunol* 2014, **192**:4210-20.

    F1000Prime RECOMMENDED

25. Chambers ES, Suwannasaen D, Mann EH, Urry Z, Richards DF, Lertmemongkolchai G, Hawrylowicz CM: **1alpha,25-dihydroxyvitaminD3 in combination with TGFbeta increases the frequency of Foxp3+ Tregs through preferential expansion and usage of IL-2.** *Immunology* 2014, **143**:52-60.

    F1000Prime RECOMMENDED

26. Gorman S, Judge MA, Hart PH: **Topical 1,25-dihydroxyvitamin D3 subverts the priming ability of draining lymph node dendritic cells.** *Immunology* 2010, **131**:415-25.

27. Kongsbak M, Levring TB, Geisler C, von Essen MR: **The vitamin D receptor and T cell function.** *Front Immunol* 2013, **4**:148.

28. Baeke F, Korf H, Overbergh L, Verstuyf A, Thorrez L, Van Lommel L, Waer M, Schuit F, Gysemans C, Mathieu C: **The vitamin D analog, TX527, promotes a human CD4+CD25highCD127low regulatory T cell profile and induces a migratory signature specific for homing to sites of inflammation.** *J Immunol* 2011, **186**:132-42.

29. Grishkan IV, Fairchild AN, Calabresi PA, Gocke AR: **1,25-Dihydroxyvitamin D3 selectively and reversibly impairs T helper-cell CNS localization.** *Proc Natl Acad Sci USA* 2013, **110**:21101-6.

    F1000Prime RECOMMENDED

30. Chang JH, Cha HR, Lee DS, Seo KY, Kweon MN: **1,25-Dihydroxyvitamin D3 inhibits the differentiation and migration of T(H) 17 cells to protect against experimental autoimmune encephalomyelitis.** *PLoS One* 2010, **5**:e12925.

31. Riek AE, Oh J, Bernal-Mizrachi C: **1,25(OH)2 vitamin D suppresses macrophage migration and reverses atherogenic cholesterol metabolism in type 2 diabetic patients.** *J Steroid Biochem Mol Biol* 2013, **136**:309-12.

32. Gorman S, Judge MA, Burchell JT, Turner DJ, Hart PH: **1,25-dihydroxyvitamin D3 enhances the ability of transferred CD4+ CD25+ cells to modulate T helper type 2-driven asthmatic responses.** *Immunology* 2010, **130**:181-92.

33. Gorman S, Judge MA, Hart PH: **Gene regulation by 1,25-dihydroxyvitamin D3 in CD4+CD25+ cells is enabled by IL-2.** *The Journal of investigative dermatology* 2010, **130**:2368-76.

34. Yip KH, Kolesnikoff N, Yu C, Hauschild N, Taing H, Biggs L, Goltzman D, Gregory PA, Anderson PH, Samuel MS, Galli SJ, Lopez AF, Grimbaldeston MA: **Mechanisms of vitamin D3 metabolite repression of IgE-dependent mast cell activation.** *J Allergy Clin Immunol.* 2014, **133**:1356-64 e1314.

    F1000Prime RECOMMENDED

35. Jeffery LE, Wood AM, Qureshi OS, Hou TZ, Gardner D, Briggs Z, Kaur S, Raza K, Sansom DM: **Availability of 25-hydroxyvitamin D(3) to APCs controls the balance between regulatory and inflammatory T cell responses.** *J Immunol* 2012, **189**:5155-64.

    F1000Prime RECOMMENDED

36. Davenport C: **Multiple sclerosis from the standpoint of geographic distribution and race.** *Arch Neurol Psychiat* 1922, **8**:51.

37. Acheson ED, Bachrach CA: **The distribution of multiple sclerosis in U.S. veterans by birthplace.** *Am J Hyg* 1960, **72**:88-99.

38. Acheson ED, Bachrach CA, Wright FM: **Some comments on the relationship of the distribution of Multiple Sclerosis to latitude, solar radiation and other variables.** *Acta Psychiatr Scand Suppl* 1960, **35**(S147):132-47.

39. Kampman MT, Wilsgaard T, Mellgren SI: **Outdoor activities and diet in childhood and adolescence relate to MS risk above the Arctic Circle.** *J Neurol* 2007, **254**:471-7.

40. van der Mei IA, Ponsonby AL, Dwyer T, Blizzard L, Simmons R, Taylor BV, Butzkueven H, Kilpatrick T: **Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study.** *Bmj* 2003, **327**:316-21.

41. Lucas RM, Ponsonby AL, Dear K, Valery PC, Pender MP, Taylor BV, Kilpatrick TJ, Dwyer T, Coulthard A, Chapman C, van der Mei I, Williams D, McMichael AJ: **Sun exposure and vitamin D are independent risk factors for CNS demyelination.** *Neurology* 2011, **76**:540-8.

42. Burton JM, Kimball S, Vieth R, Bar-Or A, Dosch HM, Cheung R, Gagne D, D'Souza C, Ursell M, O'Connor P: **A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis.** *Neurology* 2010, **74**:1852-9.

43. Soilu-Hanninen M, Aivo J, Lindstrom BM, Elovaara I, Sumelahti ML, Farkkila M, Tienari P, Atula S, Sarasoja T, Herrala L, Herrala L, Keskinarkaus I, Kruger J, Kallio T, Rocca MA, Filippi M: **A randomised, double blind, placebo controlled trial with vitamin D3 as an add on treatment to interferon beta-1b in patients with multiple sclerosis.** *J Neurol Neurosurg Psychiatry* 2012, **83**:565-71.

    F1000Prime RECOMMENDED

44. Becklund BR, Severson KS, Vang SV, Deluca HF: **UV radiation suppresses experimental autoimmune encephalomyelitis independent of vitamin D production.** *Proc Nat Acad Sci USA* 2010, **107**:6418-23.

    F1000Prime RECOMMENDED

45. Breuer J, Schwab N, Schneider-Hohendorf T, Marziniak M, Mohan H, Bhatia U, Gross CC, Clausen BE, Weishaupt C, Luger TA, Meuth SG, Loser K, Wiendl H: **Ultraviolet B light attenuates the systemic immune response in central nervous system autoimmunity.** *Ann Neurol* 2014, **75**:739-58.

46. Zivadinov R, Treu CN, Weinstock-Guttman B, Turner C, Bergsland N, O'Connor K, Dwyer MG, Carl E, Ramasamy DP, Qu J, Ramanathan M: **Interdependence and contributions of sun exposure and vitamin D to MRI measures in multiple sclerosis.** *J Neurol Neurosurg Psychiatry* 2013, **84:**1075-81.

47. McLeod JG, Hammond SR, Kurtzke JF: **Migration and multiple sclerosis in immigrants to Australia from United Kingdom and Ireland: a reassessment.** *I. Risk of MS by age at immigration. J Neurol* 2011, **258:**1140-9.

48. Kurtzke JF, Beebe GW, Norman JE, Jr.: **Epidemiology of multiple sclerosis in US veterans: III. Migration and the risk of MS.** *Neurology* 1985, **35:**672-8.

49. Disanto G, Chaplin G, Morahan JM, Giovannoni G, Hypponen E, Ebers GC, Ramagopalan SV: **Month of birth, vitamin D and risk of immune mediated disease: a case control study.** *BMC Med* 2012, **10:**69.

F1000Prime RECOMMENDED

50. Saastamoinen KP, Auvinen MK, Tienari PJ: **Month of birth is associated with multiple sclerosis but not with HLA-DR15 in Finland.** *Multiple sclerosis (Houndmills, Basingstoke, England)* 2012, **18:**563-8.

F1000Prime RECOMMENDED

51. Staples J, Ponsonby AL, Lim L: **Low maternal exposure to ultraviolet radiation in pregnancy, month of birth, and risk of multiple sclerosis in offspring: longitudinal analysis.** *BMJ* 2010, **340:**c1640.

F1000Prime RECOMMENDED

52. Fernandes de Abreu DA, Landel V, Feron F: **Seasonal, gestational and postnatal influences on multiple sclerosis: the beneficial role of a vitamin D supplementation during early life.** *J Neurol Sci* 2011, **311:**64-8.

53. Ascherio A, Munger KL, White R, Kochert K, Simon KC, Polman CH, Freedman MS, Hartung HP, Miller DH, Montalban X *et al*: **Vitamin D as an early predictor of multiple sclerosis activity and progression.** *JAMA neurology* 2014, **71:**306-14.

F1000Prime RECOMMENDED

54. Hewison M: **Antibacterial effects of vitamin D.** *Nat Rev Endocrinol* 2011, **7:**337-45.

55. Gallo RL, Hooper LV: **Epithelial antimicrobial defence of the skin and intestine.** *Nat Rev Immunol* 2012, **12:**503-16.

56. Bals R, Wilson JM: **Cathelicidins–a family of multifunctional antimicrobial peptides.** *Cell Mol Life Sci* 2003, **60:**711-20.

57. Kahlenberg JM, Kaplan MJ: **Little peptide, big effects: the role of LL-37 in inflammation and autoimmune disease.** *J Immunol* 2013, **191:**4895-901.

58. Gombart AF, Bhan I, Borregaard N, Tamez H, Camargo CA, Jr., Koeffler HP, Thadhani R: **Low plasma level of cathelicidin antimicrobial peptide (hCAP18) predicts increased infectious disease mortality in patients undergoing hemodialysis.** *Clin Infect Dis* 2009, **48:**418-24.

59. Bals R, Weiner DJ, Moscioni AD, Meegalla RL, Wilson JM: **Augmentation of innate host defense by expression of a cathelicidin antimicrobial peptide.** *Infection and immunity* 1999, **67:**6084-9.

60. Bhan I, Camargo CA, Jr., Wenger J, Ricciardi C, Ye J, Borregaard N, Thadhani R: **Circulating levels of 25-hydroxyvitamin D and human cathelicidin in healthy adults.** *J Allergy Clin Immunol* 2011, **127:**1302-4 e1301.

61. Ralph AP, Lucas RM, Norval M: **Vitamin D and solar ultraviolet radiation in the risk and treatment of tuberculosis.** *Lancet Infect Dis* 2013, **13:**77-88.

62. Kamada N, Seo SU, Chen GY, Nunez G: **Role of the gut microbiota in immunity and inflammatory disease.** *Nat Rev Immunol* 2013, **13:**321-35.

63. Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, Davies J, Ervine A, Poulter L, Pachter L, Moffatt MF, Cookson WO: **Disordered microbial communities in asthmatic airways.** *PLoS One* 2010, **5:** e8578.

F1000Prime RECOMMENDED

64. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, Program NCS, Bouffard GG, Blakesley RW, Murray PR, Green ED, Turner ML, Segre JA: **Topographical and temporal diversity of the human skin microbiome.** *Science* 2009, **324:**1190-2.

F1000Prime RECOMMENDED

65. Gorman S, Weeden CE, Tan DH, Scott NM, Hart J, Foong RE, Mok D, Stephens N, Zosky G, Hart PH: **Reversible control by vitamin D of granulocytes and bacteria in the lungs of mice: an ovalbumin-induced model of allergic airway disease.** *PLoS One* 2013, **8:**e67823.

66. Lagishetty V, Misharin AV, Liu NQ, Lisse TS, Chun RF, Ouyang Y, McLachlan SM, Adams JS, Hewison M: **Vitamin D deficiency in mice impairs colonic antibacterial activity and predisposes to colitis.** *Endocrinology* 2010, **151:**2423-32.

67. Ooi JH, Li Y, Rogers CJ, Cantorna MT: **Vitamin D regulates the gut microbiome and protects mice from dextran sodium sulfate-induced colitis.** *J Nutr* 2013, **143:**1679-86.

F1000Prime RECOMMENDED

68. Scott CL, Aumeunier AM, Mowat AM: **Intestinal CD103+ dendritic cells: master regulators of tolerance?** *Trends in immunology* 2011, **32:**412-9.

69. Mai V, McCrary QM, Sinha R, Glei M: **Associations between dietary habits and body mass index with gut microbiota composition and fecal water genotoxicity: an observational study in African American and Caucasian American volunteers.** *J Nutr* 2009, **8:**49.

F1000Prime RECOMMENDED

70. Zeeuwen PL, Kleerebezem M, Timmerman HM, Schalkwijk J: **Microbiome and skin diseases.** *Curr Opin Allergy Clin Immunol* 2013, **13:**514-20.

71. Tremezaygues L, Reichrath J: **Vitamin D analogs in the treatment of psoriasis: Where are we standing and where will we be going?** *Dermatoendocrinol* 2011, **3:**180-6.

72. Gorman S, Kuritzky LA, Judge MA, Dixon KM, McGlade JP, Mason RS, Finlay-Jones JJ, Hart PH: **Topically applied 1,25-dihydroxyvitamin D3 enhances the suppressive activity of CD4+CD25+ cells in the draining lymph nodes.** *J Immunol* 2007, **179:**6273-83.

73. Reinholz M, Ruzicka T, Schauber J: **Cathelicidin LL-37: an antimicrobial peptide with a role in inflammatory skin disease.** *Annals of dermatology* 2012, **24:**126-35.

74. Bisgaard H, Vissing NH, Carson CG, Bischoff AL, Folsgaard NV, Kreiner-Moller E, Chawes BL, Stokholm J, Pedersen L, Bjarnadottir E, Thysen AH, Nilsson E, Mortensen LJ, Olsen SF, Schjørring S, Krogfelt KA, Lauritzen L, Brix S, Bønnelykke K: **Deep phenotyping of the unselected COPSAC2010 birth cohort study.** *Clin Exp Allergy* 2013, **43:**1384-94.

75. Appleyard CB, Cruz ML, Isidro AA, Arthur JC, Jobin C, De Simone C: **Pretreatment with the probiotic VSL#3 delays transition from inflammation to dysplasia in a rat model of colitis-associated cancer.** *Am J Physiol Gastrointest Liver Physiol* 2011, **301:**G1004-13.

F1000Prime RECOMMENDED

76. Rebel H, der Spek CD, Salvatori D, van Leeuwen JP, Robanus-Maandag EC, de Gruijl FR: **UV exposure inhibits intestinal tumor growth and progression to malignancy in intestine-specific Apc mutant mice kept on low vitamin D diet.** *Int J Cancer* 2014, **136:**217-7.

F1000Prime RECOMMENDED

77. Wada K, Tanaka H, Maeda K, Inoue T, Noda E, Amano R, Kubo N, Muguruma K, Yamada N, Yashiro M, Sawada T, Nakata B, Ohira M,

Hirakawa K: **Vitamin D receptor expression is associated with colon cancer in ulcerative colitis.** *Oncol Rep* 2009, **22:**1021-5.

F1000Prime RECOMMENDED

78. Korf H, Wenes M, Stijlemans B, Takiishi T, Robert S, Miani M, Eizirik DL, Gysemans C, Mathieu C: **1,25-Dihydroxyvitamin D3 curtails the inflammatory and T cell stimulatory capacity of macrophages through an IL-10-dependent mechanism.** *Immunobiology* 2012, **217:**1292-300.

F1000Prime RECOMMENDED

79. Waterhouse M, Tran B, Armstrong BK, Baxter C, Ebeling PR, English DR, Gebski V, Hill C, Kimlin MG, Lucas RM, Venn A, Webb PM, Whiteman DC, Neale RE: **Environmental, personal, and genetic determinants of response to vitamin D supplementation in older adults.** *J Clin Endocrinol Metab* 2014, **99:**E1332-40.

80. Liu PT, Wheelwright M, Teles R, Komisopoulou E, Edfeldt K, Ferguson B, Mehta MD, Vazirnia A, Rea TH, Sarno EN, Graeber TG, Modlin RL: **MicroRNA-21 targets the vitamin D-dependent antimicrobial pathway in leprosy.** *Nat Med* 2012, **18:**267-73.

F1000Prime RECOMMENDED

81. Adams JS, Hewison M: **Extrarenal expression of the 25-hydroxyvitamin D-1-hydroxylase.** *Arch Biochem Biophys* 2012, **523:**95-102.

F1000Prime RECOMMENDED

82. Zerwekh JE: **Blood biomarkers of vitamin D status.** *Am J Clin Nutr* 2008, **87:**1087S-91S.

83. Ginde AA, Liu MC, Camargo CA, Jr.: **Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004.** *Arch Intern Med* 2009, **169:**626-32.

84. Stokes CS, Volmer DA, Grunhage F, Lammert F: **Vitamin D in chronic liver disease.** *Liver Int* 2013, **33:**338-52.

85. Blanton D, Han Z, Bierschenk L, Linga-Reddy MV, Wang H, Clare-Salzler M, Haller M, Schatz D, Myhr C, She JX, Wasserfall C, Atkinson M: **Reduced serum vitamin D-binding protein levels are associated with type 1 diabetes.** *Diabetes* 2011, **60:**2566-70.

86. Moller UK, Streym S, Heickendorff L, Mosekilde L, Rejnmark L: **Effects of 25OHD concentrations on chances of pregnancy and pregnancy outcomes: a cohort study in healthy Danish women.** *Eur J Clin Nutr* 2012, **66:**862-8.

87. Armas LA, Hollis BW, Heaney RP: **Vitamin D2 is much less effective than vitamin D3 in humans.** *J Clin Endocrinol Metab* 2004, **89:**5387-91.

88. Houghton LA, Vieth R: **The case against ergocalciferol (vitamin D2) as a vitamin supplement.** *Am J Clin Nutr* 2006, **84:**694-7.

89. Lucas R, Group AI: **Lower vitamin D status at first clinical diagnosis of central nervous system demyelination compared to matched controls.** *J Multiple Sclr* 2009, **15:**1393.

90. Mata-Granados JM, Vargas-Vasserot J, Ferreiro-Vera C, Luque de Castro MD, Pavon RG, Quesada Gomez JM: **Evaluation of vitamin D endocrine system (VDES) status and response to treatment of patients in intensive care units (ICUs) using an on-line SPE-LC-MS/MS method.** *J Steroid Biochem Mol Biol* 2010, **121:**452-5.

91. Lappe JM, Heaney RP: **Why randomized controlled trials of calcium and vitamin D sometimes fail.** *Dermatoendocrinol* 2012, **4:**95-100.

F1000Prime RECOMMENDED

92. Durup D, Jorgensen HL, Christensen J, Schwarz P, Heegaard AM, Lind B: **A reverse J-shaped association of all-cause mortality with serum 25-hydroxyvitamin D in general practice: the CopD study.** *J Clin Endocrinol Metab* 2012, **97:**2644-52.

F1000Prime RECOMMENDED

93. Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, Nicholson GC: **Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial.** *JAMA* 2010, **303:**1815-22.