Editorial from Guest Editor

Vitamin D and Respiratory Health

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This Hot Topic edition of *Current Respiratory Medicine Reviews* considers the literature suggesting that vitamin D status is a key determinant of respiratory health. At first sight, the proposition that deficiency of a single micronutrient could be implicated in the pathogenesis of conditions as diverse as asthma, allergy, respiratory infection and lung cancer may seem biologically implausible. However, a large and growing body of evidence from both the laboratory and the clinic suggests that associations between vitamin D deficiency and susceptibility to these conditions may well be causal.

Humans acquire vitamin D *via* cutaneous synthesis as a result of the action of solar ultraviolet B radiation on 7-dehydrocholesterol in the skin, or from the diet, principally by consumption of oily fish or dietary supplements. Vitamin D from either source is metabolised by the liver to form 25-hydroxyvitamin D (25[OH]D), the major circulating vitamin D metabolite whose serum concentration defines vitamin D status. 25(OH)D is metabolised by the enzyme 25(OH)D-1α-hydroxylase (CYP27B1) to form 1,25-dihydroxyvitamin D ($1,25[OH]_2D$), the steroid hormone and active metabolite of vitamin D. $1,25(OH)_2D$ induces biological actions by ligating vitamin D receptor (VDR), which acts as a transcription factor, regulating the expression of vitamin D-responsive genes [1].

The clinical features of vitamin D deficiency were first described in 1651, when Glisson, Bate and Regemorter published 'A treatise of the rickets: being a disease common to children' [2]. For many years, bone disease was regarded as the only clinical manifestation of inadequate vitamin D status; CYP27B1 was thought to be expressed predominantly in the kidney and $1,25(OH)_2D$ was regarded primarily as a hormone of calcium homeostasis. This perception changed in 1981, when Barbour and colleagues reported a case of hypercalcaemia in an anephric patient with sarcoidosis who was found to have an elevated serum $1,25(OH)_2D$ concentration [3]; this observation provided the first evidence that 1-alpha hydroxylation of 25(OH)D could occur extra-renally. CYP27B1 activity has subsequently been demonstrated in macrophages [4], dendritic cells [5], lymphocytes [5] and pulmonary epithelial cells [6]. In the same year, Abe and colleagues reported expression of VDR in a murine myeloid leukaemia cell line [7], raising the possibility that vitamin D might possess immunomodulatory activity. VDR has subsequently been shown to be expressed in human monocytes, macrophages, B and T lymphocytes [8], in dendritic cells [9] and in both normal and malignant lung tissue [10]; moreover, $1,25(OH)_2D$ has been shown to exert antimicrobial, anti-inflammatory and anti-proliferative actions on these cells *in vitro* [11]. Taken together, these observations indicate that $1,25(OH)_2D_3$ can be synthesised by, and can exert biological activity in, many cell populations in the lung.

The articles presented here encompass the effects of vitamin D status on respiratory health throughout the lifespan. Steven Goldring, John Warner, Seif Shaheen and Robert Boyle open with a review of the clinical evidence linking antenatal vitamin D status with subsequent risk of asthma and atopy. Virender Rehan and John Torday proceed to enlarge on the molecular mechanisms by which perinatal vitamin D deficiency may contribute to asthma pathogenesis in the developing lung, before Graham Devereux and James Wagner discuss insights into the actions of vitamin D provided by animal models and consider the evidence that vitamin D status may influence incidence and severity of asthma in children and adults. Abigail Jackson and Nicholas Hopkinson then review the potential implications of vitamin D deficiency for respiratory, musculoskeletal and cardiovascular health in patients with COPD. Emerging evidence of the potential importance of vitamin D deficiency in susceptibility to acute respiratory infection is then reviewed by Yasmeen Hanifa and Robert Walton, before Kirsten Mitchell, Chris Griffiths and myself provide an update of the evidence linking vitamin D deficiency and susceptibility to tuberculosis. Helga Rhein presents the case for conducting trials to determine whether vitamin D supplementation may prolong survival in patients with lung cancer, before Anna Coussens wraps up the issue with an overview of vitamin D metabolism and of the immunomodulatory actions of $1,25(OH)_2D$ that underpin its many functions.

The evidence reviewed above is tantalising, but much of it comes from *in vitro* studies and observational epidemiology; randomised clinical trials are now needed to determine whether this scientific promise will translate into a clinical benefit. The challenges in conducting trials of vitamin D supplementation are significant: prevention trials, in particular, will require large sample sizes and prolonged

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follow-up in order to demonstrate clinically plausible effect sizes. Even modest effects are worthy of detection however: vitamin D supplementation is safe, simple to administer and inexpensive, and if it is eventually shown to benefit patients with common and debilitating respiratory disease then the benefits to public health will be very significant.

REFERENCES

[1] Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357(3): 266-81.

[2] Glisson F, Bate G, Regemorter A. A treatise of the rickets: being a disease common to children. London: P. Cole; 1651.

[3] Barbour GL, Coburn JW, Slatopolsky E, Norman AW, Horst RL. Hypercalcemia in an anephric patient with sarcoidosis: evidence for extrarenal generation of 1,25-dihydroxyvitamin D. N Engl J Med 1981; 305(8): 440-3.

[4] Adams JS, Sharma OP, Gacad MA, Singer FR. Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis. J Clin Invest 1983; 72(5): 1856-60.

[5] Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. Curr Opin Pharmacol 2010; 10(4): 482-96.

[6] Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. J Immunol 2008; 181(10): 7090-9.

[7] Abe E, Miyaura C, Sakagami H, *et al*. Differentiation of mouse myeloid leukemia cells induced by 1 alpha,25-dihydroxyvitamin D3. Proc Natl Acad Sci USA 1981; 78(8): 4990-4.

[8] Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. 1,25-dihydroxyvitamin D3 receptors in human leukocytes. Science 1983; 221(4616): 1181-3.

[9] Brennan A, Katz DR, Nunn JD, *et al*. Dendritic cells from human tissues express receptors for the immunoregulatory vitamin D3 metabolite, dihydroxycholecalciferol. Immunology 1987; 61(4): 457-61.

[10] Menezes RJ, Cheney RT, Husain A, *et al*. Vitamin D receptor expression in normal, premalignant, and malignant human lung tissue. Cancer Epidemiol Biomarkers Prev 2008; 17(5): 1104-10.

[11] Hughes DA, Norton R. Vitamin D and respiratory health. Clin Exp Immunol 2009; 158(1): 20-5.

Early Life Vitamin D Status and Lung Development

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Abstract: There is evidence of an association between early life vitamin D insufficiency and future risk of developing asthma. Given the high prevalence of vitamin D insufficiency in women during pregnancy when developmental programming is occurring, this may be of critical public health importance. There are plausible biological mechanisms for an association. Vitamin D is the pro-hormone of calcitriol, a secosteroid hormone with widespread pleiotropic actions. It is a powerful immune modulator and has been shown in animal and *in vitro* work to have a role in early lung development. Calcitriol may influence lung development through expression of the vitamin D receptor on lung and immune cells, and through epigenetic mechanisms. If the association between early life vitamin D status and childhood respiratory disease is shown to be causal, then this could have significant implications for public health policy. This hypothesis is currently being tested in a number of prospective intervention trials. The aim of this article is to review the evidence that vitamin D status influences early lung development, with a focus on early life mechanisms.

Keywords: Asthma, diet, lung development, pregnancy, vitamin D.

INTRODUCTION

Abnormal early lung development is seen in respiratory diseases that present in childhood or later life [1]. For asthma in particular, there is a large body of evidence suggesting that early life influences are important in disease pathogenesis, and micronutrient status may be particularly important. Changing environmental factors acting on genetically susceptible individuals are likely to explain the increase in asthma prevalence in the last century; however the precise environmental factors which increase asthma risk remain unidentified [2]. Early life vitamin D status is one such proposed environmental factor. As discussed elsewhere in this supplement, there are intriguing parallels in the prevalence of asthma and vitamin D deficiency, and mechanistic reasons why the relationship may be causal. Apparently contradictory hypotheses argue that either high [3], or low [4] vitamin D status, at critical times in development have contributed to the asthma epidemic. The nature of calcitriol's influence on early immune and lung development, and the dose dependency of this influence illustrate how these hypotheses may not be mutually exclusive, and how such biological effects may be possible. The purpose of this article is to review the potential role of vitamin D in early lung development, on the basis that vitamin D status is an easily modifiable variable which might influence lung development and specifically asthma risk.

EARLY LIFE NUTRITIONAL INFLUENCES ON LUNG DEVELOPMENT

The respiratory system originates as an outgrowth of the primitive foregut between the 4th and 7th week of human gestation [5]. Through timely orchestration of a complex array of transcription factors, growth factors and physical forces, [6] epithelial tubules invaginate the surrounding splanchnic mesenchyme to form the bronchial tree. Key processes are branching morphogenesis, angiogenesis, epithelial cell differentiation, sacculation, surfactant production and alveolarisation, the latter being predominantly a postnatal event in humans [1]. Epithelial-mesenchyme interactions play a crucial role [7].

Early life influences on this process include both genetic and environmental factors, and minor alterations during foetal and perinatal life may have significant postnatal consequences. For example they may alter responses to early life infections and other airway insults that are implicated in the aetiology of asthma [8, 9]. Several long term cohort studies have established that infant lung function tracks into later life, suggesting that successful modification of early lung development may influence lung function in the long term [10, 11].

Known environmental, and nutritional factors which may influence lung development [12, 13] include cigarette smoking [14], polyunsaturated fatty acids [15, 16] milk fat [17], vitamin E [18], and vitamin A [19]. The data regarding vitamin A are particularly relevant to this article, due to the common signalling pathway shared by the activated forms of vitamins A and D. The active metabolite of vitamin A, retinoic acid (RA), is a critical signalling molecule in early lung development with maternal deficiency causing profound abnormalities of the infant respiratory system, including tracheoesophageal fistula, lung hypoplasia and lung agenesis [20]. Mechanisms include RA effects on TGF-β and FGF10 pathways at prospective sites of lung formation [21]. RA also influences alveolarisation [1] so that postnatal treatment with RA increases the number of pulmonary alveoli in rats [22]. Results of a recent randomized controlled trial of vitamin A supplementation in a population of married women of childbearing age with chronic vitamin A deficiency in rural Nepal confirm the relevance of these

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mechanistic findings to respiratory health in humans. At age 9 to 13 years, offspring of mothers who received vitamin A before, during and for 6 months after pregnancy had significantly improved lung function compared with a placebo group (mean increase in FEV_1 46mls, 95% CI 6-86mls) [19]. All children in the trial received supplemental vitamin A from 6 months of age, suggesting that vitamin A status from preconception through to age 6 months influences long term lung development. It should be noted that data regarding potential confounders such as later nutritional status were not reported in this trial. However the study does provide evidence that maternal micronutrient status can influence fetal/infant lung development with significant consequences for longer term respiratory health in humans.

VITAMIN D

Vitamin D is a pro-hormone obtained from either diet or photosynthesis in the skin by the action of ultraviolet B light on 7-dihydrocholesterol. To become metabolically active vitamin D undergoes two hydroxylations. The first occurs primarily in the liver to form 25-hydroxyvitamin D (25(OH)D3) and the second, more tightly regulated hydroxylation occurs mainly in the kidney to form the biologically active hormone 1,25-dihydroxyvitamin D ($1,25(OH)_2D3$), also known as calcitriol, *via* the action of a mitochondrial P450 hydroxylase, CYP27B1. Many extra-renal cells also express both the vitamin D receptor (VDR) and CYP27B1 so that $1,25(OH)_2D3$ is able to act as an intracrine and paracrine hormone in many tissues. This is the basis of calcitriol's widespread pleiotropic actions including effects on the pulmonary and immune systems [23].

The actions of $1,25(OH)_2D3$ are mediated *via* two pathways, a slow genomic response and a rapid, non-genomic response [24, 25]. Both involve binding to its nuclear receptor, the vitamin D receptor (VDR). In the genomic pathway VDR functions as a heterodimer with the retinoid X receptor (RXR). After binding, VDR undergoes a conformation change that promotes RXR-VDR heterodimerization. The bound heterodimer translocates to the nucleus where VDR binds to vitamin D responsive elements and ultimately modifies gene transcription [26, 27]. The expression of over 200 genes are controlled *via* $1,25(OH)_2D3$/ VDR dependant pathways acting on vitamin D responsive elements [28]. In the non -genomic pathway, $1,25(OH)_2D3$ binds to VDR present in the caveolae of the plasma membrane, forming ligand bound VDR which activates signalling cascades including protein kinase C and phospholipase C [29]. Rapid responses triggered by this pathway include intestinal absorption of calcium and the secretion of insulin by pancreatic β-cells [25].

VITAMIN D METABOLISM IN EARLY LIFE

The vitamin D status of a pregnant women directly influences that of her foetus/infant [30]. During the course of pregnancy, maternal levels of 25(OH)D3 modestly decline and at delivery, cord 25(OH)D3 levels are typically ≤ 20% lower than maternal levels [31, 32]. The decidua and placenta have large amounts of CYP27B1 enzyme activity resulting in generation of $1,25(OH)_2D3$ [33] During pregnancy methylation of placental CYP24A1, the enzyme responsible for catabolism of $1,25(OH)_2D3$ to inactive metabolites, represses transcription of this gene. Thus maternal $1,25(OH)_2D3$ levels increase as high as two fold in late pregnancy compared to postpartum or non pregnant controls [34].

$1,25(OH)_2D3$ does not readily cross the placenta and combined with foetal suppression of renal CYP27B1 by low PTH and phosphorous levels, circulating foetal 1,25(OH)D3 concentrations are significantly lower than maternal values in humans [31]. Postnatally, foetal dependency on maternal vitamin D may manifest in infants of deficient mothers with hypocalcaemia and rickets. Breast milk has a low vitamin D and 25(OH)D3 content unless the mother takes supplements [31] and so exclusively breastfed infants are largely dependent on their prenatally acquired vitamin D stores or supplementation. For this reason infant formula is supplemented with vitamin D, and many countries have a policy of routine vitamin D supplementation for breastfed infants [35].

PERINATAL VITAMIN D DEFICIENCY – PREVALENCE AND IMPACT ON RESPIRATORY OUTCOMES

Early life hypovitaminosis D is common and increasingly recognized as a global public health issue [36]. The optimum level for health is not known but a consensus statement has identified concentrations ≥75nmol/L as ideal for healthy bones [37]. Table **1** lists recent studies describing the prevalence of hypovitaminosis D amongst pregnant women and their newborn infants in different populations. At risk groups include ethnicities with pigmented skin, wearing concealing clothing [38-40], poor uptake of vitamin D supplementation [36, 41-44] and prolonged breast feeding [35]. Hypovitaminosis D is also common in pregnant white women with no additional 'risk factors' [45]. A recent genome wide association study has identified common genetic variants that identify individuals at increased risk of vitamin D insufficiency [46].

Current recommendations for vitamin D supplementation vary worldwide. In the UK, pregnant women and at risk groups are advised to take 400IU daily, However, it is acknowledged that current guidance from health care providers to pregnant women is inconsistent and a barrier to effective primary prevention of hypovitaminosis D [45]. A recent editorial has called for urgent intervention to address early life hypovitaminosis D [47].

A number of studies have shown a relationship between early life vitamin D status and respiratory outcomes (Table **2**). Studies of maternal dietary intake of vitamin D in pregnancy have suggested a protective effect of a higher intake on early childhood wheezing [48-50], asthma and allergic rhinitis [51], and eczema [50]. In contrast, in another birth cohort study, the highest maternal blood 25(OH)D3 concentrations were associated with an increased risk of asthma at 9 years of age in the offspring [52]. It should be noted that the gold standard measure of vitamin D status is the blood concentration of 25(OH)D3, as it captures vitamin D exposure from diet and sunlight combined. In contrast, dietary intake of vitamin D makes a relatively small contribution to overall vitamin D status, and apparent effects

Table 1. Vitamin D Status of Pregnant Women and their Offspring: Recent Studies

Setting	Population	Cord Blood 25(OH)D3 Levels	Maternal 25(OH)D3 Levels
US national population survey [100]	928 unselected pregnant women	NA	33%<50nmol/L
US antenatal clinic [101]	433 unselected pregnant women; 376 newborns	38%<37.5nmol/L	23%<37.5nmol/L
US antenatal clinic [102]	80 pregnant black adolescents	NA	21%<37.5nmol/L
UK antenatal clinic [52]	466 pregnant Caucasian women	NA	49.5% <50nmol/L
UK antenatal clinic [103]	263 pregnant adolescents	NA	30% <25nmol/L
UK antenatal clinic [104]	180 pregnant women at 27 weeks gestation 45 Asian 45 Middle Eastern 45 Black 45 Caucasian	NA	% <25nmol/L: 47% 64%; 58%; 13%
Netherlands antenatal clinic [44]	86 pregnant women: 48 'high risk' (dark skin or veiled), 38 'controls' (light skin, not veiled)	%<25nmol/L 63% 16%	NA
US antenatal clinic [41]	400 pregnant women 200 black 200 white	<37.5nmol/L 46% 10%	<37.5nmol/L 45% 2%
Australian antenatal clinic [43]	971 unselected women at 28 weeks gestation; 901 neonates	11%<25nmol/L	15% <25nmol/L [Veiled 71%; Non veiled 11%]
New Zealand primary care antenatal clinic [105]	90 unselected pregnant women		61% deficient

All values have been converted to nmol/L where 1 ng/ml = 2.5 nmol/L.

of vitamin D intake may be confounded by other correlated nutrients. Finally, vitamin D supplementation with 2000IU during infancy in Finland was associated with an increased risk of atopy and allergic rhinitis in adulthood [53]. The finding of an increase in serum total IgE at both low (<25nmol/L) and high (>135nmol/L) blood concentrations of 25(OH)D3 in a recent large British adult cohort suggests a possible mechanism by which both high and low vitamin D status could be associated with increased asthma risk [54].

MECHANISMS THROUGH WHICH EARLY LIFE VITAMIN D STATUS MIGHT INFLUENCE LATER RESPIRATORY HEALTH

Effects of Vitamin D on Early Lung Development: Animal Data

A number of separate observations from animal work suggest that alveolarisation may be influenced in part by a complex growth axis which involves $1,25(OH)_2D3$. First, an early study of the offspring of vitamin D deprived rats showed a significant decrease in lung compliance (CL) and CL to dry lung weight ratio compared with the offspring of vitamin D sufficient rats, suggesting vitamin D deficiency may decrease lung distensibility through an effect on interstitial tissue modelling [55]. Second, it is known that the VDR is expressed during late intrauterine life in rat pulmonary tissues [56, 57]. This is during the period of maturation of alveolar type two (ATII) cells which synthesise and release surfactant [58, 59]. $1,25(OH)_2D3$ stimulates DNA synthesis in ATII cells [60], and the discovery that lung fibroblasts are capable of metabolising 25(OH)D3 to $1,25(OH)_2D3$ identified a paracrine system for vitamin D in the developing rat lung [61].

Other data suggest a role for $1,25(OH)_2D3$ in lung fibroblast development, which may in turn influence alveolarisation. $1,25(OH)_2D3$ significantly increases proliferation of rat perinatal lung fibroblasts, an effect which is greatest in combination with RA on postnatal day 4 (PN4) [62]. The same authors showed that this effect is dependent on platelet derived growth factor-AB (PDGF-AB). The PDGF null mouse has a bronchopulmonary dysplasia (BPD) like picture with an absence of fibroblasts and abnormal septation, and PDGF appears to be essential for normal alveolarisation of the developing lung [63]. PDGF contains a transcriptional control region containing response elements to $1,25(OH)_2D3$ [64] and vitamin A [65]. Thus a proposed mechanism of action for $1,25(OH)_2D3$ in promoting lung development is *via* a synergistic effect with RA in stimulating growth of immature fibroblasts in the postnatal lung, with PDGF as the critical effector [62]. Further experiments have demonstrated a role for the vitamin D analogue EB1089 on fibroblast proliferation but suggested, contrary to other work, that relatively high doses of this vitamin D analogue may disrupt alveolarisation [66]. Histological findings in EB1089-treated rats include increased alveolar chord length and prominent regions of fibroblast hypercellularity. These stained strongly for alveolarisation growth factors PDGF-AA and vascular endothelial growth factor (VEGF) and the overall effect was

Table 2. Observational Associations Between Early Life Vitamin D Status and Respiratory Health

Setting	Design	Follow Up	Method	Outcome	Result	Comment
Boston, USA [49]	Birth cohort n=1194	Age 3	Maternal diet FFQ	Recurrent wheezing	OR 0.39 (95%CI 0.25, 0.62) highest *vs* lowest quartile	Protective
Aberdeen [48]	Birth cohort n=1212	Age 5	Maternal diet FFQ	Ever wheezing Wheeze in previous year Persistent wheeze	OR 0.48 (95%CI 0.25, 0.91) highest *vs* lowest quintile OR 0.35 (95%CI 0.15, 0.83) OR 0.33 (95%CI 0.11, 0.98).	Protective
Finland [51]	Birth cohort n=1669	Age 5	Maternal diet FFQ	Asthma Allergic rhinitis	HR 0.80 (95%CI 0.64, 0.99) HR 0.85 (95%CI 0.75, 0.97)	Protective
Japan [50]	Birth cohort n=764 mother child pairs	Age 16-24 months	Maternal diet FFQ	Wheeze	OR 0.64 [95%CI 0.43, 0.97]	Protective
Southampton [52]	Birth cohort n=178	Age 9	Maternal serum 25(OH)D	Asthma	OR 5.40 (1.09, 26.65) For children of mothers with vitamin D >75nmol/L *vs* <30nmol/L	Increased risk
Finland [53]	Infant cohort n=12,058	Age 31 (63% f/up)	Infant vitamin D supplement	Allergic rhinitis Asthma Atopy	OR 1.66 (1.10, 1.60) OR 1.35 (0.99-1.80) OR 1.46 (1.4-2.0)	Increased risk

to promote fibroblast proliferation but disrupt alveolarisation [66]. Whether this disruptive effect is dose dependant or related to other effects of the vitamin D analogue in question is not known.

A third mechanism through which $1,25(OH)_2D3$ may influence alveolarisation is *via* the inhibition of apoptosis in lipid laden interstitial lung fibroblasts (LF) [67]. LF cells become highly apoptotic during the first 1-2 weeks of postnatal life in rats, a developmental process that contributes to alveolar wall thinning and alveolarisation [68]. Both *in vitro* treatment (at embryological day 19, [ED19]) and *in vivo* treatment (postnatal days 0 to 14, [PN0-14]) with $1,25(OH)_2D3$ resulted in dose-dependent increases in LF and ATII cell proliferation and decreased apoptosis, accompanied by increases in the expression of epithelial mesenchyme differentiation markers [67]. These data suggest a role of $1,25(OH)_2D3$ in alveolar thinning. $1,25(OH)_2D3$ may have a specific role in transcriptional regulation of Lgl1 (late gestation lung 1) during alveolarisation [69]. Lgl1 is a mesenchymal protein found in foetal lung that regulates epithelial airway branching and is maximally expressed in late gestation and early postnatal life [70]. In Lgl1 null mice, absence of Lgl1 is lethal prior to lung formation. Heterozygotes also display an abnormal respiratory phenotype. *In vitro* experiments in rat lung fibroblasts from E18 to PN14 suggest $1,25(OH)_2D3$ has a complex interplay with glucocorticoid (GC) and RA in the control of Lgl1. $1,25(OH)_2D3$ directly inhibits Lgl1 mRNA production, and inhibits GC induction of Lgl1. When combined with RA there was no effect on GC induction of Lgl1 gene transcription. Lgl1 may be a mesenchymal mediator of $1,25(OH)_2D3$ effects on epithelial maturation, with regulation of the target gene promoter by GC and RA as well as $1,25(OH)_2D3$ [69].

Further, less direct, evidence of a role for vitamin D status in foetal lung development come from studies of VDUP1 (vitamin D up regulated protein 1) expression in the ovine foetal lung. The basal level of lung expansion is known to be an important determinant of airway epithelial cell phenotype [71]. VDUP1 is an intracellular protein whose expression is upregulated by vitamin D3 administration [72]. In sheep lung, VDUP1 is localized to airway epithelium in small bronchioles, AEC's and mesenchymal cells. Expression of VDUP1 mRNA increase significantly during the alveolar stage of lung development [73]. Furthermore, VDUP1 mRNA correlate with different levels of lung expansion, with increased levels in under expanded lungs and decreased levels in over expanded lungs compared to normal controls [73]. It is well known that pressure effects in the airways induced by respiratory movements, contraction and relaxation of smooth muscle in the airway wall and the pressure of amniotic fluid have a potent influence on airway and alveolar development. Reduced amniotic fluid is associated with lung hypoplasia, and reduced foetal respiratory effort (eg opiate-dependent mothers) has a profound effect on post-natal lung function. Thus it is possible that VDUP1 is a vitamin D status dependent moderator of foetal lung growth and development in response to changes in foetal lung expansion [73].

Finally, in mature rats, VDR expression is present in lung fibroblasts and $1,25(OH)_2D3$ appears to have a role. Recent work shows that $1,25(OH)_2D3$ inhibited TGFβ1-induced fibroblast proliferation, and blunted TGFβ1-induced upregulation of mesenchymal cell markers and abnormal expression of epithelial cell markers. TGFβ is expressed during branching morphogenesis and has an important role in modelling of the airway wall by stimulating fibroblast collagen production [74]. Thus vitamin D status may have a homeostatic role in the developing lung, preventing some of the effects of excess TGFβ on the developing epithelial-mesenchymal trophic unit, which are thought to lead to changes such as the airway remodelling commonly seen in asthma.

Effects of Vitamin D on Early Lung Development: Human Data

Rachitic respiratory distress has been described very early in infancy, for example in small preterm infants [75]. This suggests that vitamin D deficiency of prenatal origin can have a profound acute effect on early lung function. As with the animal studies, there is also evidence in human studies that $1,25(OH)_2D3$ influences different components of lung development including fibroblast proliferation and alveolarisation. The VDR has been detected in human foetal lung fibroblasts from 16 weeks gestation, [76, 77] and in ATII cells from the second trimester [78]. In ATII cells, VDR expression is largely dependent on induction by $1,25(OH)_2D3$ [78]. After incubation of a human ATII cell line with $1,25(OH)_2D3$, a number of metabolites are also formed including 1,25-(OH)2-3-epi-VD3, which can increase surfactant phospholipid synthesis, surfactant SP-B mRNA gene expression and surfactant SP-B protein synthesis in pulmonary ATII cells [79]. However the effects of vitamin D metabolites on human lung development are likely to be complex since decreased, increased and no effect on surfactant protein synthesis have been observed on treating human fetal lung explants and ATII cells with calcitriol [78].

In the mature human lung, data are now emerging as to the cellular targets that may be influenced by vitamin D status. The VDR is expressed in the epithelia of normal and malignant bronchial tissue [80], in lung epithelial cells treated with vitamin D metabolites [81], and in airway smooth muscle (ASM) cells which demonstrate a large array of changes in gene expression in response to stimulation by $1,25(OH)_2D3$ [82]. In ASM cells that were passively sensitized with serum from an asthmatic, $1,25(OH)_2D3$ suppressed proliferation and significantly down regulated expression of matrix metalloproteinases-9 (MMP-9) and a disintegrin and metalloprotease 33 (ADAM33); both of these being significant promoters of airway remodelling [83-85]. Polymorphisms of ADAM 33 are associated with a higher risk of asthma particularly in relation to bronchial hyper-responsiveness. In British Bangladeshi adults, 25(OH)D3 status has been found to be inversely associated with plasma MMP-9 levels, with supplementation in vitamin D deficient individuals leading to reduced MMP-9 levels *in vivo* [86]. Invitro $1,25(OH)_2D3$ inhibits MMP-9 expression by M. Tuberculosis in cell culture [87]. Therefore any effects of vitamin D status on lung development may be mediated through effects on MMP-9 levels in plasma or lung tissue. Finally, in bronchial ASM samples taken during surgery, $1,25(OH)_2D3$ decreased PDGF induced cell proliferation [88].

Whilst speculative, if such relationships are present in the developing lung this could explain potential benefits of vitamin D metabolites on future lung health. In light of current concepts that molecular regulators originally associated with developmental processes may be implicated in adult diseases such as chronic obstructive airway disease [1] this is of great interest, and suggests a potential role for maintaining adequate vitamin D repletion to ensure 25(OH)D3 and 1,25(OH)D3 are available to the tissues for treatment, or prevention, of airway remodelling in asthma [89].

Effects of Vitamin D Status on Early Immune Development

$1,25(OH)_2D3$ is now widely recognized as having a key role in immune signalling, with well defined actions as a potent promoter of tolerogenic dendritic cells and regulatory T cells, and an inducer of antimicrobial peptide secretion in response to innate immune stimuli [23]. These effects occur through the local conversion of 25(OH)D3 to the biologically active $1,25(OH)_2D3$ [90] by CYP27B1 in immune cells [90]. It is possible that global deficiency or excess of 25(OH)D3 in the whole organism may lead to adverse consequences on immune development. Healthy immune development is in turn critical for lung development, since immune deficiency increases the risk of damaging pulmonary infections, and allergic airway inflammation is strongly associated with the development and persistence of asthma [91]. In animal models the principle that vitamin D deficiency at a critical time point may adversely affect immune development has been supported - developmental deficiency of vitamin D in rats induces alterations in immune organ morphology and function in adult offspring, with an increase in both immune organ size and in the pro-inflammatory IL-2 response by lymphocytes [92]. Other animal work has shown decreased allergen-induced airway hyper-responsiveness (AHR) in mice deficient in VDR, and VDR knockout mice fail to develop experimental allergic airway disease suggesting that VDR expression may be necessary for lung inflammation to occur [93, 94]. However other authors have reported that $1,25(OH)_2D3$ inhibits airway inflammation and decreases IL-4 levels in bronchoalveolar lavage (BAL) fluid and may do this through impairing T cell migration [95]. In separate experiments irradiation with a single minimal erythemal dose of UVB light prior to sensitization with antigen blunted airway hyper-responsiveness and inflammation [96]. In a murine model of allergic airway disease, treatment with calcitriol during immunizations enhanced IL-4 and IL-13 responses, whereas later treatment inhibited Il-5 and airway eosinophilia [97]. These data illustrate the complexity of 1,25(OH)D3's role in inflammatory responses involved in the pathogenesis of asthma. Their contradictory nature may relate to the differences between mouse models of allergic asthma and the human disease, or to the critical importance of dose, type of vitamin D supplement and timing of administration for optimal immune effects.

CURRENT VITAMIN D INTERVENTION TRIALS

Five prospective randomized controlled trials of vitamin D supplementation during pregnancy are currently in progress, and will shed some light on the relevance of the mechanisms described above to human health. In the United States, the 'Maternal Vitamin D Supplementation to Prevent Childhood asthma' (VDAART) study, will randomise 870 women carrying a foetus at high risk of asthma, to receive 4000IU of cholecalciferol vitamin D daily, or placebo from 10-18 weeks gestation to delivery. The primary outcome measure is asthma or recurrent wheeze at 1 year and 3 years. Also in the united States, Hollis *et al.* are conducting a three arm randomised trial of ether 400, 2000 or 4000IU of vitamin D per day from 12 weeks gestation in healthy women (NCT00292591). Primary outcomes are 25(OH)D3

levels throughout pregnancy and bone mineral density in mother and infant. To our knowledge this trial is not evaluating respiratory or atopic outcomes in the offspring. In the Copenhagen Vitamin D Supplementation During Pregnancy for Prevention of Asthma in Childhood (ABCvitaminD; ISRCTN NCT00856947), 600 women will be randomised to receive either 2400IU daily of cholecalciferol vitamin D or placebo in addition to 400IU of vitamin D daily from 24 weeks gestation to 1 week after delivery. The primary outcome is recurrent wheeze in the first 3 years. This is a factorial randomised controlled trial with a second intervention of prenatal fish oil or no fish oil. In Southampton the Maternal Vitamin D Osteoporosis Study (MAVIDOS; ISRCTN 82927713) is randomising 1074 pregnant women with a 25(OH)D3 concentration of 25-100nmol/l during pregnancy who receive vitamin D supplementation at a dose determined in pilot work, or placebo from 14 weeks gestation to delivery. Primary outcome measures relate to bone health, but the trial may also yield information on pulmonary and atopic outcomes at follow up. In our own study (ISRCTN 68645785) 180 women in London with a high prevalence of vitamin D deficiency, were randomised to no additional vitamin D in pregnancy, 200,000IU of calciferol once at 27 weeks gestation or 800IU daily of ergocalciferol from 27 weeks to delivery. The primary outcome measure is history of wheezing during the first 3 years of life, and this study is due to report in 2011.

CONCLUSIONS

The precise relationship between vitamin D status and asthma, and the impact of dietary vitamin D supplementation on this risk, are unclear. Potential mechanisms through which perinatal vitamin D may influence asthma or lung development include increased immune regulation preventing atopic immune responses, increased microbial killing capacity limiting the host consequences of viral infections, and direct effects on lung growth and development particularly on alveolarisation, fibroblast proliferation and airway smooth muscle. Interactions with correlated nutrients such as vitamin A, which interacts with vitamin D metabolites in essential ways but can antagonise in excess, may complicate this relationship [98, 99].

Current intervention trials evaluating the effects of early life vitamin D supplementation at a range of doses, in a range of populations and using a range of treatment schedules will play a critical role in establishing the possible role that vitamin D may play in this regard.

REFERENCES

[1] Shi W, Chen F, Cardoso WV. Mechanisms of lung development: contribution to adult lung disease and relevance to chronic obstructive pulmonary disease. Proc Am Thorac Soc 2009; 6(7): 558-63.

[2] Warner JO. Early life nutrition and allergy. Early Hum Dev; 83(12): 777-83.

[3] Wjst M, Dold S. Genes, factor X, and allergens: what causes allergic diseases? Allergy 1999; 54(7): 757-9.

[4] Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? J Allergy Clin Immunol 2007; 120(5): 1031-5.

[5] Groenman F, Unger S, Post M. The molecular basis for abnormal human lung development. Biol Neonate 2005; 87(3): 164-77.

[6] Maeda Y, Dave V, Whitsett JA. Transcriptional control of lung morphogenesis. Physiol Rev 2007; 87(1): 219-44.

[7] Shannon JM, Hyatt BA. Epithelial-mesenchymal interactions in the developing lung. Annu Rev Physiol 2004; 66: 625-45.

[8] Holgate ST. The airway epithelium is central to the pathogenesis of asthma. Allergol Int 2008; 57(1): 1-10.

[9] Bisgaard H, Loland L, Holst KK, Pipper CB. Prenatal determinants of neonatal lung function in high-risk newborns. J Allergy Clin Immunol 2009; 123(3): 651-7, 7 e1-4.

[10] Stern DA, Morgan WJ, Wright AL, Guerra S, Martinez FD. Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. Lancet 2007; 370(9589): 758-64.

[11] Turner SW, Palmer LJ, Rye PJ, *et al*. The relationship between infant airway function, childhood airway responsiveness, and asthma. Am J Respir Crit Care Med 2004; 169(8): 921-7.

[12] Devereux G. Session 1: Allergic disease: Nutrition as a potential determinant of asthma. Proc Nutr Soc 2010; 69(1): 1-10.

[13] Kajekar R. Environmental factors and developmental outcomes in the lung. Pharmacol Ther 2007; 114(2): 129-45.

[14] Dezateux C, Stocks J, Dundas I, Fletcher ME. Impaired airway function and wheezing in infancy: the influence of maternal smoking and a genetic predisposition to asthma. Am J Respir Crit Care Med 1999; 159(2): 403-10.

[15] Marks GB, Mihrshahi S, Kemp AS, *et al*. Prevention of asthma during the first 5 years of life: a randomized controlled trial. J Allergy Clin Immunol 2006; 118(1): 53-61.

[16] Olsen SF, Osterdal ML, Salvig JD, *et al*. Fish oil intake compared with olive oil intake in late pregnancy and asthma in the offspring: 16 y of registry-based follow-up from a randomized controlled trial. Am J Clin Nutr 2008; 88(1): 167-75.

[17] Wijga AH, Smit HA, Kerkhof M, *et al*. Association of consumption of products containing milk fat with reduced asthma risk in pre-school children: the PIAMA birth cohort study. Thorax 2003; 58(7): 567-72.

[18] Litonjua AA, Rifas-Shiman SL, Ly NP, *et al*. Maternal antioxidant intake in pregnancy and wheezing illnesses in children at 2 y of age. Am J Clin Nutr 2006; 84(4): 903-11.

[19] Checkley W, West KP, Jr., Wise RA, *et al*. Maternal vitamin A supplementation and lung function in offspring. N Engl J Med 2010; 362(19): 1784-94.

[20] Wilson JG, Roth CB, Warkany J. An analysis of the syndrome of malformations induced by maternal vitamin A deficiency. Effects of restoration of vitamin A at various times during gestation. Am J Anat 1953; 92(2): 189-217.

[21] Chen F, Desai TJ, Qian J, Niederreither K, Lu J, Cardoso WV. Inhibition of Tgf beta signaling by endogenous retinoic acid is essential for primary lung bud induction. Development 2007; 134(16): 2969-79.

[22] Massaro GD, Massaro D. Postnatal treatment with retinoic acid increases the number of pulmonary alveoli in rats. Am J Physiol 1996; 270(2 Pt 1): L305-10.

[23] Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab 2009; 94(1): 26-34.

[24] Ordez-Morn P, Muoz A. Nuclear receptors: genomic and non-genomic effects converge. Cell Cycle 2009; 8(11): 1675-80.

[25] Norman A. Minireview: vitamin D receptor: new assignments for an already busy receptor. Endocrinology 2006; 147(12): 5542-8.

[26] Artaza JN, Sirad F, Ferrini MG, Norris KC. 1,25(OH)2vitamin D3 inhibits cell proliferation by promoting cell cycle arrest without inducing apoptosis and modifies cell morphology of mesenchymal multipotent cells. J Steroid Biochem Mol Biol 2010; 119(1-2): 73-83.

[27] Margolis RN, Christakos S. The nuclear receptor superfamily of steroid hormones and vitamin D gene regulation. An update. Ann N Y Acad Sci 2010; 1192(1): 208-14.

[28] Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357(3): 266-81.

[29] Huhtakangas JA, Olivera CJ, Bishop JE, Zanello LP, Norman AW. The vitamin D receptor is present in caveolae-enriched plasma membranes and binds 1 alpha,25(OH)2-vitamin D3 *in vivo* and *in vitro*. Mol Endocrinol 2004; 18(11): 2660-71.

[30] Wagner CL, Greer FR. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. Pediatrics 2008; 122(5): 1142-52.

[31] Kovacs CS. Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. Am J Clin Nutr 2008; 88(2): 520S-8S.

[32] Mulligan ML, Felton SK, Riek AE, Bernal-Mizrachi C. Implications of vitamin D deficiency in pregnancy and lactation. American Journal of Obstetrics and Gynecology 2010; 202(5): 429.e1-.e9.

[33] Weisman Y, Harell A, Edelstein S, David M, Spirer Z, Golander A. 1 alpha, 25-Dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3 *in vitro* synthesis by human decidua and placenta. Nature 1979; 281(5729): 317-9.

[34] Shin JS, Choi MY, Longtine MS, Nelson DM. Vitamin D effects on pregnancy and the placenta. Placenta 2010; 31(12): 1027-34.

[35] Pearce SH, Cheetham TD. Diagnosis and management of vitamin D deficiency. BMJ 2010; 340: b5664.

[36] Prentice A. Vitamin D deficiency: a global perspective. Nutr Rev 2008; 66(10 Suppl 2): S153-64.

[37] Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int 2005; 16(7): 713-6.

[38] Alagol F, Shihadeh Y, Boztepe H, *et al*. Sunlight exposure and vitamin D deficiency in Turkish women. J Endocrinol Invest 2000; 23(3): 173-7.

[39] Mishal AA. Effects of different dress styles on vitamin D levels in healthy young Jordanian women. Osteoporos Int 2001; 12(11): 931-5.

[40] Lips P. Worldwide status of vitamin D nutrition. J Steroid Biochem Mol Biol 2010; 121(1-2):297-300.

[41] Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM. High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. J Nutr 2007; 137(2): 447-52.

[42] Basile LA, Taylor SN, Wagner CL, Quinones L, Hollis BW. Neonatal vitamin D status at birth at latitude 32 degrees 72': evidence of deficiency. J Perinatol 2007; 27(9): 568-71.

[43] Bowyer L, Catling-Paull C, Diamond T, Homer C, Davis G, Craig ME. Vitamin D, PTH and calcium levels in pregnant women and their neonates. Clin Endocrinol (Oxf) 2009; 70(3): 372-7.

[44] Dijkstra SH, van Beek A, Janssen JW, de Vleeschouwer LH, Huysman WA, van den Akker EL. High prevalence of vitamin D deficiency in newborn infants of high-risk mothers. Arch Dis Child 2007; 92(9): 750-3.

[45] Hypponen E, Boucher BJ. Avoidance of vitamin D deficiency in pregnancy in the United Kingdom: the case for a unified approach in National policy. Br J Nutr 2010; 104(3): 309-14.

[46] Wang TJ, Zhang F, Richards JB, *et al*. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. The Lancet 2010; 376(9736): 180-8.

[47] Lanham-New S. Vitamin D in the spotlight ? time for urgent action? British Journal of Nutrition 2010; 104(03): 315-7.

[48] Devereux G, Litonjua AA, Turner SW, *et al*. Maternal vitamin D intake during pregnancy and early childhood wheezing. Am J Clin Nutr 2007; 85(3): 853-9.

[49] Camargo CA, Jr., Rifas-Shiman SL, Litonjua AA, *et al*. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. Am J Clin Nutr 2007; 85(3): 788-95.

[50] Miyake Y, Sasaki S, Tanaka K, Hirota Y. Dairy food, calcium, and vitamin D intake in pregnancy and wheeze and eczema in infants. Eur Respir J 2010; 35(6): 1228-34.

[51] Erkkola M, Kaila M, Nwaru BI, *et al*. Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children. Clin Exp Allergy 2009; 39(6): 875-82.

[52] Gale CR, Robinson SM, Harvey NC, *et al*. Maternal vitamin D status during pregnancy and child outcomes. Eur J Clin Nutr 2008; 62(1): 68-77.

[53] Hypponen E, Sovio U, Wjst M, *et al*. Infant vitamin d supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. Ann N Y Acad Sci 2004; 1037: 84-95.

[54] Hypponen E, Berry DJ, Wjst M, Power C. Serum 25-hydroxyvitamin D and IgE - a significant but nonlinear relationship. Allergy 2009; 64(4): 613-20.

[55] Gaultier C, Harf A, Balmain N, Cuisinier-Gleizes P, Mathieu H. Lung mechanics in rachitic rats. Am Rev Respir Dis 1984; 130(6): 1108-10.

[56] Nguyen M, Guillozo H, Garabedian M, Balsan S. Lung as a possible additional target organ for vitamin D during fetal life in the rat. Biol Neonate 1987; 52(4): 232-40.

[57] Nguyen TM, Guillozo H, Marin L, *et al*. 1,25-dihydroxyvitamin D3 receptors in rat lung during the perinatal period: regulation and immunohistochemical localization. Endocrinology 1990; 127(4): 1755-62.

[58] Marin L, Dufour ME, Tordet C, Nguyen M. 1,25(OH)2D3 stimulates phospholipid biosynthesis and surfactant release in fetal rat lung explants. Biol Neonate 1990; 57(3-4): 257-60.

[59] Marin L, Dufour ME, Nguyen TM, Tordet C, Garabedian M. Maturational changes induced by 1 alpha,25-dihydroxyvitamin D3 in type II cells from fetal rat lung explants. Am J Physiol 1993; 265(1 Pt 1): L45-52.

[60] Edelson JD, Chan S, Jassal D, Post M, Tanswell AK. Vitamin D stimulates DNA synthesis in alveolar type-II cells. Biochim Biophys Acta 1994; 1221(2): 159-66.

[61] Nguyen TM, Guillozo H, Marin L, Tordet C, Koite S, Garabedian M. Evidence for a vitamin D paracrine system regulating maturation of developing rat lung epithelium. Am J Physiol 1996; 271(3 Pt 1): L392-9.

[62] Liebeskind A, Srinivasan S, Kaetzel D, Bruce M. Retinoic acid stimulates immature lung fibroblast growth *via* a PDGF-mediated autocrine mechanism. Am J Physiol Lung Cell Mol Physiol 2000; 279(1): L81-L90.

[63] Bostrom H, Willetts K, Pekny M, *et al*. PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. Cell 1996; 85(6): 863-73.

[64] Pedigo N, Zhang H, Koszewski NJ, Kaetzel DM. A 5'-distal element mediates vitamin D-inducibility of PDGF-A gene transcription. Growth Factors 2003; 21(3-4): 151-60.

[65] Pedigo NG, Zhang H, Mishra A, McCorkle JR, Ormerod AK, Kaetzel DM. Retinoic acid inducibility of the human PDGF-a gene is mediated by 5'-distal DNA motifs that overlap with basal enhancer and vitamin D response elements. Gene Expr 2007; 14(1): 1-12.

[66] Ormerod A, Xing Z, Pedigo N, Mishra A, Kaetzel D. The calcitriol analogue EB1089 impairs alveolarization and induces localized regions of increased fibroblast density in neonatal rat lung. Experimental lung research 2008; 34(4): 155-82.

[67] Sakurai R, Shin E, Fonseca S, *et al*. 1alpha,25(OH)2D3 and its 3-epimer promote rat lung alveolar epithelial-mesenchymal interactions and inhibit lipofibroblast apoptosis. Am J Physiol Lung Cell Mol Physiol 2009; 297(3): L496-L505.

[68] Awonusonu F, Srinivasan S, Strange J, Al-Jumaily W, Bruce MC. Developmental shift in the relative percentages of lung fibroblast subsets: role of apoptosis postseptation. Am J Physiol 1999; 277(4 Pt 1): L848-59.

[69] Nadeau K, Montermini L, Mandeville I, *et al*. Modulation of Lgl1 by steroid, retinoic acid, and vitamin D models complex transcriptional regulation during alveolarization. Pediatr Res 2010; 67(4): 375-81.

[70] Oyewumi L, Kaplan F, Sweezey NB. Lgl1, a mesenchymal modulator of early lung branching morphogenesis, is a secreted glycoprotein imported by late gestation lung epithelial cells. Biochem J 2003; 376(Pt 1): 61-9.

[71] Flecknoe SJ, Wallace MJ, Harding R, Hooper SB. Determination of alveolar epithelial cell phenotypes in fetal sheep: evidence for the involvement of basal lung expansion. J Physiol 2002; 542(Pt 1): 245-53.

[72] Chen KS, DeLuca HF. Isolation and characterization of a novel cDNA from HL-60 cells treated with 1,25-dihydroxyvitamin D-3. Biochim Biophys Acta 1994; 1219(1): 26-32.

[73] Filby CE, Hooper SB, Sozo F, Zahra VA, Flecknoe SJ, Wallace MJ. VDUP1: a potential mediator of expansion-induced lung growth and epithelial cell differentiation in the ovine fetus. American journal of physiology Lung cellular and molecular physiology 2006; 290(2): L250-L8.

[74] Ramirez A, Wongtrakool C, Welch T, Steinmeyer A, Zgel U, Roman J. Vitamin D inhibition of pro-fibrotic effects of transforming growth factor beta1 in lung fibroblasts and epithelial cells. The Journal of steroid biochemistry and molecular biology 2010; 118(3): 142-50.

[75] Glasgow JF, Thomas PS. Rachitic respiratory distress in small preterm infants. Arch Dis Child 1977; 52(4): 268-73.

[76] Lunghi B, Meacci E, Stio M, *et al*. 1,25-dihydroxyvitamin D3 inhibits proliferation of IMR-90 human fibroblasts and stimulates pyruvate kinase activity in confluent-phase cells. Mol Cell Endocrinol 1995; 115(2): 141-8.

[77] Stio M, Celli A, Lunghi B, Raugei G, Modesti A, Treves C. Vitamin D receptor in IMR-90 human fibroblasts and antiproliferative effect of 1,25-dihydroxyvitamin D3. Biochem Mol Biol Int 1997; 43(6): 1173-81.

[78] Phokela SS, Peleg S, Moya FR, Alcorn JL. Regulation of human pulmonary surfactant protein gene expression by 1alpha,25-dihydroxyvitamin D3. Am J Physiol Lung Cell Mol Physiol 2005; 289(4): L617-26.

[79] Rehan VK, Torday JS, Peleg S, *et al*. 1Alpha,25-dihydroxy-3-epi-vitamin D3, a natural metabolite of 1alpha,25-dihydroxy vitamin D3: production and biological activity studies in pulmonary alveolar type II cells. Mol Genet Metab 2002; 76(1): 46-56.

[80] Menezes RJ, Cheney RT, Husain A, *et al*. Vitamin D receptor expression in normal, premalignant, and malignant human lung tissue. Cancer Epidemiol Biomarkers Prev 2008; 17(5): 1104-10.

[81] Hansdottir S, Monick MM, Lovan N, Powers L, Gerke A, Hunninghake GW. Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. J Immunol 2010; 184(2): 965-74.

[82] Bosse Y, Maghni K, Hudson TJ. 1alpha,25-dihydroxy-vitamin D3 stimulation of bronchial smooth muscle cells induces autocrine, contractility, and remodeling processes. Physiol Genomics 2007; 29(2): 161-8.

[83] Song Y, Qi H, Wu C. Effect of 1,25-(OH)2D3 (a vitamin D analogue) on passively sensitized human airway smooth muscle cells. Respirology 2007; 12(4): 486-94.

[84] Atkinson J, Senior R. Matrix metalloproteinase-9 in lung remodeling. American journal of respiratory cell and molecular biology 2003; 28(1): 12-24.

[85] Crosby LM, Waters CM. Epithelial repair mechanisms in the lung. Am J Physiol Lung Cell Mol Physiol 2010; 298(6): L715-31.

[86] Timms PM, Mannan N, Hitman GA, *et al*. Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? QJM 2002; 95(12): 787-96.

[87] Coussens A, Timms P, Boucher B, *et al*. 1alpha,25-dihydroxyvitamin D3 inhibits matrix metalloproteinases induced by Mycobacterium tuberculosis infection. Immunology 2009; 127(4): 539-48.

[88] Damera G, Fogle HW, Lim P, *et al*. Vitamin D inhibits growth of human airway smooth muscle cells through growth factor-induced phosphorylation of retinoblastoma protein and checkpoint kinase 1. Br J Pharmacol 2009; 158(6): 1429-41.

[89] Clifford RL, Knox AJ. Vitamin D - a new treatment for airway remodelling in asthma? Br J Pharmacol 2009; 158(6): 1426-8.

[90] Hewison M, Burke F, Evans KN, *et al*. Extra-renal 25-hydroxyvitamin D3-1alpha-hydroxylase in human health and disease. J Steroid Biochem Mol Biol 2007; 103(3-5): 316-21.

[91] Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. Lancet 2006; 368(9537): 763-70.

[92] Harvey L, Burne TH, McGrath JJ, Eyles DW. Developmental vitamin D3 deficiency induces alterations in immune organ morphology and function in adult offspring. J Steroid Biochem Mol Biol 2010; 121(1-2): 239-42.

[93] Wittke A, Chang A, Froicu M, *et al*. Vitamin D receptor expression by the lung micro-environment is required for maximal induction of lung inflammation. Arch Biochem Biophys 2007; 460(2): 306-13.

[94] Wittke A, Weaver V, Mahon BD, August A, Cantorna MT. Vitamin D receptor-deficient mice fail to develop experimental allergic asthma. J Immunol 2004;173(5): 3432-6.

[95] Topilski I, Flaishon L, Naveh Y, Harmelin A, Levo Y, Shachar I. The anti-inflammatory effects of 1,25-dihydroxyvitamin D3 on Th2 cells *in vivo* are due in part to the control of integrin-mediated T lymphocyte homing. Eur J Immunol 2004; 34(4): 1068-76.

[96] McGlade JP, Gorman S, Zosky GR, *et al*. Suppression of the asthmatic phenotype by ultraviolet B-induced, antigen-specific regulatory cells. Clin Exp Allergy 2007; 37(9): 1267-76.

[97] Matheu V, Back O, Mondoc E, Issazadeh-Navikas S. Dual effects of vitamin D-induced alteration of TH1/TH2 cytokine expression: enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease. J Allergy Clin Immunol 2003; 112(3): 585-92.

[98] Rohde CM, DeLuca HF. All-trans retinoic acid antagonizes the action of calciferol and its active metabolite, 1,25-dihydroxycholecalciferol, in rats. J Nutr 2005; 135(7): 1647-52.

[99] Rohde CM, Manatt M, Clagett-Dame M, DeLuca HF. Vitamin A antagonizes the action of vitamin D in rats. J Nutr 1999; 129(12): 2246-50.

[100] Ginde AA, Sullivan AF, Mansbach JM, Camargo CA, Jr. Vitamin D insufficiency in pregnant and nonpregnant women of childbearing age in the United States. Am J Obstet Gynecol 2010; 202: 436.e1-8.

[101] Merewood A, Mehta SD, Grossman X, *et al*. Widespread vitamin D deficiency in urban Massachusetts newborns and their mothers. Pediatrics 2010; 125(4): 640-7.

[102] Davis LM, Chang SC, Mancini J, Nathanson MS, Witter FR, O'Brien KO. Vitamin D insufficiency is prevalent among pregnant African American adolescents. J Pediatr Adolesc Gynecol 2010; 23(1): 45-52.

[103] Baker PN, Wheeler SJ, Sanders TA, *et al*. A prospective study of micronutrient status in adolescent pregnancy. Am J Clin Nutr 2009; 89(4): 1114-24.

[104] Yu CK, Sykes L, Sethi M, Teoh TG, Robinson S. Vitamin D deficiency and supplementation during pregnancy. Clin Endocrinol (Oxf) 2009; 70(5): 685-90.

[105] Judkins A, Eagleton C. Vitamin D deficiency in pregnant New Zealand women. N Z Med J 2006; 119(1241): U2144.

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Perinatal Vitamin D Deficiency and Childhood Asthma: A Molecular Perspective

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Abstract: There is a large body of literature suggesting that the recent increase in the incidence of childhood asthma might be associated with vitamin D deficiency during lung development. There are also strong experimental animal data showing that vitamin D is one of the local alveolar paracrine factors that spatiotemporally modulates perinatal pulmonary maturation. However, the mechanistic link between vitamin D deficiency during pregnancy and childhood asthma is not known. In this review, we demonstrate how perinatal vitamin D deficiency could mechanistically drive both the proximal and distal airways to a myogenic phenotype, molecularly and structurally, predisposing the offspring to asthma. More specifically, we will review how perinatal vitamin D deficiency results in an increased abundance of mesenchymal myofibroblasts, a feature that is highly consistent with the pathophysiology of asthma. We also provide evolutionary insights as to how vitamin D deficiency might (re)activate the atavistic host defense mechanisms that could precipitate a lung phenotype consistent with asthma. Since vitamin D deficiency seems to alter the normal homeostatic epithelial-mesenchymal signaling pathways in the developing lung, it offers a distinct translational opportunity to prevent this process through targeted molecular manipulations. While we wait for the results of on-going trials of vitamin D supplementation during pregnancy to get some definitive answers on its role in the pathogenesis of childhood asthma, we advocate studies to discover new vitamin D analogs and/or metabolites with optimal respiratory effects and without any significant side effects.

Keywords: Vitamin D, asthma, pregnancy, epithelial-mesenchymal interactions, PTHrP, PPARγ, Wnt signaling.

INTRODUCTION

Asthma is the most common chronic disease of childhood in the world [1], resulting in a significant medical burden and healthcare costs [2]. The burden of this disease is increasing rapidly, with over 300 million people affected worldwide [1]. A large body of literature suggests that the recent increase in the incidence of asthma might be associated with vitamin D deficiency during lung development. For example, many recent studies demonstrate a high prevalence of vitamin D deficiency among women of child-bearing age, and in pregnant women, resulting in a large number of infants being born deficient in vitamin D [3-8]. Additionally, a number of recent studies have demonstrated that a higher maternal vitamin D intake during pregnancy or early life is inversely correlated with risk of asthma and allergy in later life [9-12]. However, the mechanism by which vitamin D deficiency during gestation and early lung development is linked to childhood asthma is not well established. And, in fact, some studies also suggest a detrimental effect of vitamin D supplementation on the development of allergy [13, 14], suggesting that a more detailed understanding of vitamin D's role in lung development and asthma is necessary.

If there is a real causal relationship between perinatal vitamin D deficiency and childhood asthma, then we would expect that vitamin D would promote signaling pathways that are known to be important for normal lung development, and its deficiency would dysregulate these pathways, leading to a lung molecular and structural phenotype which predisposes to asthma. In this review, we demonstrate how perinatal vitamin D deficiency could mechanistically drive both the proximal and distal airways to a myogenic phenotype, molecularly and structurally, predisposing the offspring to asthma. More specifically, we will describe how perinatal vitamin D deficiency results in an increased abundance of mesenchymal myofibroblasts, a feature that is highly consistent with the pathophysiology of asthma [15, 16]. Fig. (**1**) outlines the proposed effects of vitamin D on lung development that might modulate asthma. Understanding the molecular mechanisms underlying vitamin D-associated childhood asthma could be crucial in designing effective preventive and therapeutic strategies against childhood asthma.

Vitamin D's role in Proximal Airway Development: During lung development, mesodermal fibroblasts, which provide the scaffolding for both the proximal and distal airways, are dominated by the Wnt pathway, which is the default program for the muscle phenotype [17]. The Wnt pathway actively down-regulates CCAAT/enhancer-binding protein α (C/EBPα) and its downstream targets [18]. The functionally relevant molecular intermediates in the Wnt pathway are glycogen synthase kinase β (GSK3β), β-catenin, lymphoid enhancer-binding factor 1/T cell protein (LEF-1/TCP), C/EBPα and peroxisome proliferator-activated receptor γ (PPARγ). These molecules interact canonically, including phosphorylation, proteolysis, and ubiquitination. This cascade culminates in the expression of myogenic genes

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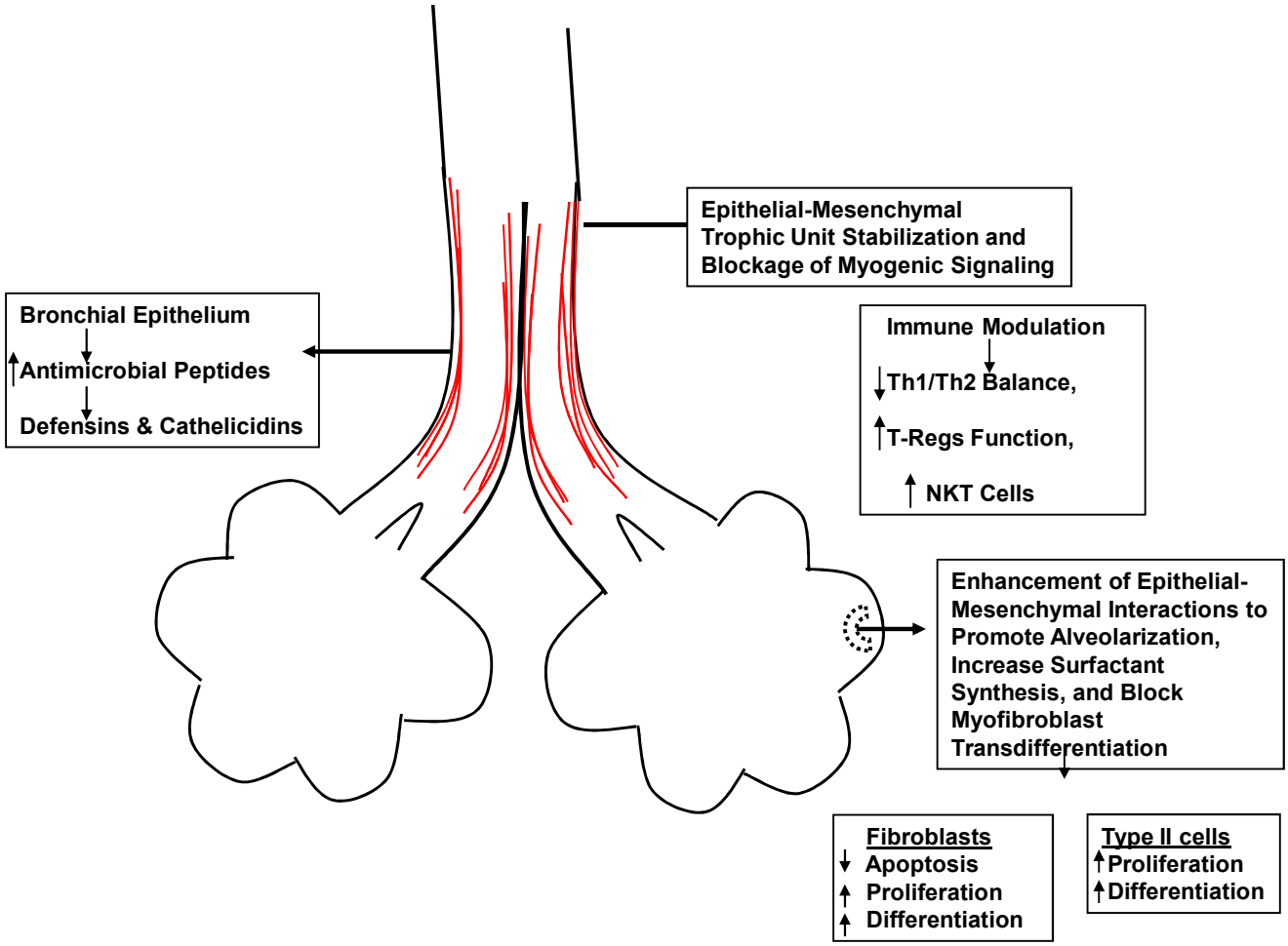

Fig. (1). Proposed effect of vitamin D that might modulate asthma.

such as myosin heavy chain, α smooth muscle actin and calponin. Recently, the vitamin D receptor (VDR) has been shown to be expressed in airway smooth muscle, and its activation inhibits smooth muscle proliferation [19]. Persistent expression of some of the early markers of myogenic differentiation has also been reported in VDR knockout mice [20], suggesting that vitamin D deficiency during development could lead to an enhanced myogenic phenotype of the proximal airways. Furthermore, the epithelium of the asthmatic airways is known to be structurally and functionally abnormal, with increased susceptibility to injury by endogenous and exogenous exposures and an impaired ability to repair [21].

Vitamin D's Role in Distal Airway Development: Alveolar epithelial-mesenchymal interactions play a critical role in perinatal pulmonary maturation, which is characterized by an increase in surfactant synthesis and alveolar wall thinning. Adepithelial lipid-laden interstitial lung fibroblasts, or lipofibroblasts (LIFs), which are normally present in abundance at birth, and which undergo apoptosis during the perinatal period, play a significant role in both surfactant synthesis and alveolar septal thinning [22, 23]. Recently, vitamin D has been shown to spatio-temporally coordinate alveolar epithelial and LIF proliferation and differentiation, leading to increased surfactant synthesis and alveolar septal wall thinning [24]. These effects are mediated by vitamin D's stimulatory effect on homeostatic Parathyroid Hormone-related Protein/ Peroxisome Proliferator-Activated Receptor γ (PTHrP/ PPARγ) epithelial-mesenchymal signaling, and inhibitory effect on myogenic Wnt signaling.

During normal lung development, under the influence of Sonic Hedgehog, the developing endoderm expresses PTHrP, along with its receptor, on the adjacent mesenchyme [25]. PTHrP binding to its receptor on the mesenchyme activates cAMP-dependent PKA signaling [26], which down-regulates the mesenchymal default Wnt pathway [27, 28], and up-regulates the adipogenic pathway through the key nuclear transcription factor PPARγ [29] and its down-stream regulatory genes such as Adipocyte Differentiation-Related Protein (ADRP) [30] and leptin [31, 32]. ADRP is necessary for the transit of neutral lipids from the LIF to the alveolar type II (ATII) cell for surfactant phospholipid synthesis [30]. Lipofibroblasts, in turn, secrete leptin, which acts on its receptor on ATII cells, stimulating both surfactant phospholipid and protein synthesis [32]. Since PTHrP stimulates leptin production by LIFs, it provides a complete growth factor-mediated homeostatic paracrine loop for the synthesis of pulmonary surfactant. Hence, alveolar PTHrP/PPARγ signaling, by inhibiting Wnt signaling,

inhibits the default myogenic phenotype, and induces the lipogenic phenotype, which, through its autocrine effect on LIFs and its paracrine effect on ATII cells, is necessary for alveolar development and homeostasis. This mechanism appears to apply to both rodents [33] and primates [34] alike. The net result of alveolar vitamin D epithelial-mesenchymal paracrine signaling is increased substrate for surfactant phospholipid synthesis, enhanced epithelial and mesenchymal differentiation and septal wall thinning [22, 24].

These data clearly demonstrate vitamin D's critical role in the development of both the proximal and distal airways, and show how its deficiency during pregnancy could interfere with homeostatic lung signaling pathways, resulting in the up-regulation of the default Wnt signaling-driven myogenic pathway in both the proximal and distal airways, thereby predisposing offspring to bronchial hyper-responsiveness. The association of Wnt signaling genes and impaired lung function in two childhood asthma cohorts reported in a recent study further validates the link between vitamin D deficiency/Wnt signaling and childhood asthma [35].

Vitamin D's Role in the 'Evolution' of Asthma: Even deeper insights into the etiology of asthma come from the all-encompassing evolutionary overlap between PTHrP/PPARγ, Wnt signaling and environmental factors. We have previously shown how and why lung evolution has been driven by the interaction between external and internal factors that can be traced all the way back to the swim bladder of fish [36], raising the question as to how and why vitamin D evolved to promote lung development and homeostasis. The mechanisms of lung development, environmental selection pressure and vitamin D intersect functionally through vitamin D's roles in maintaining epithelial host defense by stimulating both surfactant proteins and antimicrobial peptides. In vertebrates, antimicrobial peptides are inhibited by salinity, and in fish, the salinity of the water environment activates epithelial vitamin D by increasing its hydroxylation [37], counterbalancing the loss of antimicrobial peptide activity. As is the case for many such counterbalancing selection pressures, vitamin D hydroxylation has evolved as a constitutive cis regulatory mechanism through selection pressure. Therefore, vitamin D deficiency may (re)activate the atavistic antimicrobial peptide mechanism, promoting host defense, but may inadvertently precipitate dysmorphogenesis, as we have observed with the cytokine agonist lipopolysaccharide, which disrupts normal cell-cell interactions [38]. The recrudescence of such deep evolutionary homologies in complex diseases has been termed decanalization [39], referring to the reversal of the mechanism of canalization that Waddington used to describe evolution [40]. Such evolutionary insight to the genetic 'history' of asthma offers novel, counterintuitive ways to effectively preempt the asthma phenotype using the evolutionary strategy. As a note in proof of the overlap between vitamin D, host defense and asthma, antimicrobial peptides expressed in airway epithelial cells and skin are regulated by vitamin D [41]. These antimicrobial peptides fail to up-regulate in atopical dermatititis [42], which is closely associated with asthma. Therefore, vitamin D deficiency may precipitate both atopy and asthma through its effect on antimicrobial peptide production in lung and skin.

In conclusion, this review introduces a novel concept that vitamin D deficiency during pregnancy and the perinatal period interferes with normal developmental, homeostatic lung signaling pathways, resulting in up-regulation of the default myogenic pathways of both the proximal and distal airways, thereby predisposing offspring to bronchial hyper-responsiveness. This structural predisposition to asthma due to perinatal vitamin D deficiency is fundamentally different from the previously proposed allergy hypothesis, which, with or without increased frequency and/or severity of respiratory infections, has been linked to vitamin D deficiency and childhood asthma [43, 44]. In experimental allergic models of asthma, either no benefit [13], some benefit [45, 46], or even harm [13, 45] has been reported with vitamin D supplementation, undermining the validity of the allergy hypothesis. However, it is important to emphasize that the concept proposed in this review and the previously proposed allergy hypothesis may not be mutually exclusive, but may act in tandem.

The hypothesis that vitamin D deficiency alters the normal homeostatic epithelial-mesenchymal signaling pathways in the developing lung affords a unique molecular/mechanistic perspective on asthma pathogenesis, and provides a direction for translational research to prevent this process through targeted molecular manipulations. Though the results of on-going trials of vitamin D supplementation during pregnancy are likely to provide some definitive answers on the role of vitamin D deficiency in the pathogenesis of childhood asthma, its optimal dose may still not be known in the near future. Moreover, the discovery of a vitamin D analog or metabolite that exerts beneficial respiratory effects without inducing adverse effects associated with the physiologically active hormone dihydroxycholecalciferol is a research priority. Lastly, since asthma is both genetic and environmental in origin, it is possible that genetic variants in PTHrP/PPARγ/Wnt signaling might influence the effects of vitamin D on childhood asthma phenotype.

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REFERENCES

[1] World health organization. Asthma- Fact sheet N°307.
[2] National Heart, Lung and Blood Institute Chartbook on cardiovascular, lung and blood diseases, U.S. Department of Health and Human Services, National Institute of Health 2007.
[3] Hollis BW, Wagner CL. Vitamin D deficiency during pregnancy: an ongoing epidemic. Am J Clin Nutr 2006; 84: 273.
[4] Lee JM, Smith JR, Philipp BL, Chen TC, Mathieu J, Holick MF. Vitamin D deficiency in a healthy group of mothers and newborn infants. Clin Pediatr (Phila) 2007; 46: 42-4.
[5] Bassir M, Laborie S, Lapillonne A, Claris O, Chappuis MC, Salle BL. Vitamin D deficiency in Iranian mothers and their neonates: a pilot study. Acta Pediatr 2001; 90: 577-9.
[6] Sachan A, Gupta R, Das V, Agarwal A, Awasthi PK, Bhatia V. High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India. Am J Clin Nutr 2005; 81: 1060-4.

[7] Judkins A, Eagleton C. Vitamin D deficiency in pregnant New Zealand women. N Z Med J 2006;119: U2144.

[8] Van der Meer IM, Karamali NS, Boeke AJ, *et al*. High prevalence of vitamin D deficiency in pregnant non-Western women in The Hague, Netherlands. Am J Clin Nutr 2006; 84: 350-3.

[9] Devereux G, Litonjua AA, Turner SW, *et al.* Maternal vitamin D intake during pregnancy and early childhood wheezing. Am J Clin Nutr 2007; 85(3): 853-9.

[10] Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? J Allergy Clin Immunol 2007; 120(5): 1031-5.

[11] Camargo CA Jr, Rifas-Shiman SL, Litonjua AA, *et al*. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 yrs of age. Am J Clin Nutr 2007; 85(3): 788-95.

[12] Black PN, Scragg RS. Relationship between serum 25 hydroxyvitamin D and pulmonary function in the third national health and nutrition examination survey. Chest 2005; 128(6): 3792-8.

[13] Witte A, Weaver C, Mahon BD, August A, Cantorna MT. Vitamin-D receptor-deficient mice fail to develop experimental allergic asthma. J Immunol 2004; 173: 3432-6.

[14] Topilski I, Flaishon L, Naveh Y, Harmelin A, Levo Y, Shachar I. The anti-inflammatory effects of 1,25-dihydroxyvitamin D3 on Th2 cells *in vivo* are due in part to the control of integrin-mediated T lymphocyte homing. Eur J Immunol 2004; 34(4): 1068-76.

[15] Elias JA, Zhu Z, Chupp G, Homer RJ. Airway remodeling in asthma. J Clin Invest 1999; 104: 1001-6.

[16] Woodruff PG, Dolganov GM, Ferrando RE, *et al.* Hyperplasia of smooth muscle in mild to moderate asthma without changes in cell size or gene expression. Am J Respir Crit Care Med 2004; 169: 1001-6.

[17] Cossu G, Tajbakhsh S, Buckingham M. How is myogenesis initiated in the embryo? Trends Genet 1996; 12: 218-23.

[18] Ross SE, Hemati N, Longo KA, *et al*. Inhibition of adipogenesis by Wnt signaling. Science 2000; 289: 950-3.

[19] Gosens R, Roscioni, SS, Bart GJ, *et al*. Pharmacology of airway smooth muscle proliferation. Eur J Pharmacol 2008; 585(2-3): 385-97.

[20] Marie Demay. Muscle: A nontraditional 1,25-Dihydroxyvitamin D target tissue exhibiting classic hormone-dependent vitamin D receptor actions. Endocrinology 2003; 144(12): 5135-37.

[21] Holgate ST. Asthma: more than an inflammatory disease. Curr Opin Allergy Clin Immunol 2002; 2: 27-9.

[22] Torday JS, Rehan VK. A paracrine model for lung development, disease, and treatment-perspective. Pediatr Res 2007; 62(1): 2-7.

[23] Awonusonu F, Srinivasan S, Strange J, Al-Jumaily W, Bruce MC. Developmental shift in the relative percentages of lung fibroblast subsets: role of apoptosis postseptation. Am J Physiol 1999; 277(4 Pt 1): L848-59.

[24] Sakurai R, Shin E, Fonseca S, *et al*. 1alpha,25(OH)2D3 and its 3-epimer promote rat lung alveolar epithelial-mesenchymal interactions and inhibit lipofibroblast apoptosis. Am J Physiol Lung Cell Mol Physiol 2009; 297(3): L496-505.

[25] Lee K, Deeds JD, Segre GV. Expression of parathyroid hormone-related peptide and its receptor messenger ribonucleic acids during fetal development of rats. Endocrinol 1995; 136: 453-63.

[26] Rubin LP, Kovacs CS, De Paepe ME, Tsai SW, Torday JS, Kronenberg HM. Arrested pulmonary alveolar cytodifferentiation and defective surfactant synthesis in mice missing the gene for parathyroid hormone-related protein. Dev Dyn 2004; 230: 278-89.

[27] Kovala T, Lorimer IA, Brickenden AM, Ball EH, Sanwal BD. Protein kinase A regulation of cAMP phosphodiesterase expression in rat skeletal myoblasts. J Biol Chem 1994; 269: 8680-5.

[28] Ross SE, Hemati N, Longo KA, *et al*. Inhibition of adipogenesis by Wnt signaling. Science 2000; 289: 950-3.

[29] Tontonoz P, Hu E, Spiegelman BM. Regulation of adipocyte gene expression and differentiation by peroxisome proliferator activated receptor gamma. Curr Opin Genet Dev 1995; 5: 571-6.

[30] Schultz CJ, Torres E, Londos C, Torday JS. Role of adipocyte differentiation-related protein in surfactant phospholipid synthesis by type II cells. Am J Physiol Lung Cell Mol Physiol 2002; 283: L288-96.

[31] Torday JS, Sun H, Wang L, Torres E, Sunday ME, Rubin LP. Leptin mediates the parathyroid hormone-related protein paracrine stimulation of fetal lung maturation. Am J Physiol Lung Cell Mol Physiol 2002; 282(3): L405-10.

[32] Torday JS, Rehan VK. Stretch-stimulated surfactant synthesis is coordinated by the paracrine actions of PTHrP and leptin. Am J Physiol Lung Cell Mol Physiol 2002; 283(1): L130-5.

[33] Torday JS, Rehan VK. Up-regulation of fetal rat lung parathyroid hormone-related protein gene regulatory network down-regulates the Sonic Hedgehog/Wnt/beta catenin gene regulatory network. Pediatr Res 2006; 60: 382-8.

[34] Cullen A, Emanuel RL, Torday JS, Asokananthan N, Sikorski KA, Sunday ME. Bombesin-like peptide and receptors in lung injury models: diverse gene expression, similar function. Peptides 2000; 21: 1627-38.

[35] Sharma S, Tantisira K, Carey V, *et al*. A role for Wnt signaling genes in the pathogenesis of impaired lung function in asthma. Am J Respir Crit Care Med 2010; 181(4): 328-36.

[36] Torday JS, Rehan VK. Exploiting cellular-developmental evolution as the scientific basis for the preventive medicine. Med hypothesis 2009; 72(5): 596-602.

[37] Sundh H, Larsson D, Sundell K. Environmental salinity regulates the *in vitro* production of [^{3}H]-1,25-dihydroxyvitamin D3 and [3H]-24,25 dihydroxyvitamin D3 in rainbow trout (Oncorhynchus mykiss). Gen Comp Endocrinol 2007; 152(2-3): 252-8.

[38] Rehan VK, Dargan-Batra SK, Wang Y, *et al*. A paradoxical temporal response of the PTHrP/PPARgamma signaling pathway to lipopolysaccharide in an *in vitro* model of the developing rat lung. Am J Physiol Lung Cell Mol Physiol 2007; 293(1): L182-90.

[39] Gibson G. Decanalization and the origin of complex disease. Nat Rev Genet 2009; 10(2): 134-40.

[40] Waddington CH. Canalization of development and the inheritance of acquired characters. Nature 1942; 150: 563-5.

[41] Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. J Immunol 2008; 181(10): 7090-9.

[42] Ong PY, Hamid QA, Travers JB, *et al*. Decreased IL-15 may contribute to elevated IgE and acute inflammation in atopic dermatitis. J Immunol 2002; 168(1): 505-10.

[43] Lange NE, Litonjua A, Hawrylowicz CM, Weiss S. Vitamin D, the immune system and asthma. Expert Rev Clin Immunol 2009; 5(6): 693-702.

[44] Ginde AA, Mansbach JM, Camargo CA. Vitamin D, respiratory infections, and asthma. Curr Allergy Asthma Rep 2009; 9: 81-7.

[45] Topilski I, Flaishon L, Naveh Y, Harmelin A, Levo Y, Shachar I. The anti-inflammatory effects of 1,25-dihydroxyvitamin D3 on Th2 cells *in vivo* are due in part to the control of integrin-mediated T lymphocyte homing. Eur J Immunol 2004; 34(4): 1068-76.

[46] Matheu V, Back O, Mondoc E, Issazadeh-Navikas S. Dual effects of vitamin D-induced alteration of TH1/TH2 cytokine expression: enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease. J Allergy Clin Immunol 2003; 112: 585-59.

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Vitamin D and Asthma: Scientific Promise and Clinical Reality

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Abstract: Widespread vitamin D insufficiency and vitamin D supplementation (even low dose rickets prophylaxis) have been hypothesised as contributory factors to the recent increase in asthma. These hypotheses are supported by reports of immunomodulatory effects of vitamin D on antigen presenting cells, regulatory T cells and T-helper cells and evidence that vitamin D influences fetal lung differentiation and epithelial-mesenchymal function. Studies of vitamin D in animal models confirm complex effects of vitamin D on asthma immunopathogenesis. In humans a majority of epidemiological studies support the hypothesis that vitamin D insufficiency during pregnancy increases the likelihood of childhood wheeze and possibly asthma, although some studies do report the converse. In children and adults with asthma, reduced serum vitamin 25-hydroxyvitamin D levels have been associated with parameters of increased asthma severity. Clinical trials are underway addressing whether maternal vitamin D supplementation during pregnancy reduces the likelihood of childhood asthma and if there is a role for vitamin D supplementation in established asthma.

Keywords: Vitamin D, asthma, pregnancy, T cells, lung development, prevention, treatment.

INTRODUCTION

The recognition that changing environmental exposures underlie the recent increase in asthma and allergic disease has stimulated research attempting to identify responsible factors with the ultimate aim of implementing primary preventive interventions. In the last five years there has been increasing interest in the possible association between changes in vitamin D status and asthma prevalence, with a remarkably rapid transition from basic science and observational studies to the setting up of clinical trials investigating the potential of vitamin D supplementation to prevent asthma and to complement conventional asthma treatment. In this review we will outline the hypotheses relating vitamin D to asthma and allergy, summarise the biological properties of vitamin D considered to be pertinent, discuss studies of vitamin D in rodent models and detail epidemiological studies of vitamin D and asthma.

VITAMIN D AND ASTHMA HYPOTHESES

Three somewhat contradictory but not irreconcilable hypotheses link vitamin D to wheezing symptoms, asthma and allergy:

In 1999 Wjst and Dold hypothesised an adverse effect of vitamin D on the likelihood of asthma and allergy [1]. They observed that the increase in asthma and allergy prevalence followed, to some extent, the geographical and temporal use of vitamin D supplementation as rickets prophylaxis from the 1950s onwards in many westernised countries in the form of cod liver oil and fortification of milk, cereal and margarine [2]. It was suggested that the lower prevalence of asthma and allergy reported in anthroposophic communities and farms could be explained by the lifestyle-associated avoidance of vitamin D supplements. A further suggestion was that the vitamin D fortification policies in the USA, UK and Germany may have contributed to the higher prevalence of asthma in these countries [2].

In 2007 Litonjua and Weiss hypothesised that the recent increase in asthma and allergy was a consequence of the widespread vitamin D insufficiency that has been well documented in westernised countries [3]. This insufficiency is believed to be a consequence of an increasing tendency to stay indoors for the purposes of recreation and employment and the health promotion message of covering up and using sun block whenever exposed to direct sunshine in order to reduce the risk of melanoma.

In 2009, Camargo and colleagues [4] questioned whether there is any association between vitamin D status at physiologic levels and the risk of asthma. They suggested that the reported associations between reduced vitamin D status and increased childhood wheezing illness (see below) reflect adverse effects of reduced vitamin D status on innate immune responses with consequential increases in the frequency and/or severity of acute respiratory infections. It was speculated that any associations with asthma reflected the diagnostic difficulties associated with recurrent wheezing episodes consequent upon an increased susceptibility to respiratory infections because of vitamin D insufficiency.

All of these hypotheses are supported by studies that have investigated the effects of vitamin D on innate and adaptive immune processes.

IMMUNOMODULATORY EFFECTS OF VITAMIN D

Asthma and allergic diseases are inflammatory conditions initiated and perpetuated by $CD4^+$ T-helper (Th) cells of the Th2 phenotype. Regulatory T cells that can directly inhibit both Th1 and Th2 responses have been implicated in the immunopathogenesis of asthma and allergic disease with several studies highlighting IL-10

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secreting Tr1 regulatory T cells and $CD25^+$ regulatory T cells [5]. Given that effector Th-cells and regulatory T cells are critical to the immunopathogenesis of asthma and allergy it seems likely that any environmental factor contributing to the increase in these conditions will have immunomodulatory properties to alter the balance of Th1/Th2 differentiation, either directly and/or indirectly *via* effects on antigen presenting cells and/or regulatory T cells.

Vitamin D has potent immunomodulatory properties affecting cells of the innate and adaptive immune system. 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) induces expression of antimicrobial peptides such as defensins and cathelicidin by myeloid-derived cells [6] and bronchial epithelial cells [7]. The most well-established effects of vitamin D on the adaptive immune system are its actions on Th-cells and regulatory T cells. $1,25(OH)_2D_3$ directly inhibits Th1 interferon (IFN)-γ responses in murine [8] and human [9] Th1 cells and in the presence of the corticosteroid dexamethasone the inhibitory effect of $1,25(OH)_2D_3$ on IFN-γ release is augmented [10]. The effect of $1,25(OH)_2D_3$ on Th2 responses is more variable with reports of negligible effects on IL-4 release from human Th2 clones [9] and inhibition of IL-4, IL-13 and IFN-γ by $1,25(OH)_2D_3$ in *ex vivo* studies of cord blood T cells obtained from neonates [11]. An increase in IL-5 and IL-13 release upon the addition of 10^{-6}M $1,25(OH)_2D_3$ to *ex vivo* Th-cells has also been reported in the absence and presence of dexamethasone [10]. A more recent study has demonstrated dose-dependent inhibition of both Th1 (IFN-γ) and Th2 (IL-13) responses with $1,25(OH)_2D_3$, except at the highest concentration tested (10^{-6}M), where less suppression but no enhancement of either Th1 or Th2 responses was observed [12]. These studies suggest that vitamin D exerts variable dose-dependent effects on Th1/Th2 balance, possibly augmenting Th2 differentiation at higher concentrations.

The effects of $1,25(OH)_2D_3$ on the immune response are more complex than direct influences on Th-cells alone, and include actions on regulatory T cells and antigen presenting cells. When stimulated in the presence of dexamethasone, human regulatory T cells usually secrete high levels of IL-10, which is a potent anti-inflammatory and immunosuppressive cytokine [10]. In contrast, the *ex vivo* addition of dexamethasone to regulatory T cells from subjects with corticosteroid resistant asthma fails to induce the IL-10 response observed in cells from normal subjects [13]. However, IL-10 responsiveness to dexamethasone is nearly completely restored by treatment of the regulatory T cells from corticosteroid-resistant asthmatics with $1,25(OH)_2D_3$ either *in vitro* or by the oral administration of $1,25(OH)_2D_3$ to subjects [13]. More recently it has been reported that $1,25(OH)_2D_3$ at concentrations $\leq 10^{-7}$M induces dose-dependent changes in *ex vivo* cytokine responses with suppression of Th1 (IFN-γ) and Th2 (IL-13) responses but stimulation of IL-10 secretion. However, at a higher concentration (10^{-6}M) of $1,25(OH)_2D_3$, IL-10 responses declined substantially [12]. Vitamin D has been shown to promote a tolerogenic phenotype in human dendritic cells with *in vitro* $1,25(OH)_2D_3$ treatment of dendritic cells leading to the induction of Foxp3+ regulatory T cells with suppressive activity [14]. The complex effects of vitamin D on immune responses encompass effects on antigen presenting cells, regulatory T cells and Th-cells that appear to be dose- and possibly age-dependent. In addition to its immunomodulatory properties, vitamin D has also been implicated in fetal lung development and interactions between bronchial epithelial and mesenchymal elements involved in asthma pathogenesis.

VITAMIN D AND AIRWAY EPITHELIAL-MESENCHYMAL DEVELOPMENT AND FUNCTION

Although central to asthma pathogenesis, immunologically driven inflammation alone does not explain many features of asthma [15]. Holgate has highlighted evidence that the epithelium of asthmatic airways is structurally and functionally abnormal, with an increased susceptibility to injury by endogenous and exogenous exposures and an impaired ability to repair [15, 16]. The intrinsic airway epithelial abnormality, particularly the interaction with smooth muscle cells, is believed to contribute to airway remodelling and also increases the likelihood of asthmatic airway inflammation, particularly if there is a parallel development of a Th2-biased immune response [15, 17]. It is likely that intrinsic airway epithelial abnormalities develop in early life, probably *in utero* [17]. Vitamin D has been implicated in fetal airway development, the processes of airway remodelling and airway defence - processes known to be abnormal in asthma. In fetal rat lung explants there is expression of vitamin D receptor (VDR) sites during the process of type II pneumocyte differentiation [18, 19]. In addition, in fetal rat lung $1,25(OH)_2D_3$ has been reported to play a critical role in perinatal lung maturation by stimulating epithelial-mesenchymal differentiation and proliferation of lipofibroblasts and type II pneumocytes [20]. Further evidence for an effect of vitamin D on epithelial-mesenchymal function comes from the demonstration that VDR is present in human airway smooth muscle cells and that stimulation of these cells by $1,25(OH)_2D_3$ upregulates genes implicated in morphogenesis and cell growth as well as genes encoding proteins implicated in airway remodelling such as vascular endothelial growth factor (VEGF) and fibronectin FN1 [21]. Vitamin D also regulates airway epithelial cell secretion of antimicrobial peptides such as defensins and cathelicidin [22], the secretion of which is known to be reduced in asthma [23]. Clearly vitamin D has the potential to influence the immunological and airway developmental processes central to the airway inflammatory and remodelling processes characteristic of asthma. However, it is not possible to predict reliably whether vitamin D adversely or beneficially influences the development of asthma and allergic disease in humans. These issues have been addressed in a series of investigations in rodent models of asthma that induce eosinophilic airway inflammation by sensitisation and airway challenge with ovalbumin as an antigenic stimulus.

VITAMIN D RECEPTOR KNOCKOUT MICE

VDR–dependent modulation of experimental asthma has been described in a series of studies using VDR knockout (KO) mice, which are deficient in the nuclear receptor that binds and mediates $1,25(OH)_2D_3$ effects. Ovalbumin sensitization and challenge induced airway hyper-reactivity, mucus production and inflammation in wild type (WT), but not VDR knockout, mice [24]. This lack of airway response

occurred despite significant production of IgE in KO mice compared to allergic WT mice. Even adoptive transfer of activated splenocytes from allergic WT mice could not induce the allergic airway phenotype in VDR KO mice [25]. Conversely, immune-activated splenocytes derived from VDR KO mice were able to induce allergic airway inflammation when transferred to WT mice. These results suggest that, despite appropriate immune activation of T cells in VDR KO mice, their recruitment to the lung is compromised. Interestingly, a similar deficit in T cell homing is proposed to underlie the susceptibility of VDR KO mice to develop experimental inflammatory bowel disease [26]. In these later studies, the severity of disease was associated with lack of recruitment and homing of CD4 and CD8 cells to gut epithelium. Notably, deficits in airway recruitment in VDR KO mice are not limited to Th2 signals, but are also compromised in response to airway exposure to lipopolysaccharide [25].

STUDIES OF VITAMIN D DEFICIENCY AND SUPPLEMENTATION IN RODENTS

Few studies of allergic responses have examined naturally occurring deficiency by feeding mice vitamin D depleted diets. Wittke and co-workers [25] reported that mice fed vitamin D deficient diets started in late gestation could still respond with an allergic airway phenotype after ovalbumin sensitization and challenge in adolescence. However these responses in deficient mice were not compared with allergic mice fed a normal vitamin D sufficient diet. In rodent models of developmental vitamin D deficiency, wherein females are placed on deficient diets well before breeding, the offspring display altered immune function. For example, contact hypersensitivity in male (but not female) BALB/c mice is enhanced in animals derived from vitamin D deficient colonies [27]. In similar studies of 10 week-old Sprague Dawley rats derived from gestational vitamin D deficiency, spleen and thymus weights were increased and cytokine production from blood mononuclear cells were enhanced compared to rats derived from normal gestational diets [28]. These dietary regimens more closely model the reported associations of an increased likelihood of wheeze and asthma in children from mothers with maternal dietary vitamin D deficiency (see below). However the effects of vitamin D deficiency *in utero* have yet to be tested in rodent models of eosinophilic airway inflammation.

INTERVENTION WITH VITAMIN D IN ALLERGIC MICE

Several animal studies have investigated the effects of vitamin D supplementation in established asthma and allergy. Mice require approximately 125 ng of vitamin D_3 (cholecalciferol) daily [29], of which approximately 50% is absorbed [30]. Most treatment studies in rodents however, have used the VDR-binding metabolite $1,25(OH)_2D_3$, or calcitriol. These intervention studies in allergic mice typically employ doses of 100-200ng of calcitriol, which is similar to the ingested amount of the nonactive parent compound, and is usually delivered by bolus injection by subcutaneous (s.c.) and intraperitoneal (i.p.) routes. As such these protocols rely on pharmacologic doses of the bioactive, VDR-binding hormone, rather than addressing food intake of vitamin D_3. For example daily administration of 100 ng calcitriol can decrease allergic pulmonary inflammation and histopathological lesions when given to mice by i.p. [31] or s.c. injections [32]. Conversely, daily ingestion of 200ng calcitriol in rodent chow has no effect on the development of allergic airway disease in the ovalbumin sensitized BALB/c mouse [24]. However this same dietary regimen is effective in IBD mouse models [33]. Given that these doses of calcitriol are on the order of 150-200 times greater than therapeutic doses in humans, the translational value of these studies to understand allergic airway responses is limited. Meanwhile, approaches that modify vitamin D_3 (cholecalciferol) intake, *via* either supplements or food choices, have not been reported in experimental asthma models. As such, while the results from animal studies corroborate in part the immunomodulatory ability of vitamin D demonstrated *in vitro*, significant problems of pharmacokinetics, molecular formulations, and toxicity need to be addressed.

OBSERVATIONAL STUDIES OF VITAMIN D AND ASTHMA IN HUMANS

Epidemiological investigations of the association between vitamin D and asthma lag somewhat behind scientific investigations of the effects of vitamin D on immune function and airway cell biology *in vitro* and in animal models. Epidemiological studies of vitamin D and asthma can be broadly divided into those investigating associations between asthma and early life vitamin D status during fetal development and infancy, and those that have investigated vitamin D status in subjects with asthma.

Early Life Vitamin D Status

Five birth cohort studies have reported associations between maternal vitamin D status during pregnancy and childhood wheeze and asthma outcomes [34-38]. Whilst the studies have been conducted prospectively in populations that differ in dietary patterns, smoking habits, socio-economic profiles and probably genetic susceptibility all have methodological weaknesses. Four studies did not fully assess maternal vitamin D status during pregnancy, reporting associations with maternal vitamin D intake, thus failing to account for the substantial contribution of sunlight derived vitamin D [34-37]. Project Viva in the US reported reduced maternal vitamin D intake during pregnancy to be associated with an increased likelihood of wheezing outcomes in 1194 children aged 3 (e.g. recurrent wheeze, highest *vs* lowest quartile of maternal intake OR 0.39, 95% confidence interval 0.25-0.62) [34]. In Scotland reduced maternal vitamin D intake during pregnancy has been reported to be associated with an increased likelihood of wheezing outcomes in 1212 children aged 5 (e.g. current wheeze highest *vs* lowest quintile of maternal intake OR 0.35, 95% confidence interval 0.15-0.83); however this study was notable for reporting no association between maternal vitamin D intake and asthma, lung function or exhaled nitric oxide in children aged 5 years [35]. A Japanese birth cohort study similarly reported reduced maternal vitamin D intake during pregnancy to be associated with an increased likelihood of wheezing in 762 children aged 16-24 months (e.g. current wheeze highest *vs* lowest maternal intakes (divided at 25^{th} centile) OR 0.64,

95% confidence interval 0.43-0.97); however it is likely that this association reflects the isolated finding of a markedly reduced likelihood of wheezing in children born to mothers in the second quartile of vitamin D intake [36]. Reduced maternal vitamin D intake during pregnancy has been reported to be associated with an increased likelihood of asthma in 1669 Finnish children aged 5, hazard ratio 0.76, 95% confidence interval 0.59–0.99 [37]. However this study quantified maternal diet postnatally and the children were selected for their increased risk of type I diabetes because of their HLA-DQB1 genotype. A weakness of these studies is that vitamin D intake alone was measured, failing to quantify vitamin D generated from sunlight exposure. Serum 25-hydroxyvitamin D (25[OH]D) concentration is considered to be the best circulating biomarker of vitamin D status, reflecting contributions from dietary and sunlight exposure. An adverse association between maternal serum 25(OH)D concentration during the third trimester of pregnancy and asthma in 178 children aged 9 years has been reported in a UK birth cohort study (maternal serum 25(OH)D>75nmol/l *vs* <30nmol/l OR 5.40, 95% confidence interval 1.09-26.65); however the loss to follow up (62%) was substantial [38]. High dose (2000 IU/day) vitamin D supplementation during infancy has also been adversely associated with allergic outcomes (atopic sensitisation, allergic rhinitis) in 7,648 adults aged 31 years participating in a Finnish birth cohort survey [39]. The likelihood of asthma in these adults was also increased in those supplemented during infancy (OR 1.33, 95% confidence interval 0.97-1.82), but this was not statistically significant (p=0.08). Taken as a whole these studies suggest that reduced maternal vitamin D intake during pregnancy is associated with a higher likelihood of childhood wheezing and maybe asthma. However, despite methodological limitations it should be noted that the likelihood of childhood allergic and asthma outcomes has been adversely associated with high maternal serum 25(OH)D during pregnancy and high-dose (2000 IU/day) vitamin D supplementation during infancy. The possible dose-dependent associations with vitamin D are analogous to those reported in some immunological studies [12].

Vitamin D in Asthma

The immunomodulatory properties of vitamin D justify studies of vitamin D status in people with asthma because of the potential use of vitamin D supplements to complement conventional asthma therapy; conversely, an adverse association could lead to a re-evaluation of osteoporosis vitamin D prophylaxis in asthma. A number of studies have investigated the association between serum 25(OH)D concentration and parameters of asthma severity in adults and children. Brehm and colleagues reported low serum 25(OH)D concentrations to be associated with elevated total IgE and eosinophil counts and increased likelihood of methacholine airway responsiveness, asthma-related hospitalization and use of anti-inflammatory medication in 616 Costa Rican children with asthma aged 6-14 [40]. Brehm and colleagues have also quantified the serum 25(OH) D concentrations of the 1024 children with mild to moderate asthma participating in the Childhood Asthma Management Program [41]. In cross-sectional and prospective analyses, vitamin D insufficiency (25(OH)D ≤75 nmol/L) was associated with an increased likelihood of an asthma exacerbation necessitating hospitalisation or a visit to the Emergency Department in the year prior to (OR 1.7, 95% CI 1.2-2.7), and the four years after (OR 1.4 95% CI 1.0-1.9) measurement of serum 25(OH)D concentration. Similar associations between reduced serum 25(OH)D concentration and increased use of inhaled corticosteroids, use of oral corticosteroids, raised serum IgE and number of positive aeroallergen skin prick tests have also been reported in 100 children with asthma aged 0-18 years (median age 7 years, interquartile range 4-10 years) [42]. The same study also reported positive associations between increased serum 25(OH)D concentration and increased FEV1 and FEV1:FVC ratio. The interpretation of this study is likely to be complicated by a number of design features. Some very young children with asthma were included; furthermore there is a high likelihood of significant selection bias because the studied children were identified by the fact that they had had a serum 25(OH)D measurement as part of their clinical management. In 54 adults with asthma reduced serum 25(OH)D concentration has been reported to be associated with reduced FEV1, and in the same study vitamin D insufficiency (serum 25(OH)D <75 nmol/L) was associated with an increased likelihood of methacholine airway responsiveness [43]. Whilst these studies suggest an association between severity of asthma and vitamin D, it is not possible to conclude from these studies that people with asthma have a reduced vitamin D status because the studies outlined above did not include control subjects. A recent case-control study of 80 subjects with asthma aged 15-50 years and 80 closely matched control subjects reported no difference in serum total 25(OH)D between subjects with asthma (mean 10.1 ng/ml, 95% CI 8.3-11.9) and those without (mean 10.1 ng/ml, 95% CI 8.4-11.7) [44]. In the subjects with asthma serum total 25(OH)D was not associated with FEV1 or asthma severity. This study suggests that serum 25(OH)D concentration is not reduced in asthma, and does not support the widespread use of vitamin D to supplement conventional asthma therapy. A notable feature of this study were the very low 25(OH)D levels (mean 10.1ng/ml 95% CI 8.9-11.3) and this may have limited the ability of the study to detect a difference; of note, there was no difference in serum total 25(OH)D concentrations between cases and controls in the two participating centres, one in the North East of Scotland (mean 7.4 ng/ml, 95% CI 5.9-9.0) and the South East of England (mean 14.1ng/ml, 95% CI 12.6-15.5). It is worth noting that an association between maternal vitamin D intake during pregnancy and childhood wheeze has been reported in women of the same age living in the same Scottish Region [35], Although the very limited data to date suggest that universal vitamin D supplementation of people with asthma is not currently justified, the possibility remains that vitamin D supplementation may be beneficial in select groups of people with asthma e.g. corticosteroid resistant, severe, and indeed randomised controlled trials are underway to directly address these issues.

Two studies of vitamin D in adults, whilst not directly relevant to asthma warrant discussion. In NHANES III the highest quartile of serum 25(OH)D (>85.7 nmol/l) was associated with an increased FEV1 (106ml) and FVC (142ml) in 14,091 adults [45]. The absence of any association between serum 25(OH)D and FEV1:FVC ratio

suggests that in adults vitamin D is not associated with airflow obstruction, but rather the associations are in keeping with an effect of vitamin D on lung growth and/or rate of decline of lung function. A non-linear association between serum 25(OH)Dand serum IgE has been reported in 7288 adults aged 45 participating in the UK 1958 birth cohort study [46]. Serum IgE was elevated by 29% in those with a serum 25(OH)D in the lowest quartile and by 56% in those in the highest quartile. This non-linear "U" shaped association is analogous to the dose-dependent associations with vitamin D status reported in some immunological studies.

CONCLUSIONS

The increasing interest in vitamin D and asthma mirrors a generalised increase in interest in vitamin D in relation to many conditions such as cancer, infection, cardiovascular disease, schizophrenia, multiple sclerosis, inflammatory bowel disease and diabetes [47]. The scientific debate surrounding vitamin D and asthma is somewhat unusual in that beneficial, adverse and null effects have been hypothesised, with all being supported to some extent by studies of cellular immunology, airway cell biology, animal models and epidemiological studies. Currently the major questions in the field are whether vitamin D supplementation during pregnancy reduces the likelihood of childhood asthma, and whether there is a role for vitamin D supplementation in asthma. The available evidence suggest that whilst people with asthma appear not to be more deficient in vitamin D than normal subjects [44] there is some evidence suggesting that children and adults with severe asthma have reduced vitamin D status when compared with those with less severe asthma [40-43]. Trials are currently underway to ascertain whether vitamin D supplementation increases the therapeutic effectiveness of corticosteroids in asthma and is beneficial in severe asthma.

Based on the results of epidemiological studies and consideration of biological properties, vitamin D supplementation during pregnancy has been for advocated for pregnant women as a preventative for childhood asthma [48]. Intervention trials of vitamin D supplementation during pregnancy with the intention of primary asthma prevention raise a number of difficult scientific, ethical and regulatory issues. Whilst indeed a majority of observational studies suggest that vitamin D supplementation during pregnancy should reduce the likelihood of childhood wheeze and possibly asthma, it is important to highlight the significant minority of studies suggesting that perhaps vitamin D supplementation during pregnancy and infancy may have an adverse effect on asthma. In mice, higher doses with the precursor vitamin D2 can result in malformed fetuses [49]. It has only been recently described that the endometrium during pregnancy expresses VDR and synthesizes $1,25(OH)_2D$ [50, 51]. That vitamin D can suppress signals of normal cell cycling [52] and inhibit myometrial cell growth *in vitro* [53], suggests the *in utero* environment during pregnancy could be sensitive to high concentrations of vitamin D, and therefore defining the therapeutic index for adverse versus beneficial effects is critical. Despite these concerns the evidence to date is that high dose vitamin D supplementation (up to 4,000 IU/day) during pregnancy is safe [54]. It still remains to be seen whether safety in a relatively small trial of women is translated into safety for millions of pregnant women and fetuses.

Further observational, immunological, cellular and animal studies are unlikely to resolve the issues surrounding the potential for vitamin D supplementation to prevent asthma and therefore scientific, ethical and economic considerations justify the interventional trials of vitamin D supplementation in pregnant women that are underway in the US, Denmark and New Zealand. The results of these trials are likely to provide definitive answers in the near future as to whether childhood asthma is reduced, increased or unaffected by maternal vitamin D supplementation during pregnancy.

REFERENCES

[1] Wjst M, Dold S. Genes, factor X, and allergens: what causes allergic diseases? Allergy 1999; 54: 757-9.

[2] Wjst M. The vitamin D slant on allergy. Pediatr Allergy Immunol 2006: 17: 477-83.

[3] Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? J Allergy Clin Immunol 2007; 120: 1031-5.

[4] Ginde AA, Mansbach JM, Camargo CA. Vitamin D, respiratory infections, and asthma. Curr Allergy Asthma Rep 2009; 9: 81-7.

[5] Lloyd CM. Hawrylowicz CM. Regulatory T cells in asthma. Immunity 2009; 31: 438-49.

[6] Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. FASEB J 2005; 19: 1067-77.

[7] Yim S, Dhawan P, Ragunath C, Christakos S, Diamond G. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3). J Cyst Fibros 2007; 6: 403-10.

[8] Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HFJ, O'Garra A. 1α,25-Dihydroxyvitamin D3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. J Immunol 2001; 167: 4974-80.

[9] Lemire JM, Archer DC, Beck L, Speigelberg HL. Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions. J Nutr 1995; 125: 1704S-8S.

[10] Jirapongsananuruk O, Melamed I, Leung DY. Additive immunosuppressive effects of 1,25-dihydroxyvitamin D3 and corticosteroids on TH1, but not TH2, responses. J Allergy Clin Immunol 2000; 106: 981-5.

[11] Pichler J, Gerstmayr M, Szépfalusi Z, Urbanek R, Peterlik M, Willheim M. 1 alpha,25(OH)2D3 inhibits not only Th1 but also Th2 differentiation in human cord blood T cells. Pediatr Res 2002; 52: 3-5.

[12] Urry Z, Xystrakis E, Richards DF, *et al*. Ligation of TLR9 induced on human IL-10-secreting Tregs by 1alpha,25-dihydroxyvitamin D3 abrogates regulatory function. J Clin Invest 2009; 119: 387-98.

[13] Xystrakis E, Kusumakar S, Boswell S, *et al*. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. J Clin Invest 2006; 116: 146-55.

[14] Penna G, Roncari A, Amuchastegui S, *et al*. Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for induction of CD4+Foxp3+ regulatory T cells by 1,25-dihydroxyvitamin D3. Blood 2005; 106: 3490-7

[15] Holgate ST. Asthma: more than an inflammatory disease. Curr Opin Allergy Clin Immunol 2002; 2: 27-9.

[16] Puddicombe SM, Polosa R, Richter A, *et al*. Involvement of the epidermal growth factor receptor in epithelial repair in asthma. FASEB J 2000; 14: 1362-74.

[17] Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST. Airway remodelling in asthma: new insights. J Allergy Clin Immunol 2003; 111: 215-25.

[18] Nguyen M, Guillozo H, Garabedian M, Balsan S. Lung as a possible target organ for Vitamin D during fetal life in the rat. Biol Neonate 1987; 52: 232-40.

[19] Nguyen M, Trubert CL, Rizk-Rabin M, *et al*. 1,25-Dihydroxyvitamin D3 and fetal lung maturation: immunogold detection of VDR expression in pneumocytes type II cells and effect on fructose 1,6 bisphosphatase. J Steroid Biochem Mol Biol 2004; 89-90: 93-7.

[20] Sakurai R, Shin E, Fonseca S, *et al.* 1α,25(OH)2D3 and its 3-epimer promote rat lung alveolar epithelial-mesenchymal interactions and inhibit lipofibroblast apoptosis. Am J Physiol Lung Cell Mol Physiol 2009; 297: L496 - L505.

[21] Bossé Y, Maghni K, Hudson TJ. 1α,25-Dihydroxy-vitamin D3 stimulation of bronchial smooth muscle cells induces autocrine, contractility, and remodeling processes. Physiol Genomics 2007; 29: 161-8.

[22] Pieter S. Hiemstra The role of epithelial β-defensins and cathelicidins in host defense of the lung. Exp Lung Res 2007; 33: 537-42.

[23] Xiao W, Hsu YP, Ishizaka A, Kirikae T, Richard B. COPD, and Asthma Inflammation Cytokines Discriminate Cystic Fibrosis, Activation System Components, and Sputum Cathelicidin, Urokinase Plasminogen. Chest 2005; 128: 2316-26.

[24] Wittke A, Weaver V, Mahon BD, August A, Cantorna MT. Vitamin D receptor-deficient mice fail to develop experimental allergic asthma. J Immunol 2004; 173: 3432-6.

[25] Wittke A, Chang A, Froicu M, *et al*. Vitamin D receptor expression by the lung micro-environment is required for maximal induction of lung inflammation. Arch Biochem Biophys 2007; 460: 306-13.

[26] Yu S, Bruce D, Froicu M, Weaver V, Cantorna MT. Failure of T cell homing, reduced CD4/CD8alphaalpha intraepithelial lymphocytes, and inflammation in the gut of vitamin D receptor KO mice. Proc Natl Acad Sci USA 2008; 105: 20834-9.

[27] Malley RC, Muller HK, Norval M, Woods GM. Vitamin D3 deficiency enhances contact hypersensitivity in male but not in female mice. Cell Immunol 2009; 255: 33-40.

[28] Harvey L, Burne TH, McGrath JJ, Eyles DW. Developmental vitamin D(3) deficiency induces alterations in immune organ morphology and function in adult offspring. J Steroid Biochem Mol Biol 2010; 121(1-2): 239-42.

[29] NRC. Nutrient Requirements for Laboratory Animals, 4th rev. edn. in National Academy Press (National Research Council, Washington, DC, 1995).

[30] Schachter D, Finkelstein J, Kowarski S. Metabolism of Vitamin D. I. Preparation of Radioactive Vitamin D and its Intestinal Absorption in the Rat. J Clin Invest 1964; 43: 787-96.

[31] Topilski I, Flaishon L, Naveh Y, Harmelin A, Levo Y, Shachar I. The anti-inflammatory effects of 1,25-dihydroxyvitamin D3 on Th2 cells *in vivo* are due in part to the control of integrin-mediated T lymphocyte homing. Eur J Immunol 2004; 34: 1068-76.

[32] Matheu V, Back O, Mondoc E, Issazadeh-Navikas S. Dual effects of vitamin D-induced alteration of TH1/TH2 cytokine expression: enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease. J Allergy Clin Immunol 2003; 112: 585-92.

[33] Cantorna MT, Munsick C, Bemiss C, Mahon BD. 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. J Nutr 2000; 130: 2648-52.

[34] Camargo CA, Rifas-Shiman SL, Litonjua AA, *et al*. Maternal intake of Vitamin D during pregnancy and reports of recurrent wheezing in children at 3 y of age. Am J Clin Nutrition 2007; 85: 788-95.

[35] Devereux G, Litonjua AA, Turner SW, *et al*. Maternal vitamin D intake during pregnancy and early childhood wheeze. Am J Clin Nutrition 2007; 85: 853-9.

[36] Miyake Y, Sasaki S, Tanaka K, Hirota Y. Dairy food, calcium, and vitamin D intake in pregnancy and wheeze and eczema in infants. Eur Respir J 2010; 35: 1228-34.

[37] Erkkola M, Kaila M, Nwaru BI, *et al*. Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children. Clin Exp Allergy 2009; 39: 875-82.

[38] Gale CR, Robinson SM, Harvey NC, *et al*, Princess Anne Hospital Study Group. Maternal vitamin D status during pregnancy and childhood outcomes. Eur J Clin Nutrition 2008; 62: 68-77.

[39] Hypponen E, Sovio U, Wjst M, *et al*. Infant vitamin D supplementation and allergic conditions in adulthood: northern Finland birth cohort. 1966. Ann N York Acad Sciences 2004; 1037: 84-95.

[40] Brehm J, Celedón JC, Soto-Quiros ME, *et al*. Serum Vitamin D levels and markers of severity of childhood asthma in Costa Rica. Am J Respir Crit Care Med 2009; 179; 765-71.

[41] Brehm JM, Schuemann B, Fuhlbrigge AL, *et al*. Childhood Asthma Management Program Research Group. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. J Allergy Clin Immunol 2010; 126: 52-8.

[42] Searing DA, Zhang Y, Murphy JR, Hauk PJ, Goleva E, Leung DY. Decreased serum vitamin D levels in children with asthma are associated with increased corticosteroid use. J Allergy Clin Immunol 2010; 125(5): 995-1000.

[43] Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DY. Vitamin D levels, lung function, and steroid response in adult asthma. Am J Respir Crit Care Med 2010; 181(7): 699-704.

[44] Devereux G, Wilson A, Avenell A, McNeill G, Fraser WD. A case control study of vitamin D status and asthma in adults. Allergy 2010; 65: 666-7.

[45] Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin D and pulmonary function in the third national health and nutrition examination survey. Chest 2005; 128: 3792-8.

[46] Hypponen E, Berry DJ, Wjst M, Power P. Serum 25-hydroxyvitamin D and IgE – a significant but nonlinear relationship. Allergy 2009: 64: 613-20.

[47] Hollick MF. Medical Progress: Vitamin D Deficiency. New Eng J Med 2007: 357; 266-81.

[48] Weiss ST, Litonjua AA. Maternal diet *vs* lack of exposure to sunlight as the cause of the epidemic of asthma, allergies and other autoimmune diseases. Thorax 2007; 62; 745-6.

[49] Zane CE. Assessment of Hypervitaminosis D during the first trimester of pregnancy on the mouse embryo. Preliminary report. Arzneimittelforschung 1976; 26: 1589-90.

[50] Vigano P, Lattuada D, Mangioni S, *et al*. Cycling and early pregnant endometrium as a site of regulated expression of the vitamin D system. J Mol Endocrinol 2006; 36: 415-24.

[51] Evans KN, Nguyen L, Chan J, *et al*. Effects of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 on cytokine production by human decidual cells. Biol Reprod 2006; 75: 816-22.

[52] Jensen SS, Madsen MW, Lukas J, Binderup L, Bartek J. Inhibitory effects of 1alpha, 25-dihydroxyvitamin D(3) on the G(1)-S phase-controlling machinery. Mol Endocrinol 2001; 15: 1370-80.

[53] Blauer M, Rovio PH, Ylikomi T, Heinonen PK. Vitamin D inhibits myometrial and leiomyoma cell proliferation *in vitro*. Fertil Steril 2009; 91: 1919-25.

[54] Wagner CL, Johnson D, Hulsey TC, *et al*. Vitamin D supplementation during Pregnancy Part I NICHD/CTSA Randomized Clinical Trial (RCT): Safety Considerations. Pediatric Academic Societies 2010; Abstract 2630.7.

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Vitamin D in COPD - A Pleiotropic Micronutrient in a Multisystem Disease

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Abstract: Chronic Obstructive Pulmonary Disease (COPD) is a debilitating disease affecting an estimated 3 million people in the United Kingdom. It is characterised by progressive and irreversible airway obstruction and lung parenchymal damage, and by multisystem involvement including skeletal muscle impairment, systemic inflammation, and an increased prevalence of osteoporosis, cardiovascular disease and lung cancer.

Patients with COPD have reduced dietary intake of vitamin D, spend a reduced amount of time outdoors and have been shown to have lower levels of 25-hydroxyvitamin D (25[OH]D) than age-matched subjects without COPD. The active metabolite of vitamin D, 1,25-dihydoxyvitamin D ($1,25[OH]_2D$), is a pleiotropic hormone with effects on lung development and function, the immune system and musculoskeletal function; vitamin D deficiency also associates with increased prevalence of cardiovascular disease and cancer. This article focuses on the evidence that vitamin D deficiency is highly prevalent in patients with COPD and reviews associations between vitamin D status and lung function, muscle function and risk of osteoporosis, cardiovascular disease and cancer. The potential pathological mechanisms which may be involved are also discussed.

Keywords: COPD, immune system, lung function, osteoporosis, skeletal muscle, vitamin D.

INTRODUCTION

COPD is a chronic and under-diagnosed disease affecting an estimated 1.4 million people in England [1] and it is predicted to be the 3rd leading cause of death worldwide by 2020 [2]. It is predominantly caused by cigarette smoking and is characterised by progressive and irreversible airflow obstruction due to airway inflammation and destruction of lung parenchyma. Although classified as a disease of the respiratory system, COPD has important systemic manifestations. Patients with COPD have a high prevalence of osteoporosis and osteopenia [3], leading to an increased risk of fracture. Skeletal muscle weakness is a common feature of the condition [4], which is associated with decreased quality of life and an increase in mortality [5, 6]. There is systemic as well as lung inflammation [7], and an increased susceptibility to infection with recurrent exacerbations causing rapid decline in lung function [8] and body mass index (BMI) [9]. COPD patients also have an increased prevalence of lung cancer [10] and cardiovascular disease [11].

Vitamin D is a pro-hormone which is produced by the action of sunlight on the skin, although it can also be ingested in the diet. It is metabolised in the liver to 25(OH)D, which is transported and stored in the blood bound to vitamin D binding protein (DBP). 25(OH)D is metabolised to the active form, $1,25(OH)_2D$, by the enzyme 1α-hydroxlase which is produced predominantly in the kidney, although it has now been identified in a number of other target organs where $1,25(OH)_2D$ is thought to work in a paracrine fashion. In the kidney, it has been demonstrated that 25(OH)D bound to DBP is delivered to the site of the enzyme 1α-hydroxlase by megalin/cubilin mediated receptor endocytosis, although it is not clear whether this process occurs in all tissues [12]. Vitamin D deficiency has been implicated in the development of a wide range of medical conditions associated with COPD as well as decline in lung function itself. Considering that in the mouse, vitamin D directly or indirectly regulates over 3% of the genome [13] and the widening areas of research into vitamin D, the growing interest in the role of vitamin D in respiratory disease is not surprising [14]. This review will focus on the evidence that vitamin D deficiency occurs in COPD, and review associations between vitamin D deficiency and lung function, muscle strength and risk of osteoporosis as well as summarising other important actions of vitamin D on the immune and cardiovascular systems which are likely to be relevant in patients with COPD.

25-HYDOXYVITAMIN D LEVELS IN COPD

Over half of the elderly population in the UK and USA have vitamin D deficiency depending on the definition used [15, 16]. Debate is still ongoing about the optimal definition of vitamin D deficiency, which could be based on levels required to suppress parathyroid hormone (PTH), levels required for optimal calcium absorption in the intestine and levels required to maintain bone mineral density (BMD) and prevent falls and fractures. Current consensus is that levels greater than 75 nmol/l are required for optimal bone health [17]. Factors associated with 25(OH)D levels in normal subjects are sunlight exposure, latitude and season, skin type, dietary intake of vitamin D, BMI [16] and genetic influences, in particular polymorphisms in the vitamin D binding protein (DBP) [18-20].

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Vitamin D can be obtained from exposure to sunlight or in the diet. In the United Kingdom, sunlight is only sufficiently strong to produce vitamin D between April and November [21]. A recent study in city-dwelling subjects in the UK suggests that current recommendations for brief episodes of sun exposure in the summer months will place most people in the 'sufficient' range of 25(OH)D, but not the optimal range above 75nmol/l. COPD patients are less active [22] and spend less time outdoors [23, 24] compared to healthy elderly subjects due to breathlessness and leg fatigue. They are therefore less likely to have adequate sunlight exposure although this has not been measured directly. They can also have poor nutritional intake, particularly with severe disease. One study in Spain showed that only 4% of COPD subjects consumed the recommended daily intake of 10μg of vitamin D [25]. These factors combined put them at increased risk of having vitamin D deficiency.

One published cross-sectional study has investigated 25(OH)D levels specifically in COPD patients compared to a control population [26]. This study reported that COPD patients had significantly lower levels of 25(OH)D than controls, and that 25(OH)D levels decreased with increasing GOLD Stage of disease. The control population consisted of smokers and ex-smokers who were matched for age and sex, and no subjects were taking any vitamin D supplementation. This evidence is supported by an earlier study that investigated 25(OH)D levels in patients with severe lung disease who were referred for lung transplantation, of whom approximately 50% had a diagnosis of COPD [27]. Although there was no control group, vitamin D deficiency was common: 52% of underweight patients and 55% of those with normal weight had serum 25(OH)D levels < 37.5 nmol/L.

There is an inverse relationship between BMI and serum 25(OH)D concentration [16, 28], which is dependent on fat mass rather than fat free mass (FFM) [29, 30], and which is more pronounced in women than men [31]. This is important in the subset of patients with COPD who are obese; however COPD subjects are often cachectic, which is likely to be due to a combination of decreased calorific intake, increased work of breathing, systemic inflammation and increased resting energy expenditure. Loss of FFM rather than loss of fat is an important prognostic indicator [5]. Even in underweight subjects with lung disease though, fat mass is an important determinant of 25(OH)D levels [27].

25(OH)D and 1,25(OH)D_2 levels are affected by genetic factors. DBP is the main transport and storage protein for vitamin D. The DBP wildtype allele is Gc1F, which over time has undergone 2 mutations to produce a Gc1S allele and a Gc2 allele. Both of the latter alleles have a lower affinity for 25(OH)D and 1,25$(OH)_2$D and are associated with lower circulating levels of both vitamin D metabolites [18]. Interestingly, in COPD the Gc2 allele has been shown to have a protective effect on the development of COPD due to cigarette smoking [32], and the frequency of Gc1F homozygotes has been found to be increased in COPD versus control populations in two other studies [33, 34]. In one of these studies, Gc1F homozygotes had a faster decline in lung function and more severe changes of emphysema seen on CT scan [34]. A more recent study however, found that Gc1S homozygotes had an increased risk for COPD, and this risk appeared to be due to the effects of the Gc1S allele in reducing 25(OH)D levels [26]. Large genetic association studies have not confirmed a clear-cut relationship between DBP polymorphisms and COPD [35, 36]. Studies looking at the effects of polymorphisms in the vitamin D receptor (VDR) [37, 38] or the 1α-hydroxylase enzyme CYP27B1 [39], have not shown any consistent relationship with serum 25(OH)D or 1,25$(OH)_2$ levels.

STUDIES OF VITAMIN D AND LUNG FUNCTION

The 3rd National Health and Nutrition survey (NHANES) was a large US population-based study involving over 14,000 people. It demonstrated convincing evidence that 25(OH)D levels are independently associated with both FEV_1 and FVC [40]. Between the lowest and highest quartiles of serum 25(OH)D concentration there was a difference of 126mls in FEV_1 and 172mls in FVC, after correcting for confounding factors. No association was seen with FEV_1/FVC however, which might suggest that vitamin D deficiency does not contribute to airflow obstruction. However, when looking at the patients in the survey who had a diagnosis of emphysema or bronchitis, the difference in FEV_1 and FVC across the quintiles of 25(OH)D concentration was larger.

Although there appears to be a clear-cut relationship between vitamin D status and lung function in normal subjects, the evidence in COPD is less extensive. The previously mentioned study by Janssens *et al*. did find a correlation between circulating 25(OH)D concentration and % predicted FEV_1 in COPD patients, but not in controls. However, they do not report on whether 25(OH)D was an independent predictor of FEV_1 or FVC [26]. A Norwegian study involving unselected patients referred for lung transplant found only an association between 25(OH)D and the FEV_1/FVC ratio [27].

POTENTIAL PATHOLOGICAL MECHANISMS

The pathological mechanisms of COPD are complex and poorly understood. There is evidence for alteration of the protease/antiprotease balance mediated by an increase in neutrophils and macrophages [41], increased oxidative stress [42], autoimmune dysfunction [43] and dysregulation of lung development pathways including retinoic acid, notch and hedgehog signalling [44]. Although cigarette smoking is the predominant cause of COPD, not all of those who smoke develop the disease. Genetic influences must therefore be important, as illustrated by the case of DBP.

There has been limited research to date looking at potential mechanisms of vitamin D action in the lung. VDR has been identified in bronchial smooth muscle cells [45] and lung fibroblasts [46]. The enzyme 1α-hydroxlase is expressed in respiratory epithelial cells [47] and in alveolar macrophages where increased expression has been demonstrated in disease states such as lung cancer and sarcoidosis [48]. 1,25$(OH)_2$D has been shown to inhibit matrix metalloproteinases which are important in lung remodelling and repair [49]. It has also been shown to mediate the pro-fibrotic response to TGFβ in lung fibroblasts and epithelial cells [46]. A potential role in lung

development is also emerging with $1,25(OH)_2D$ stimulating surfactant production in type II alveolar cells [50], and calcitriol analogues stimulating actin and collagen production in neonatal rat lung fibroblast cultures [51]. However in the latter study the calcitriol analogues impaired the overall alveolarisation process, supporting the hypothesis that excessive vitamin D may have adverse consequences.

VITAMIN D AND THE IMMUNE SYSTEM

There are no studies to our knowledge looking directly at the effects of vitamin D on immune function in COPD. However, vitamin D is an important modulator of the innate and adaptive immune systems. The VDR is expressed in most cells of the immune system including macrophages, dendritic cells, neutrophils, B cells and activated $CD4^+$ and $CD8^+$ T cells [52]. There are two important roles that vitamin D has in the regulation of the immune system which are likely to have an impact on the development and progression of COPD.

In the innate immune system, $1,25(OH)_2D$ stimulates the production of cathelicidin. This is an antimicrobial peptide which has broad spectrum activity against bacterial and viral pathogens. It acts as a chemoattractant for various inflammatory cell types, and is involved in epithelial proliferation and repair and angiogenesis [53]. Cathelicidin expression has been demonstrated in a wide variety of cells and tissues including lung, small intestine, liver, monocytes, neutrophils and myeloid cells [54]. A vitamin D response element is present in the cathelicidin gene promoter region, and direct $1,25(OH)_2D$-mediated expression of cathelicidin has been demonstrated in keratinocytes, neutrophils, monocytes, lung adenocarcinoma cells, and in airway epithelia and serous and mucous cells of the airway submucosal glands [55]. Vitamin D induces activity against *Mycobacterium tuberculosis*, an action which may be partially mediated by cathelicidin [56]. More importantly, for subjects with COPD, cathelicidin has also been shown to have activity against *Pseudomonas aeruginosa* and *Escherichia coli* [55]. There are very few studies looking at the role of cathelicidin in COPD. One study in Polish farmers with COPD found higher levels of cathelicidin in induced sputum compared to farmers without COPD and urban dwellers [57]. Another study also found higher levels of cathelicidin in induced sputum in patients with COPD and cystic fibrosis compared to control subjects, whilst an asthmatic subgroup had the lowest levels [58]. Despite the limited evidence available, cathelicidin, through its multiple actions, may play a role in the development and progression of COPD and further work is required in this area.

Another important role of vitamin D in the immune system is that of T cell regulation. $1,25(OH)_2D$ alters transcription of IL-2, IFN-γ and IL-4 and has been shown to directly influence Th1/Th2 balance in CD4+ cell populations [59, 60]. More recently it has been demonstrated that $1,25(OH)_2D$ suppresses production of IFNγ, IL-17 and IL-21 by $CD4^+$ cells and induces the development of adaptive regulatory T cells [61]. These latter actions may be relevant to the pathogenesis of COPD. The lungs of subjects who smoke but have normal lung function have been shown to have higher levels of regulatory T cells than those who have never smoked, and compared to smokers who have developed COPD [62]. Studies in mice have demonstrated an inflammatory airway response mediated by Th17 cells and characterised by increased neutrophilic inflammation and B cell influx, which was suppressed by the co- transfer of regulatory T cells [63]. Taken together, it appears that functional regulatory T cells may protect against the development of COPD by controlling the T cell response to immune stimuli.

RESPIRATORY INFECTIONS

The actions of vitamin D on the innate immune system are likely to be of relevance to the occurrence of respiratory infection in COPD. The disease is characterised by recurrent exacerbations which are associated with increased breathlessness and reduced quality of life. Recovery back to baseline levels after an exacerbation is often incomplete [64], and recurrent exacerbations are associated with a decline in lung function. Sputum is characterised by an increase in neutrophils which is related to exacerbation severity, and in 78% of cases in one study, bacterial and/or viral pathogens were isolated [65].

COPD exacerbations more commonly occur in the winter months, and a connection between 25(OH)D levels, which are lower in the winter, and susceptibility to upper respiratory tract infections (URTIs) in the population in general has been postulated. This is supported by data from the NHANES study which found an association between recent self-reported URTI and lower 25(OH)D levels [66]. This relationship was stronger in subjects with asthma than in those with COPD. It is possible that the mechanism involved in this protective effect of vitamin D is related to the production of cathelicidin [67].

It also appears that DBP influences the induction of cathelicidin through its effects on the bioavailability of 25(OH)D to monocytes. Monocytes cultured with serum containing low affinity Gc1S or Gc2 DBP had a nearly 3-fold higher induction of cathelicidin compared to monocytes cultured in serum with high affinity Gc1F DBP [68]. This finding could help to explain the protective effects of the low affinity Gc2 allele seen in COPD. DBP is itself immunologically active when it is metabolised to macrophage activating factor by enzymes released from lymphocytes, and it also has been shown to enhance neutrophil chemotaxis [69].

A trial in school children has shown a decrease in the relative risk of influenza A, but not influenza B or rapid diagnostic test negative flu-like symptoms, with vitamin D supplementation [70]. An interesting secondary outcome in this study was a reduction in asthma exacerbations in the intervention arm. Viral infection may contribute to 50% of acute COPD exacerbations attending the emergency department [71], although influenza A is not the most commonly identified pathogen. However, if a simple measure such as vitamin D supplementation can reduce the exacerbation rate in patients with COPD, this could improve quality of life and delay disease progression.

OSTEOPOROSIS IN COPD

Vitamin D has well known actions on bone which are beyond the scope of this review. Essentially, low levels of 25(OH)D contribute to the development of osteopenia and

osteoporosis as there is reduced calcium absorption from the gut, and both PTH and $1,25(OH)D_2$ increase bone resorption to maintain calcium levels [72]. Vitamin D supplementation in elderly people, when given with calcium, has been shown to improve bone mineral density (BMD) and reduce the risk of falls and fracture [73-75]. The reduction in fracture risk may be due to a combination of increases in BMD and indirectly through increases in muscle strength and a reduction in falls.

Recent studies have shown a consistently high prevalence of osteoporosis and osteopenia in patients with COPD. A systematic review showed that osteoporosis occurred in 9 to 69% of patients, and osteopenia in 27 to 67%, with an overall mean prevalence of osteoporosis from 13 studies, involving 772 patients, of 35.1% [3]. Four studies included in this review compared COPD patients and age-matched healthy control subjects and there was a significant difference in prevalence: 32.5% in COPD *vs.* 11.4% in healthy controls. Interestingly, the prevalence of osteoporosis in COPD appears to be higher than in some other chronic lung diseases, including those involving chronic corticosteroid use such as idiopathic pulmonary fibrosis [76-78]. Factors which are consistently related to osteoporosis in multivariate analysis are age, sex, BMI and other anthropometric measurements, lung function and corticosteroid use [79-81]. As well as the morbidity and mortality associated with hip and wrist fractures, vertebral compression fractures are of particular relevance in COPD patients. These cause kyphosis which has a detrimental effect on lung function [82, 83].

Only one study has investigated the association between 25(OH)D levels and BMD in COPD patients: it found no relationship between them. BMD was compared in 49 COPD and 40 healthy control subjects and found to be significantly lower in COPD patients in the lumbar spine, femoral neck and total femur. FEV_1 (l) and weight were independently associated with BMD in the multivariate model which included corticosteroid use and 25(OH)D [84]. Again, this is an area which needs further research.

VITAMIN D AND SKELETAL MUSCLE FUNCTION IN COPD

Vitamin D has a significant role in skeletal muscle function. Patients with osteomalacia develop a myopathy which is usually proximal, and skeletal muscle biopsies in these patients have shown a reduction in size and number of type II muscle fibres [85]. VDR has been demonstrated in skeletal muscle [86] and interestingly the amount of receptors has been shown to decrease with age [87]. VDR knockout mice have reduced type I and type II fibre diameter, and abnormal expression of myogenic regulatory factors which is corrected by the addition of $1,25(OH)_2D$ *in vitro* [88]. Other demonstrated actions of vitamin D in muscle include regulation of calcium transport [89] and phospholipid metabolism [86].

A number of cross-sectional studies have been carried out in elderly subjects demonstrating an association between vitamin D status and different measures of muscle strength [90-92] and a meta-analysis shows a reduction in falls with supplementation of vitamin D [93]. Supplementation with vitamin D has also been shown to increase the size and number of type II fibres [94, 95].

Approximately a quarter of patients with COPD develop skeletal muscle weakness [96] which is thought to be due mainly to inactivity, with other factors such as systemic inflammation and oxidative stress also being important [97]. The myopathy is characterised by a switch from type I to type II fibres with a subsequent reduction in the size of type II fibres. No studies to date have looked at vitamin D levels in relation to muscle function in COPD. One study by our group has looked at polymorphisms in the VDR and found an association between the *FokI* polymorphism and muscle strength in COPD patients and control subjects. People with the C allele, which produces a shorter protein product, had a reduced quadriceps strength when compared to those with one or more T alleles, and this supports previous findings in healthy elderly men [98]. Interestingly, the shorter allele has been associated with an increased risk of type I diabetes, and human monocytes homozygous for the short VDR protein product express higher levels of IL-12 protein and mRNA [99]. Thus it is possible that the reduced muscle strength associated with the C allele is due to an exaggerated response to inflammatory stimuli in muscle. Further studies are required to demonstrate the interaction of 25(OH)D and $1,25(OH)_2D$ levels and polymorphisms in the VDR and effects on muscle strength in COPD.

OTHER CO-MORBIDITIES

Vitamin D is also implicated in other co-morbidities which are more common in COPD such as cardiovascular disease and cancer. In 1α-hydroxylase knockout mice, increased blood pressure, activation of the renin-angiotensin system, myocardial hypertrophy and decreased cardiac function are seen which are prevented by the administration of $1,25(OH)_2D$ [100]. Low levels of 25(OH)D have been associated with increased risk of hypertension [101] and cardiovascular disease [102], and 8 weeks of vitamin D and calcium supplementation have been shown to reduce systolic blood pressure compared to calcium alone [103].

Several epidemiological studies have associated low levels of vitamin D with increased risk of colorectal, breast and prostate cancer risk [104]. However its effects in lung cancer have not been clearly demonstrated. VDR polymorphisms have been associated with the incidence of lung cancer [105] and *in vitro* studies have shown that vitamin D has anti-cancer effects such as suppression of angiogenesis and cell proliferation [104]. Mouse studies have shown a protective effect of $1,25(OH)_2D$ against metastases in lung cancer [106]. However epidemiological data has not shown an association between low 25(OH)D and lung cancer [107].

SUMMARY

In summary, COPD is a common and debilitating disease for which there is currently no cure, and little benefit from available treatment strategies. The widespread effects of vitamin D are peculiarly relevant to the co-morbidities associated with vitamin D deficiency, although current evidence in this field is limited. It is interesting to note that a number of the effects of vitamin D deficiency, such as

osteoporosis and skeletal muscle weakness, seem exaggerated in subjects with COPD compared to those with other lung diseases. Whether this is due to coincidence, to vitamin D deficiency, or to defects in the vitamin D pathway and/or genetic influences requires further research as most of the current evidence is cross-sectional and associative, and causality of vitamin D deficiency in the pathogenesis of COPD has not yet been proven. In addition, and regardless of any causal relationship, supplementation studies are required to see whether any important clinical effect related to COPD might be obtained, in both preventive and therapeutic strategies.

REFERENCES

[1] Nacul L, Soljak M, Samarasundera E, *et al*. COPD in England: a comparison of expected, model-based prevalence and observed prevalence from general practice data. J Public Health 2010: 2011; 33(1): 108-16.

[2] Lopez AD, Murray CC. The global burden of disease, 1990-2020. Nat Med 1998; 4(11): 1241-3.

[3] Graat-Verboom L, Wouters EF, Smeenk FW, *et al*. Current status of research on osteoporosis in COPD: a systematic review. Eur Respir J 2009; 34(1): 209-18.

[4] Seymour JM, Spruit MA, Hopkinson NS, *et al*. The prevalence of quadriceps weakness in COPD and the relationship with disease severity. Eur Respir J 2010; 36(1):81-8.

[5] Schols AM, Broekhuizen R, Weling-Scheepers CA, *et al*. Body composition and mortality in chronic obstructive pulmonary disease. Am J Clin Nutr 2005; 82(1): 53-9.

[6] Swallow EB, Reyes D, Hopkinson NS, *et al*. Quadriceps strength predicts mortality in patients with moderate to severe chronic obstructive pulmonary disease. Thorax 2007; 62(2): 115-20.

[7] Garcia-Rio F, Miravitlles M, Soriano JB, *et al*. Systemic inflammation in chronic obstructive pulmonary disease: a population-based study. Respir Res 2010; 11(1): 63.

[8] Anzueto A, Leimer I, Kesten S. Impact of frequency of COPD exacerbations on pulmonary function, health status and clinical outcomes. Int J Chron Obstruct Pulmon Dis 2009; 4: 245-51.

[9] Hopkinson NS, Tennant RC, Dayer MJ, *et al*. A prospective study of decline in fat free mass and skeletal muscle strength in chronic obstructive pulmonary disease. Respir Res 2007; 8(1): 25.

[10] Garcia Rodriguez LA, Wallander MA, Martin-Merino E, Johansson S. Heart failure, myocardial infarction, lung cancer and death in COPD patients: A UK primary care study. Respir Med 2010; 104(11): 1691-9.

[11] Finkelstein J, Cha E, Scharf SM. Chronic obstructive pulmonary disease as an independent risk factor for cardiovascular morbidity. Int J Chron Obstruct Pulmon Dis 2009; 4: 337-49.

[12] Nykjaer A, Fyfe JC, Kozyraki R, *et al*. Cubilin dysfunction causes abnormal metabolism of the steroid hormone 25(OH) vitamin D(3). Proc Natl Acad Sci USA 2001; 98(24): 13895-900.

[13] Bouillon R, Carmeliet G, Verlinden L, *et al*. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev 2008; 29(6): 726-76.

[14] Janssens W, Lehouck A, Carremans C, *et al*. Vitamin D beyond bones in chronic obstructive pulmonary disease: time to act. Am J Respir Crit Care Med 2009; 179(8): 630-6.

[15] Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357(3): 266-81.

[16] Hypponen E, Power C. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. Am J Clin Nutr 2007; 85(3): 860-8.

[17] Dawson-Hughes B, Heaney RP, Holick MF, *et al*. Estimates of optimal vitamin D status. Osteoporos Int 2005; 16(7): 713-6.

[18] Lauridsen AL, Vestergaard P, Hermann AP, *et al*. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. Calcif Tissue Int 2005; 77(1): 15-22.

[19] Taes YE, Goemaere S, Huang G, *et al*. Vitamin D binding protein, bone status and body composition in community-dwelling elderly men. Bone 2006; 38(5): 701-7.

[20] Ahn J, Yu K, Stolzenberg-Solomon R, *et al*. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet 2010; 19(13): 2739-45.

[21] Webb AR, Engelsen O. Calculated ultraviolet exposure levels for a healthy vitamin D status. Photochem Photobiol 2006; 82(6): 1697-703.

[22] Pitta F, Troosters T, Spruit MA, *et al*. Characteristics of physical activities in daily life in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2005; 171(9): 972-7.

[23] Baghai-Ravary R, Quint JK, Goldring JJ, *et al*. Determinants and impact of fatigue in patients with chronic obstructive pulmonary disease. Respir Med 2009; 103(2): 216-23.

[24] Donaldson GC, Wilkinson TM, Hurst JR, *et al*. Exacerbations and time spent outdoors in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2005; 171(5): 446-52.

[25] de Batlle J, Romieu I, Anto JM, *et al*. Dietary habits of firstly admitted Spanish COPD patients. Respir Med 2009; 103(12): 1904-10.

[26] Janssens W, Bouillon R, Claes B, *et al*. Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. Thorax 2010; 65(3): 215-20.

[27] Forli L, Bjortuft O, Boe J. Vitamin D status in relation to nutritional depletion and muscle function in patients with advanced pulmonary disease. Exp Lung Res 2009; 35(6): 524-38.

[28] Brock KE, Graubard BI, Fraser DR, *et al*. Predictors of vitamin D biochemical status in a large sample of middle-aged male smokers in Finland. Eur J Clin Nutr 2010; 64(3): 280-8.

[29] Vilarrasa N, Maravall J, Estepa A, *et al*. Low 25-hydroxyvitamin D concentrations in obese women: their clinical significance and relationship with anthropometric and body composition variables. J Endocrinol Invest 2007; 30(8): 653-8.

[30] Moschonis G, Tanagra S, Koutsikas K, *et al*. Association between serum 25-hydroxyvitamin D levels and body composition in postmenopausal women: the postmenopausal Health Study. Menopause 2009; 16(4): 701-7.

[31] Gomez JM, Maravall FJ, Gomez N, *et al*. Relationship between 25-(OH) D3, the IGF-I system, leptin, anthropometric and body composition variables in a healthy, randomly selected population. Horm Metab Res 2004; 36(1): 48-53.

[32] Schellenberg D, Pare PD, Weir TD, *et al*. Vitamin D binding protein variants and the risk of COPD. Am J Respir Crit Care Med. 1998; 157(3 Pt 1): 957-61.

[33] Ishii T, Keicho N, Teramoto S, *et al*. Association of Gc-globulin variation with susceptibility to COPD and diffuse panbronchiolitis. Eur Respir J 2001; 18(5): 753-7.

[34] Ito I, Nagai S, Hoshino Y, *et al*. Risk and severity of COPD is associated with the group-specific component of serum globulin 1F allele. Chest 2004; 125(1): 63-70.

[35] Wilk JB, Walter RE, Laramie JM, *et al*. Framingham Heart Study genome-wide association: results for pulmonary function measures. BMC Med Genet 2007; 8 Suppl 1: S8.

[36] Bakke PS, Zhu G, Gulsvik A, *et al*. Candidate genes for chronic obstructive pulmonary disease in two large data sets. Eur Respir J 2011; 37(2): 255-63.

[37] Ramos-Lopez E, Jansen T, Ivaskevicius V, *et al*. Protection from type 1 diabetes by vitamin D receptor haplotypes. Ann N Y Acad Sci 2006; 1079: 327-34.

[38] Ogunkolade BW, Boucher BJ, Prahl JM, *et al*. Vitamin D receptor (VDR) mRNA and VDR protein levels in relation to vitamin D status, insulin secretory capacity, and VDR genotype in Bangladeshi Asians. Diabetes 2002; 51(7): 2294-300.

[39] Engelman CD, Fingerlin TE, Langefeld CD, *et al*. Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in Hispanic and African Americans. J Clin Endocrinol Metab 2008; 93(9): 3381-8.

[40] Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey. Chest 2005; 128(6): 3792-8.

[41] Djekic UV, Gaggar A, Weathington NM. Attacking the multi-tiered proteolytic pathology of COPD: new insights from basic and translational studies. Pharmacol Ther 2009; 121(2): 132-46.

[42] MacNee W. Pulmonary and systemic oxidant/antioxidant imbalance in chronic obstructive pulmonary disease. Proc Am Thorac Soc 2005; 2(1): 50-60.

[43] Cosio MG, Saetta M, Agusti A. Immunologic aspects of chronic obstructive pulmonary disease. N Engl J Med 2009; 360(23): 2445-54.

[44] Shi W, Chen F, Cardoso WV. Mechanisms of lung development: contribution to adult lung disease and relevance to chronic obstructive pulmonary disease. Proc Am Thorac Soc 2009; 6(7): 558-63.

[45] Bosse Y, Maghni K, Hudson TJ. 1alpha,25-dihydroxy-vitamin D3 stimulation of bronchial smooth muscle cells induces autocrine, contractility, and remodeling processes. Physiol Genomics 2007; 29(2): 161-8.

[46] Ramirez AM, Wongtrakool C, Welch T, *et al*. Vitamin D inhibition of pro-fibrotic effects of transforming growth factor beta1 in lung fibroblasts and epithelial cells. J Steroid Biochem Mol Biol 2010; 118(3): 142-50.

[47] Hansdottir S, Monick MM, Hinde SL, *et al*. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. J Immunol 2008; 181(10): 7090-9.

[48] Yokomura K, Suda T, Sasaki S, *et al*. Increased expression of the 25-hydroxyvitamin D(3)-1alpha-hydroxylase gene in alveolar macrophages of patients with lung cancer. J Clin Endocrinol Metab 2003; 88(12): 5704-9.

[49] Koli K, Keski-Oja J. 1alpha,25-dihydroxyvitamin D3 and its analogues down-regulate cell invasion-associated proteases in cultured malignant cells. Cell Growth Differ 2000; 11(4): 221-9.

[50] Rehan VK, Torday JS, Peleg S, *et al*. 1Alpha,25-dihydroxy-3-epi-vitamin D3, a natural metabolite of 1alpha,25-dihydroxy vitamin D3: production and biological activity studies in pulmonary alveolar type II cells. Mol Genet Metab 2002; 76(1): 46-56.

[51] Ormerod AK, Xing Z, Pedigo NG, *et al*. The calcitriol analogue EB1089 impairs alveolarization and induces localized regions of increased fibroblast density in neonatal rat lung. Exp Lung Res 2008; 34(4): 155-82.

[52] Baeke F, Takiishi T, Korf H, *et al*. Vitamin D: modulator of the immune system. Curr Opin Pharmacol 2010; 10(4): 482-96.

[53] Hiemstra PS. The role of epithelial beta-defensins and cathelicidins in host defense of the lung. Exp Lung Res 2007; 33(10): 537-42.

[54] Bals R, Wang X, Zasloff M, *et al*. The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. Proc Natl Acad Sci USA 1998; 95(16): 9541-6.

[55] Wang TT, Nestel FP, Bourdeau V, *et al*. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004; 173(5): 2909-12.

[56] Martineau AR, Wilkinson KA, Newton SM, *et al*. IFN-gamma- and TNF-independent vitamin D-inducible human suppression of mycobacteria: the role of cathelicidin LL-37. J Immunol 2007; 178(11): 7190-8.

[57] Golec M, Reichel C, Mackiewicz B, *et al*. Cathelicidin LL-37, granzymes, TGF-beta1 and cytokines levels in induced sputum from farmers with and without COPD. Ann Agric Environ Med 2009; 16(2): 289-97.

[58] Xiao W, Hsu YP, Ishizaka A, *et al*. Sputum cathelicidin, urokinase plasminogen activation system components, and cytokines discriminate cystic fibrosis, COPD, and asthma inflammation. Chest 2005; 128(4): 2316-26.

[59] Alroy I, Towers TL, Freedman LP. Transcriptional repression of the interleukin-2 gene by vitamin D3: direct inhibition of NFATp/AP-1 complex formation by a nuclear hormone receptor. Mol Cell Biol 1995; 15(10): 5789-99.

[60] Boonstra A, Barrat FJ, Crain C, *et al*. 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. J Immunol 2001; 167(9): 4974-80.

[61] Jeffery LE, Burke F, Mura M, *et al*. 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. J Immunol 2009; 183(9): 5458-67.

[62] Barcelo B, Pons J, Ferrer JM, *et al*. Phenotypic characterisation of T-lymphocytes in COPD: abnormal CD4+CD25+ regulatory T-lymphocyte response to tobacco smoking. Eur Respir J 2008; 31(3): 555-62.

[63] Jaffar Z, Ferrini ME, Girtsman TA, *et al*. Antigen-specific Treg regulate Th17-mediated lung neutrophilic inflammation, B-cell recruitment and polymeric IgA and IgM levels in the airways. Eur J Immunol 2009; 39(12): 3307-14.

[64] Seemungal TA, Donaldson GC, Bhowmik A, *et al*. Time course and recovery of exacerbations in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000; 161(5): 1608-13.

[65] Papi A, Bellettato CM, Braccioni F, *et al*. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. Am J Respir Crit Care Med 2006; 173(10): 1114-21.

[66] Ginde AA, Mansbach JM, Camargo CA, Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med 2009; 169(4): 384-90.

[67] White JH. Vitamin D as an inducer of cathelicidin antimicrobial peptide expression: Past, present and future. J Steroid Biochem Mol Biol 2010; 121(1-2): 234-8

[68] Chun RF, Lauridsen AL, Suon L, *et al*. Vitamin D-Binding Protein Directs Monocyte Responses to 25-Hydroxy- and 1,25-Dihydroxyvitamin D. J Clin Endocrinol Metab 2010; J Clin Endocrinol Metab 2010; 95(7): 3368-76.

[69] Chishimba L, Thickett DR, Stockley RA, *et al*. The vitamin D axis in the lung: a key role for vitamin D-binding protein. Thorax 2010; 65(5): 456-62.

[70] Urashima M, Segawa T, Okazaki M, *et al*. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am J Clin Nutr 2010; 91(5): 1255-60.

[71] Kherad O, Kaiser L, Bridevaux PO, *et al*. Upper viral respiratory infection, biomarkers and chronic obstructive pulmonary disease (COPD) exacerbations. Chest 2010; 138(4): 896-904

[72] Suda T, Ueno Y, Fujii K, *et al*. Vitamin D and bone. J Cell Biochem 2003; 88(2): 259-66.

[73] Chapuy MC, Pamphile R, Paris E, *et al*. Combined calcium and vitamin D3 supplementation in elderly women: confirmation of reversal of secondary hyperparathyroidism and hip fracture risk: the Decalyos II study. Osteoporos Int 2002; 13(3): 257-64.

[74] Boonen S, Lips P, Bouillon R, *et al*. Need for additional calcium to reduce the risk of hip fracture with vitamin d supplementation: evidence from a comparative metaanalysis of randomized controlled trials. J Clin Endocrinol Metab 2007; 92(4): 1415-23.

[75] Patient level pooled analysis of 68 500 patients from seven major vitamin D fracture trials in US and Europe. BMJ 2010; 340: b5463.

[76] Aris RM, Neuringer IP, Weiner MA, *et al*. Severe osteoporosis before and after lung transplantation. Chest 1996; 109(5): 1176-83.

[77] Katsura H, Kida K. A comparison of bone mineral density in elderly female patients with COPD and bronchial asthma. Chest 2002; 122(6): 1949-55.

[78] Tschopp O, Boehler A, Speich R, *et al*. Osteoporosis before lung transplantation: association with low body mass index, but not with underlying disease. Am J Transplant 2002; 2(2): 167-72.

[79] Scanlon PD, Connett JE, Wise RA, *et al*. Loss of bone density with inhaled triamcinolone in Lung Health Study II. Am J Respir Crit Care Med 2004; 170(12): 1302-9.

[80] Kjensli A, Mowinckel P, Ryg MS, *et al*. Low bone mineral density is related to severity of chronic obstructive pulmonary disease. Bone 2007; 40(2): 493-7.

[81] Vrieze A, de Greef MH, Wijkstra PJ, *et al*. Low bone mineral density in COPD patients related to worse lung function, low weight and decreased fat-free mass. Osteoporos Int 2007; 18(9): 1197-202.

[82] Schlaich C, Minne HW, Bruckner T, *et al*. Reduced pulmonary function in patients with spinal osteoporotic fractures. Osteoporos Int 1998; 8(3): 261-7.

[83] Leech JA, Dulberg C, Kellie S, *et al*. Relationship of lung function to severity of osteoporosis in women. Am Rev Respir Dis 1990; 141(1): 68-71.

[84] Franco CB, Paz-Filho G, Gomes PE, *et al*. Chronic obstructive pulmonary disease is associated with osteoporosis and low levels of vitamin D. Osteoporos Int 2009; 20(11): 1881-7.

[85] Yoshikawa S, Nakamura T, Tanabe H, *et al*. Osteomalacic myopathy. Endocrinol Jpn 1979; 26(Suppl): 65-72.

[86] Boland R, de Boland AR, Marinissen MJ, *et al*. Avian muscle cells as targets for the secosteroid hormone 1,25-dihydroxy-vitamin D3. Mol Cell Endocrinol 1995; 114(1-2): 1-8.

[87] Bischoff-Ferrari HA, Borchers M, Gudat F, *et al*. Vitamin D receptor expression in human muscle tissue decreases with age. J Bone Miner Res 2004; 19(2): 265-9.

[88] Endo I, Inoue D, Mitsui T, *et al*. Deletion of vitamin D receptor gene in mice results in abnormal skeletal muscle development with

deregulated expression of myoregulatory transcription factors. Endocrinology 2003; 144(12): 5138-44.

[89] Boland R. Role of vitamin D in skeletal muscle function. Endocr Rev 1986; 7(4): 434-48.

[90] Dhesi JK, Bearne LM, Moniz C, *et al*. Neuromuscular and psychomotor function in elderly subjects who fall and the relationship with vitamin D status. J Bone Miner Res 2002; 17(5): 891-7.

[91] Stewart JW, Alekel DL, Ritland LM, *et al*. Serum 25-hydroxyvitamin D is related to indicators of overall physical fitness in healthy postmenopausal women. Menopause 2009; 16(6): 1093-101.

[92] Zamboni M, Zoico E, Tosoni P, *et al*. Relation between vitamin D, physical performance, and disability in elderly persons. J Gerontol A Biol Sci Med Sci 2002; 57(1): M7-11.

[93] Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, *et al*. Effect of Vitamin D on falls: a meta-analysis. Jama 2004; 291(16): 1999-2006.

[94] Sato Y, Iwamoto J, Kanoko T, *et al*. Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. Cerebrovasc Dis 2005; 20(3): 187-92.

[95] Sorensen OH, Lund B, Saltin B, *et al*. Myopathy in bone loss of ageing: improvement by treatment with 1 alpha-hydroxycholecalciferol and calcium. Clin Sci (Lond) 1979; 56(2): 157-61.

[96] Seymour JM, Moore L, Jolley CJ, *et al*. Outpatient pulmonary rehabilitation following acute exacerbations of COPD. Thorax 2010; 65(5): 423-8.

[97] Jackson A, Hopkinson NS. Skeletal Muscle in Chronic Obstructive Pulmonary Disease. Clin Pulmonary Med 2009; 16(2): 61-7.

[98] Roth SM, Zmuda JM, Cauley JA, *et al*. Vitamin D receptor genotype is associated with fat-free mass and sarcopenia in elderly men. J Gerontol A Biol Sci Med Sci 2004; 59(1): 10-5.

[99] van Etten E, Verlinden L, Giulietti A, *et al*. The vitamin D receptor gene FokI polymorphism: functional impact on the immune system. Eur J Immunol 2007; 37(2): 395-405.

[100] Goltzman D. Vitamin D action : Lessons learned from genetic mouse models. Ann N Y Acad Sci 2010; 1192(1): 145-52.

[101] Forman JP, Curhan GC, Taylor EN. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension among young women. Hypertension 2008; 52(5): 828-32.

[102] Kendrick J, Targher G, Smits G, *et al*. 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination Survey. Atherosclerosis 2009; 205(1): 255-60.

[103] Pfeifer M, Begerow B, Minne HW, *et al*. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. J Clin Endocrinol Metab 2001; 86(4): 1633-7.

[104] Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). Cancer Causes Control 2005; 16(2): 83-95.

[105] Dogan I, Onen HI, Yurdakul AS, *et al*. Polymorphisms in the vitamin D receptor gene and risk of lung cancer. Med Sci Monit 2009; 15(8): BR232-42.

[106] Nakagawa K, Kawaura A, Kato S, *et al*. Metastatic growth of lung cancer cells is extremely reduced in Vitamin D receptor knockout mice. J Steroid Biochem Mol Biol 2004; 89-90(1-5): 545-7.

[107] Kilkkinen A, Knekt P, Heliovaara M, *et al*. Vitamin D status and the risk of lung cancer: a cohort study in Finland. Cancer Epidemiol Biomarkers Prev 2008; 17(11): 3274-8.

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Vitamin D and Acute Respiratory Tract Infection

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Abstract: Acute respiratory tract infections (ARTI) generate a major disease burden worldwide, disproportionately affecting the elderly and the very young. A growing body of evidence supports an important immunomodulatory function for the biologically active metabolite of vitamin D,1,25-dihydroxyvitamin D (1,25$[OH]_2$D). Respiratory epithelial cells and leucocytes express both the vitamin D receptor and CYP27B1, the enzyme which converts 25-hydroxyvitamin D to 1,25$(OH)_2$D. Observational and ecological studies report associations between inadequate vitamin D status and susceptibility to ARTI, and vitamin D deficiency has been proposed as the seasonal stimulus for influenza epidemics. In children, associations between profound vitamin D deficiency and susceptibility to lower respiratory tract infection have been reported in a variety of settings. In adults, particularly amongst those with asthma and COPD, inadequate vitamin D status has been reported to be associated with susceptibility to upper respiratory tract infection. Vitamin D supplementation trials for the prevention of ARTI report conflicting results. This may reflect variation in study populations and methodology, or factors such as genetic heterogeneity within the vitamin D metabolic or signalling pathways. Further trials of vitamin D supplementation in different settings, with measurement of participants' vitamin D status and evaluation of genetic factors which might modify the effect of vitamin D supplementation are needed.

Keywords: Vitamin D, acute lower respiratory tract infection, upper respiratory tract infection, influenza, cold, immune function.

1. INTRODUCTION

Acute respiratory tract infections (ARTI) are a major global cause of morbidity and mortality. They disproportionately affect the elderly [1-4] and the very young [5, 6] and impose a significant economic burden in terms of health care usage [7]. A number of observational studies have reported an association between inadequate vitamin D status and susceptibility to ARTI in both adults and young children [8-13]. These findings are complemented by a rapidly evolving body of evidence which demonstrates that vitamin D metabolites exert immunomodulatory actions *in vitro* [14]. However, in spite of the existence of plausible biological mechanisms whereby vitamin D could enhance antimicrobial immunity [15-18], clinical trials of vitamin D supplementation for the prevention of acute respiratory infection report conflicting results [19-24]. This review article will discuss current evidence for the role of vitamin D deficiency as a risk factor for ARTI, and for vitamin D supplementation as a strategy to prevent ARTI.

1.1. Vitamin D Metabolic and Signalling Pathways

1.1.1. Metabolism of Vitamin D

Vitamin D is predominantly generated through the action of solar ultraviolet B (UVB) radiation (wavelength, 290 to 315 nm) on vitamin D precursors within the skin; dietary sources are limited. Vitamin D undergoes two successive hydroxylation steps to generate the biologically active metabolite, 1,25-dihydroxyvitamin D_3 (1,25$[OH]_2$D) [14]. The first hydroxylation step, 25-hydroxylation by CYP27A1 and CYP2R1 enzymes, occurs predominantly in the liver and generates 25-hydroxyvitamin D (25[OH]D). 25(OH)D is the major circulating vitamin D metabolite and the accepted measure of vitamin D status. The second step, 1-alpha hydroxylation, is catalysed by CYP27B1 and generates 1,25$(OH)_2$D which is transported to target cells bound to vitamin D binding protein (DBP) or deactivated by 24-hydroxylation by CYP24A1 enzyme. 1-alpha hydroxylation occurs mainly in the kidney, but respiratory epithelial cells [15] and leucocytes also express *CYP27B1* and are therefore also potentially capable of generating 1,25$(OH)_2$D [25]. 1,25$(OH)_2$D exerts its biological effects by binding a nuclear receptor, the vitamin D receptor (VDR), which acts as a transcription factor either inducing or repressing the transcription of genes possessing a vitamin D response element (VDRE) in their promoter region. VDR is expressed in a wide range of cells including respiratory epithelial cells and leucocytes [15, 25] (Fig. **1**).

1.1.2. Genetic Polymorphisms in the Vitamin D Metabolic and Signalling Pathways

VDR, DBP, CYP27A1, CYP2R1, CYP27B1 and CYP24A1 are all polymorphic. DPB and VDR polymorphisms have been studied extensively in the context of tuberculosis, and have been reported to influence susceptibility to the disease both independently [26, 27] and in association with vitamin D deficiency [28, 29] and response to antimicrobial therapy [30-32]. An association between VDR polymorphisms and susceptibility to viral bronchiolitis in young children has also been reported [33, 34].

The DBP gene is highly polymorphic and three common alleles (Gc1F, Gc1S and Gc2) result in six common phenotypes. Gc1F allele frequency is common amongst black Americans and black Africans and Gc1F protein has

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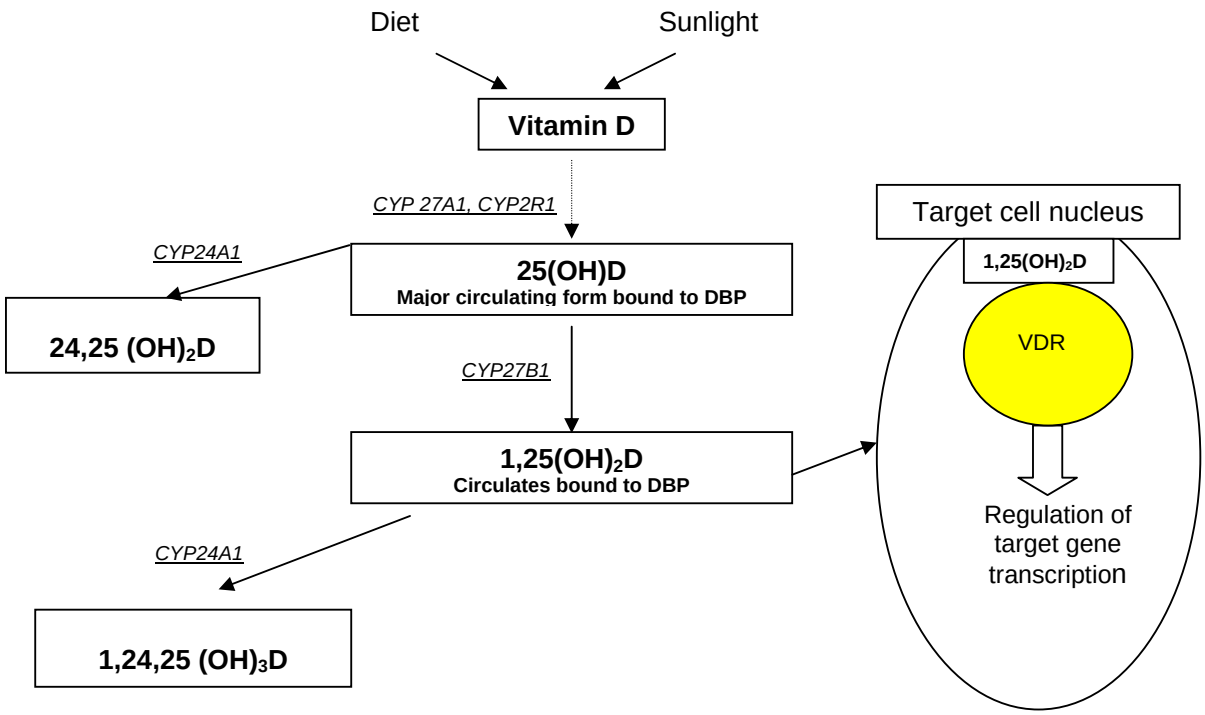

Fig. (1). Key elements in the vitamin D metabolic and signalling pathways. DBP=Vitamin D binding protein; VDR= Vitamin D receptor.

greater affinity for, and is a more efficient transporter of, vitamin D metabolites than other variants [35]. Gc2 protein has the lowest affinity for vitamin D metabolites [36] and is metabolised more quickly than Gc1 [37]. DBP polymorphisms associate with serum 25(OH)D concentrations [38, 39] and influence the 25(OH)D response to vitamin D supplementation [38]. Hence DPB genotype might influence the amount of $25(OH)_2D$ reaching target tissues, and predict which individuals would most benefit from supplementation.

There are four well characterised single nucleotide polymorphisms (SNP) in the VDR gene. Three SNPs (*Taq*I, *Apa*1 and *Bsm*1) are synonymous (i.e. they do not change the amino acid sequence of translated protein), [32] but are in linkage disequilibrium with a poly(A) length polymorphism in the 3' untranslated region (UTR) of the *VDR* gene. Carriage of the *t* allele of the *TaqI* polymorphism has been reported to associate with an increase in VDR gene expression [40]. Because the 3'-UTR is a major regulator of mRNA half-life [41], this association was originally thought to be mediated *via* an effect on mRNA stability [40]; however, subsequent studies have refuted this hypothesis [42, 43], and the mechanism for the association is not currently understood. In contrast the *f* allele of the *Fok1* VDR polymorphism generates a VDR of increased length which is less efficiently transcribed, thus reducing the amount of VDR produced [44]. *In vitro*, *Fok1* variants have been found to have differing effects on immune cell proliferation and cytokine synthesis and on the activity of transcription factors that co-regulate immune responses [44]. These findings suggest that VDR genotype could modify immunomodula-tory effects of vitamin D supplementation.

1.2. Immunomodulatory Role of Vitamin D in Respiratory Infection

Vitamin D has pleiotropic immunomodulatory functions and *in vitro* data provide plausible biological mechanisms whereby vitamin D could influence immune responses to infection. *In vitro* studies using supra-physiological concentrations of $1,25(OH)_2D$ demonstrate multiple actions within the innate immune system. Respiratory epithelial cells, activated macrophages, monocytes and dendritic cells all express CYP27B1 and are thus potentially capable of generating the high local concentrations of $1,25(OH)_2D$ required for exertion of its immune actions [15, 45]. $1,25(OH)_2D$ directly induces the expression of antimicrobial peptides (AMP) such as cathelicidin by monocytes, neutrophils, and respiratory epithelial cells, and these peptides provide a rapid and broad-spectrum defence mechanism against bacterial, viral and fungal pathogens [17, 18, 46]. Viral infection itself upregulates CYP27B1 expression by respiratory epithelial cells [15] and activation of macrophage Toll-like receptors (TLR) by pathogenic antigens also induces the expression of CYP27B1, VDR and AMPs [16]. Hence $1,25(OH)_2D$ has the potential to play an important role within the innate immune system –the first line of defence against respiratory pathogens.

In vitro, $1,25(OH)_2D$ inhibits the synthesis of pro-inflammatory Th1 cytokines such as IFN-γ and TNF-α, modulates expression of inflammatory cytokines induced by viral infection and upregulates the production of anti-inflammatory cytokines such as Interleukin-10 (IL-10) [15, 25, 45]. This anti-inflammatory action has been demonstrated in patients with congestive heart failure, where raised circulating concentrations of pro-inflammatory cytokines are

thought to contribute to disease pathogenesis, and vitamin D supplementation significantly increased the concentration of the anti-inflammatory cytokine IL-10 and prevented an increase in pro-inflammatory TNF-α [47]. In influenza infection, where host inflammatory response is implicated in the pathogenesis of severe disease [48], this anti-inflammatory action might be beneficial. However type I cytokines also play a protective role against some infections: IFN-γ may be protective against hypoxia in acute bronchiolitis [49], and infants with severe RSV bronchiolitis have been shown to have significantly lower levels of IFN-γ in nasopharyngeal aspirates compared with those with less severe disease [50]. Thus, suppression of type I cytokine responses has potential for adverse as well as favourable consequences.

1.3. Epidemiology of Vitamin D Insufficiency and Deficiency

Vitamin D status is determined by measurement of circulating 25(OH)D concentration; liquid chromatography-tandem mass spectrometry is considered the gold standard assay [51-53]. Interpretation of reported 25(OH)D concentrations is complicated by variation in performance of different assays, and inter-laboratory variability has been reported with immunoassays and high performance liquid chromatography [54-56]. Quality assurance schemes have been established in the UK (www.deqas.org) and the US (www.nist.gov) to address this problem. Some experts recommend a target level of >30 ng/ml (75 nmol/l) throughout the year for optimal skeletal and non-skeletal health outcomes, and define vitamin D deficiency and insufficiency as 25(OH)D levels of <20 ng/ml (50 nmol/l) and 20-30ng/ml (50 to 75 nmol/l) respectively [55, 57]; others regard concentration > 20 ng/ml (50 nmol/L) as adequate for optimal health [58]. As dietary sources of vitamin D are limited, the most important determinants of inadequate vitamin D status in otherwise healthy individuals are factors which reduce cutaneous synthesis of vitamin D. These include inadequate sun exposure; season and latitude (over 35 degrees from the equator very little vitamin D is produced in Winter and Spring as fewer UVB photons penetrate the atmosphere); skin pigmentation and use of sunscreen (as both melanin and sunscreen absorb UVB); and aging, which reduces the concentration of vitamin D precursors in the skin [14]. Breast milk of vitamin D deficient mothers contains little vitamin D, hence exclusively breast fed infants of vitamin D deficient mothers are at high risk of developing vitamin D deficiency [14].

Using the aforementioned definitions a current world wide epidemic of vitamin D inadequacy is reported [59]. An estimated 30-77% of European and US populations are either vitamin D insufficient or deficient [8, 55, 60], most notably during the winter and spring months [61] when incidence of ARTI peaks [62, 63]. Deficiency is common in patients with COPD and asthma, amongst whom reduced 25(OH)D levels associate with disease severity [8, 64, 65], and URTIs are common precipitants for acute exacerbations [1, 66, 67]. Profound vitamin D deficiency (serum 25(OH)D concentration <10 ng/ml [25 nmol/l]) which is frequently accompanied by osteomalacia (or rickets in young children) is particularly common amongst the institutionalised elderly, who are also most susceptible to ARTI [59, 68]. Hence observational data certainly support a link between inadequate vitamin D status and acute respiratory tract infection.

1.4. Classification of Upper and Lower Respiratory Tract Infection

The International Classification of Diseases defines acute upper respiratory tract infection (URTI) as acute infection affecting the airway at any level above the bronchi. Influenza and pneumonia with or without upper airway involvement, and acute bronchitis and bronchiolitis are categorised as acute lower respiratory tract infections (ALRI) [69]. It is useful to distinguish between viral and bacterial infections in order to provide appropriate treatment, where required. URTIs are generally viral in origin [70] and ALRI is often caused by bacteria [71, 72]. However this is not a hard and fast rule, as viral bronchiolitis is an important cause of ALRI, influenza can infect both bronchi and lungs, and up to 10% of URTIs are caused by bacteria [70]. Combinations of symptoms have been validated to distinguish between URTI [73], influenza [74] and ALRI [63, 75], but there is overlap between these symptom-complexes, in particular between those used to identify URTI and influenza. It can be difficult to accurately categorise ARTI as URTI or ALRI using symptom criteria alone. In most of the studies discussed below it has been necessary to use symptom complexes, and some studies have investigated the incidence of upper and lower acute respiratory tract infections combined. In a research context, where an intervention is being evaluated for the prevention of ARTI, the gold standard for defining a case of ARTI should ideally involve direct evidence of infection, i.e. detection of bacteria or virus, aided by indirect evidence i.e. radiographic pneumonia; this would remove the need to categorise infections as upper or lower respiratory in origin. Furthermore, the rapid advances in nucleic amplification tests have made it possible to easily directly detect the presence of respiratory virus infection [76]. However, microbiological techniques are not always effective in identifying the causative agents of ARTI, and in resource-constrained settings access to microbiological diagnostics and chest radiography remains limited.

1.4.1. Case Definitions for ALRI

In early childhood the World Health Organization (WHO) clinical case definitions for identifying ALRI have generally been used in studies evaluating the association between ALRI with rickets or inadequate vitamin D status. These are well-validated case definitions, aimed to assist health care workers in the management of ALRI, which focus on the detection of pneumonia and bronchiolitis that are responsible for the majority of ALRI globally in early childhood [77]. Children under 5 years with cough or difficulty breathing are considered to have ‘clinical

pneumonia' if their respiratory rates are raised (≥60 breaths/min in children ≤2 months; ≥50 breaths/min in children 2–11 months; ≥40breaths/min in children 1–4 years) or if lower chest wall indrawing is present [78, 79]. Radiological criteria, where available, are considered useful for defining bacterial episodes and chest radiographs are more specific than clinical criteria alone [77]. Laboratory investigations are not considered useful in establishing a diagnosis of ALRI in resource-limited settings, and these have not in general been used to identify cases in the studies discussed below.

In adults the Macfarlane criteria [63], which require the presence of a new or worsening cough together with at least one other lower respiratory tract symptom (sputum production, dyspnoea, wheeze, and chest pain or discomfort) are widely used to identify ALRI cases. These criteria have been validated in a hospital setting, but in a primary care setting, only the presence of focal chest signs was found to reliably identify patients with ALRI, against a gold standard of isolation of respiratory pathogen and/or radiographic features of pneumonia [63].

1.4.2. Case Definitions for URTI

Symptom-complex criteria are widely used to identify URTIs in research settings. The Jackson criteria were validated in young health volunteers to identify experimentally-induced colds [73]. Jackson symptoms comprise sneezing, sore throat, headache, chilliness, malaise, nasal obstruction, nasal discharge and cough which are scored in severity by patients on a scale of 0 to 3. The total symptom score is used to identify URTI [73]. Influenza-like illness is commonly identified in epidemiological studies by the presence of cough, feeling of fever or chilliness and myalgia [74]. The majority of studies discussed below use combinations of the aforementioned symptoms, some also grading severity of infection, in order to assign case definitions for URTI.

2. ACUTE LOWER RESPIRATORY INFECTION (ALRI)

Pneumonia is the leading cause of death in young children worldwide [5] and still remains the leading respiratory killer of the elderly in the UK [1]. RSV bronchiolitis in infants is also a major cause of severe respiratory infections globally and generates substantial morbidity [6, 80]. In early childhood there is a strong association between malnutrition and both incidence and mortality arising from ALRI [75, 80]. Nutritional interventions, in particular zinc supplementation and the promotion of breast feeding are proven to prevent childhood ALRI in developing countries [75], and researchers have called for the evaluation of other nutritional interventions [80] including vitamin D supplementation [75]. In spite of the burden of ALRI in the elderly [1] who are at high risk of vitamin D deficiency [59, 68], studies evaluating the association between vitamin D status and risk of ALRI [10, 11, 13, 34, 81-85] and the effectiveness of supplementation in preventing or treating these infections [23, 86] have to date only been conducted in the very young. These studies are discussed below.

2.1. Observational Studies

Table **1** summarises key details of observational studies evaluating the association between vitamin D status or nutritional rickets and risk of ALRI. In general observational studies from developing countries describe an association between either vitamin D deficiency or nutritional rickets and risk of ALRI, [10, 11, 13, 81, 84, 85] but findings differ in two Canadian case-control studies [82, 83], most likely reflecting differences in vitamin D status of the populations studied and predominance of viral bronchiolitis in the Canadian studies.

2.1.1. Studies Evaluating the Association Between Rickets and Risk of ALRI

Salimpour *et al.*, who systematically screened children admitted to a hospital in Iran for rickets, found that 43% of children with radiologically-confirmed rickets had bronchopneumonia [13]. A case-control study in Jordan, which also screened hospitalised infants for rickets, reported unadjusted odds of ALRI in rachitic infants three-fold that of non-rachitic infants, but failed to report any multivariable analysis [11]. In both of these studies, although the case definitions for rickets were rigorous and included radiological improvement after vitamin D treatment, the criteria for diagnosis of ALRI were not described. The strongest evidence for an association between rickets and ALRI comes from a large case-control study of young children admitted to a hospital in Ethiopia with pneumonia, which used case definitions incorporating validated clinical criteria and radiological features for pneumonia and rickets [10]. After adjusting for confounding factors, the odds of rickets in their pneumonia cases were 13-fold higher than in matched controls. These findings contrast with a Zambian study in which no evidence of rickets was found in 96 children who were admitted with a clinical and radiological diagnosis of pneumonia [87]. Most of the children in this latter study were malnourished, and in the absence of 25(OH)D measurement, the presence of subclinical vitamin D deficiency cannot be excluded. 25(OH)D levels were also not available in any of the aforementioned studies in which rickets was assumed to be the result of vitamin D deficiency [10, 11, 13, 87]. Although the diagnosis of rickets was supported by radiological improvement after vitamin D supplementation in two of these studies [11, 13], data from Nigeria suggest that in some parts of Africa calcium deficiency, rather than vitamin D deficiency, is the primary cause of rickets [88].

2.1.2. Studies Evaluating the Association Between Vitamin D Status and Risk of ALRI

Five recent case-control studies have explored the association between vitamin D status and susceptibility to ALRI in young children. Three studies from developing countries, in which the majority of both cases and controls were vitamin D deficient (25[OH]D< 50 nmol/l), all found

Table 1. Summary of Published Studies Evaluating an Association Between Vitamin D Status or Nutritional Rickets, and ALRI in Young Children

Study, Country, Type	Study Population	Case Definition	Predominant ALRI	Average Age in Months (Age Range)	Average 25(OH)D Concentration (nmol/L) in Cases	Average 25(OH)D Concentration (nmol/L) in Controls	Key Finding
Salimpour 1975 [13] Iran Survey	Admissions to a children's hospital were systematically screened for radiological rickets over 7 years. N=200 children with radiologically confirmed rickets.	Case definition for ALRI not described All but 8 rickets cases had radiological improvement of rickets with vitamin D treatment.	Pneumonia	(60% between 4 and 12 months)	Not available	Not available	43% of rickets cases had bronchopneumonia
Muhe 1997 [10] Ethiopia Case Control	N=1000 children admitted over 5 years n=500 Cases Children admitted with pneumonia n=500 Controls who were matched for age and admission within 3 months of cases.	Pneumonia was defined using WHO criteria, examination, and CXR Rickets was diagnosed using both clinical and radiological features.	Pneumonia	Cases: 13.6 Controls: 13.4	Not available	Not available	After adjusting for confounding factors including measures of malnutrition the odds of rickets in pneumonia cases was 13-fold higher than among controls (adjusted OR 13·37, [95%CI 8·08–24·22], p<0·001).
Najada 2004 [11] Jordan Case control	N=443 acute admissions were screened for rickets over a 9 month period n=47 Cases with rickets n=396 Controls who did not have rickets	Case definition for ALRI not described Rickets was diagnosed by radiological improvement after vitamin D treatment in infants with suggestive clinical or biochemical features.	Pneumonia	Cases: 8 Controls: 7.9 (3-24 months)	Not available	Not available	In a univariable analysis 40/47 (85.1 %) of rachitic infants were admitted due to ALRI compared with 119/396 (30%) of the control infants (p < 0.01).
Wayse 2004 [85] India Case Control	N=150 recruited in May and June n=80 cases with ALRI n=70 unmatched healthy controls attending for vaccination	ALRI defined using WHO clinical criteria. 25(OH)D measured using RIA (Diasorin)	Not reported	23.9	22.8	38.4	1. Mean 25(OH)D was significantly lower in cases compared to controls (22.8 nmol/l [95% CI 21.0-24.7] *vs* 38.4 nmol/l [95% CI 34.2-43.1]; p<0.001) 2. After adjusting for factors including measures of malnutrition 25(OH)D > 22.5nmol/ l was significantly associated with lower risk of ALRI (adjusted OR 0.09, 95% CI 0.03-0.24; p<0.001)

(Table 1) contd…..

Study, Country, Type	Study Population	Case Definition	Predominant ALRI	Average Age in Months (Age Range)	Average 25(OH)D Concentration (nmol/L) in Cases	Average 25(OH)D Concentration (nmol/L) in Controls	Key Finding
Karatekin 2009 [81] Turkey Case Control.	N=40 recruited over winter and spring n=25 cases newborns with ALRI n=15 healthy newborn controls.	ALRI defined using clinical, radiographic and biochemical findings. 25(OH)D measured using RIA (Biosource).	Not reported	0.3	22.8	40.8	1. Mean 25(OH)D was significantly lower in cases than in controls (22.8 nmol/l *vs* 40.8 nmol/l; p=0.011). 2. Mean 25(OH)D of mothers of cases was significantly lower than mothers of controls (33.5 nmol/l *vs* 57 nmol/l; p=0.012). 3. 25(OH)D levels of newborns were highly correlated with their mothers' 25(OH)D.
Roth 2009 [84] Bangladesh Case Control.	N=50 recruited in January and February n=25 cases admitted with ALRI n=25 controls matched for age, sex and village.	ALRI defined using WHO clinical criteria and examination by study physician.		4.2	29.2	39.2	1. Mean 25(OH)D was significantly lower in cases than in controls (29.1 nmol/l *vs* 39.1 nmol/l; p=0.015). 2. The adjusted odds for ALRI was approximately reduced four-fold for every 10 nmol/l increase in serum 25(OH)D (adjusted OR 0.23, 95% CI 0.06-0.81; p=0.022). This suggests that there may be a threshold of 25(OH)D level beyond which risk of ALRI increases.
Mcnally 2009 [82] Canada Case Control.	N=175 recruited over winter and spring. n=105 cases admitted with ALRI. n=92 controls matched for age.	ALRI defined using clinical and radiographic features as recommended by Canadian expert consensus. 25(OH)D measured using EIA and validated against Vitamin D External Quality Assessment Scheme (DEQAS)	N=50 pneumonia. N=55 bronchiolitis.	13.6	81 49 (Amongst cases requiring intensive care)	83	1. Mean 25(OH)D levels did not differ significantly between cases and controls. 2. In a subgroup analysis the mean 25(OH)D level was significantly lower in n=16 cases requiring paediatric intensive care compared with cases admitted to general ward (49 ±24 nmol/l *vs* 87 ± 39 nmol/l, p=0.001); and also compared with controls (49 ±24 nmol/l *vs* 83 ± 30 nmol/l, p=0.001). 3. After adjusting for confounding factors, cases requiring ICU admission had an 8-fold increased risk of vitamin D deficiency compared with cases admitted to the ward. (adjusted OR 8.23, 95% CI 1.4-48.0, p=0.02) This suggests that D deficiency is associated with more severe disease.

(Table 1) contd…..

Study, Country, Type	Study Population	Case Definition	Predominant ALRI	Average Age in Months (Age Range)	Average 25(OH)D Concentration (nmol/L) in Cases	Average 25(OH)D Concentration (nmol/L) in Controls	Key Finding
Roth 2009 [83] Canada Case Control.	N=129 recruited during winter over 2 years. n=64 cases admitted with ALRI n=65 controls matched for age.	ALRI defined using clinical criteria, and designated as pneumonia if features on CXR. 25(OH)D measured using RIA in year 1, and tandem mass spectrometry in year 2.	N=4 pneumonia N=60 bronchiolitis (81% RSV detected).	13.3	77.2	77.0	Mean 25(OH)D levels did not differ significantly between cases and controls.

Abbreviations: ALRI = Acute lower respiratory tract infection; WHO=World Health Organization; CXR= Chest radiograph.

significantly lower 25(OH)D levels amongst ALRI cases compared with controls [81, 84, 85]. Studies from India [85] and Bangladesh [84], amongst children of mean age 2 years and 4 months respectively, reported differences between controls and cases in mean 25(OH)D of 15.6 nmol/l and 10.0 nmol/l respectively. The Bangladeshi study also reported a substantial reduction in the odds of ALRI amongst cases for each 10 nmol/l increase in serum 25(OH)D concentration (OR 0.23, 95% CI 0.06–0.8; p=0.02) [84]. ALRI was diagnosed on the basis of clinical features in both of these studies. Karatekin *et al.*, using a more rigorous case definition for ALRI which included radiographic features, reported a difference in mean serum 25(OH)D concentration of 18 nmol/L between healthy control newborns and newborns admitted with ALRI in Turkey [81]. A strong correlation was found between neonatal and maternal serum 25(OH)D concentrations which were also significantly lower in the mothers of cases compared to the mothers of controls. The majority of mothers of both cases and controls were vitamin D deficient, highlighting the need for vitamin D supplementation during pregnancy in this setting [81].

Two Canadian studies of children (mean age 13 months) admitted with ALRI defined using clinical and radiological features, failed to demonstrate a significant difference in mean 25(OH)D levels between cases and controls [82, 83]. Roth *et al*. documented RSV bronchiolitis in the majority of their cases [83], whereas McNally *et al.* had approximately equal numbers of bronchiolitis and pneumonia cases [82]. In both studies, the majority of cases and controls were vitamin D replete (25[OH]D $\geq$ 75 nmol/l), most likely reflecting successful food fortification strategies in Canada [89]. The results of subgroup analyses by McNally *et al.* did however yield interesting findings. In particular, 25(OH)D levels were significantly lower in ALRI cases with severe disease requiring intensive care (49 nmol/l) when compared to controls (83 nmol/l) and cases admitted to general wards (87 nmol/l). After adjusting for confounding factors, cases admitted to the intensive care unit (ICU) were 8-fold more likely to have vitamin D deficiency than cases admitted to general wards, although the small sample size is reflected in the wide confidence intervals [82]. Pneumonia cases were twice as likely to have vitamin D deficiency compared with controls, but this difference failed to reach statistical significance. These findings may indicate that in a developed world setting vitamin D deficiency is associated with more severe ALRI.

It seems likely therefore that the contrasting findings between the Canadian and developing country setting studies reflect key differences in vitamin D status and infectious disease aetiology. The majority of cases and controls in India, Bangladesh and Turkey were vitamin D deficient with mean 25(OH)D levels amongst cases ranging from 22.8 to 29.1 nmol/l [81, 84, 85]. Furthermore in developing countries bacterial pneumonia is a major problem, compared with developed countries where most ALRI is viral in origin [90]. These studies certainly provide evidence for vitamin D deficiency as a risk factor for ALRI, and Roth *et al.* have proposed that there may be a threshold 25(OH)D level below which risk of ALRI increases above baseline [84]. However associations from observational studies do not prove causality, and confounding cannot be excluded as an explanation; in particular vitamin D deficiency may merely reflect malnutrition and socioeconomic deprivation which are known to be risk factors for infectious diseases. However studies which have adjusted for these factors and used validated case definitions for ALRI report strong associations between either rickets or low 25(OH)D levels and risk of ALRI [10, 81, 84, 85].

2.2. Studies Evaluating the Effect of Vitamin D Supplementation for the Prevention or Treatment of ALRI

A double-blind randomised clinical trial of vitamin D supplementation recently conducted in Afghanistan randomly allocated young children diagnosed with pneumonia based on clinical features, to either a single high-dose oral vitamin D supplement (100,000 IU of cholecalciferol) or placebo, in addition to routine pneumonia treatment. Although no difference was observed in time to recovery, supplementation significantly reduced the risk of a repeat episode of pneumonia during the 90 days following supplementation (RR 0.78; 95% CI 0.64, 0.94, P = 0.01), and time to a repeat episode was significantly longer in

supplemented children (72 days *vs* 59 days; HR 0.71; 95% CI 0.53-0.95; p=0.02)[5]. 25(OH)D levels were not measured in this study but it is highly likely that most participants were vitamin D deficient, as the prevalence of vitamin D deficiency amongst children in Kabul is known be very high [91]. It is possible that larger or additional bolus vitamin D doses might have influenced recovery times or further reduced the incidence of repeat pneumonia episodes, but these strategies will need to be evaluated in settings where 25(OH)D and calcium levels can be monitored. In a less rigorously conducted study Rehman treated 47 children with a history of frequent respiratory infection or febrile illness, who were diagnosed with sub-clinical rickets based on raised serum calcium and alkaline phosphatase levels, with both high dose vitamin D and calcium supplements over a 6 week period. Over a 6-month post treatment period there was no recurrence of infection in these children [86]. This study lacked any randomisation or blinding procedures or measurement of 25(OH)D levels, and the criteria used to define cases of infection were not reported. In addition the provision of calcium supplementation makes it difficult to draw any conclusion regarding whether vitamin D supplementation was responsible for preventing further respiratory infections in supplemented children. These studies are summarised in Table **2**.

2.3. Vitamin D Receptor Polymorphisms and Risk of ALRI

The association between VDR genotype and the risk of ALRI has been evaluated in the context of RSV bronchiolitis in early childhood. Two case control studies, one a larger genetic association study looking at single-nucleotide polymorphisms (SNPs) in candidate genes involved in immunity and asthma [33], and the other a smaller study focussing on VDR [34] have found a strong association between *Fok1* genotype and risk of severe bronchiolitis requiring hospitalisation. Roth *et al*. found a significantly greater (seven-fold) adjusted odds of ALRI in Canadian children with the *ff* genotype compared with those carrying the *FF* genotype, and a weaker association with the *TaqI* polymorphism [34]. These preliminary findings support a role for vitamin D in determining susceptibility to viral bronchiolitis, and further larger studies are needed to confirm these results.

Hence it appears that in developing countries, where the prevalence of vitamin D deficiency is high and pneumonia is the predominant cause of ALRI, there is a strong association between inadequate vitamin D status and risk of ALRI in young children [10, 81, 84, 85]; whereas in a developed country setting vitamin D deficiency appears to be associated with more severe ALRI [82]. One clinical trial of vitamin D supplementation has demonstrated a significant reduction in the risk of repeat episodes of pneumonia in young children in Afghanistan [23], but larger trials are needed to evaluate the role of vitamin D supplementation both for the prevention and treatment of ALRI. These trials should be conducted in both developed and developing country settings, and should have access to 25(OH)D level measurement as well diagnostic tests to identify the respiratory pathogen. Different dosing regimens should be evaluated and, if it exists, the optimal 25(OH)D level to prevent ALRI needs to be ascertained. Preliminary studies suggest VDR polymorphisms influence susceptibility to ALRI, and studies are needed to evaluate whether they also influence any effect of vitamin D supplementation on incidence of ALRI. There is very little data regarding potential associations between

Table 2. Studies of Vitamin D Supplementation for the Prevention or Treatment of Acute Respiratory Tract Infections in Young Children

Study, Country, Type	Study Population	Case Definition	Main Endpoints	Intervention	Key Findings
Rehman 1994 [86] India Case control	N=47 children Age range 3-12 years n=27 cases with ≥ 6 respiratory or other febrile illnesses needing antibiotics in preceding 6 months who did not have clinical or radiological features of rickets. n=20 age-and sex-matched 'controls' with ≤ 1 infectious episode in preceding 6 months.	No case definitions described.	Frequency of any infection.	Cases were given 60000 IU oral vitamin D/week and daily 650mg calcium for 6 weeks. Both groups were followed up for 6 months.	1. Mean baseline serum alkaline phosphatase level was significantly greater in cases compared to controls (299 ±41 *vs* 201 ±23; *P* <0.005), and this normalised after the intervention, suggesting that cases had subclinical vitamin D deficiency. 2. During the 6 month follow up period no difference was observed in the frequency of infections between the test and control groups of children.
Manaseki-Holland 2010 [23] Afghanistan Double blind, placebo-controlled, randomised clinical trial	N=453 children admitted with ALRI, mean age 13.2 months. n=224 received intervention. n=229 received placebo.	ALRI defined using WHO clinical criteria.	Duration of pneumonia Risk of repeat episode within 3 month follow-up period.	Single dose of 100,000 IU D3 or placebo given in addition to standard antibiotic treatment for pneumonia as per national protocol.	1. There was no difference in mean time to recovery from the index episode of pneumonia in the intervention group compared with the placebo group (4.74 *vs* 4.98 days respectively, p=0.17). 2. The risk of repeat episode of pneumonia was significantly lower in the intervention group compared to the placebo group (RR 0.78; 95% CI 0.64, 0.94; p=0.01).

vitamin D status and risk of ALRI in the elderly, and this inequity must be addressed.

3. UPPER RESPIRATORY TRACT INFECTION (URTI), INFLUENZA, AND STUDIES WHICH DO NOT DIFFERENTIATE BETWEEN URTI AND ALRI

An estimated 72% of adults in the United States experience at least one URTI each year which imposes a significant economic impact in terms of health care usage and absenteeism from work [7]. Older adults are particularly susceptible to influenza, which is associated with a high mortality in this population [92]. Viruses are the most common cause of URTI although many of them (including influenza and parainfluenza viruses, rhinovirus and RSV) can cause both URTIs and LRTIs. Observational data, mainly from recent studies in developed countries, describe an association between vitamin D deficiency and risk of URTI or influenza-like-illness (ILI). Five trials of vitamin D supplementation for the prevention of URTI and ILI have been conducted to date, but report contrasting findings which are discussed below.

3.1. Observational Studies

Vitamin D insufficiency is particularly common in winter and spring, particularly amongst older adults [68], when UVB available from sunlight is of insufficient intensity to stimulate cutaneous vitamin D synthesis [61]. Ecological studies demonstrate that the peak in prevalence of vitamin D insufficiency during winter and spring coincides with the peak in incidence of upper and lower respiratory tract infections caused by influenza and parainfluenza viruses and RSV, and vitamin D insufficiency has been proposed as the seasonal stimulus for influenza epidemics [62, 93-95]. A recent cross-sectional analysis of 18,883 participants in the US Third National Health and Nutrition Examination Survey (NHANES III) aged 12 years and older demonstrated an independent association between vitamin D insufficiency and susceptibility to recent URTI (OR 1.36) ascertained by self-report, which was stronger amongst participants with asthma and COPD (OR, 5.67 and 2.26, respectively) [8]. This is an interesting finding as URTIs are known to be common precipitant for acute exacerbations in patients with asthma and COPD [66, 67]. Two further studies have used case definitions for acute respiratory infection which incorporate physician diagnosis with or without microbiological evidence of infection [9, 12]. Amongst Finnish military conscripts followed for 6 months, those with vitamin D deficiency (25[OH]D < 40 nmol/l) at baseline had significantly more days of absence from duty due to respiratory tract infection, than those with baseline 25(OH)D > 40 nmol/l (median: 4 *vs* 2 days respectively) [9]. This study used documentation of physician-diagnosed respiratory infection (sinusitis, tonsillitis, otitis, pharyngitis, laryngitis, bronchitis and pneumonia) abstracted from the medical records of participants [9]. Sabetta *et al.* followed a cohort of 198 healthy adults for 5 months and found that amongst participants with a baseline serum 25(OH)D concentration ≥ 95 nmol/l the incidence of acute viral respiratory tract infection was almost 3-fold lower than in those with levels <95 nmol/l (p=0.015) [12].

3.2. Interventional Studies

The findings from five placebo-controlled randomised trials of vitamin D supplementation for the prevention of ARTI have been published to date (Table **3**). There are important methodological differences between the trials, including different supplementation regimes which influenced the proportion of participants in intervention arms who attained vitamin D repletion and the time taken to reach repletion. Case definitions for ARTI or URTI also vary, with one trial using detection of respiratory virus [24], and most of the others using patient self-report of infection or antibiotic use [19, 20, 22]. Characteristics of the study populations also vary, in particular according to baseline vitamin D status and reported compliance with study medication. Two trials report secondary analyses of trials of vitamin D supplementation for the maintenance of skeletal health [19-22, 24]. Aloia *et al.* performed a secondary analysis of adverse events reported during a randomised placebo-controlled trial of vitamin D supplementation for the prevention of bone loss in postmenopausal African-American women [19]. Participants in the intervention arm received 800 IU/day of vitamin D3 for 2 years, followed by 2000 IU/day in the final year, and self-reported cold or influenza symptoms were recorded on 6-monthly basis. Mean baseline 25(OH)D levels in both intervention and control arms were < 50 nmol/l, rising to >70 nmol/L after supplementation [96]. Significantly fewer participants receiving supplementation reported cold and influenza symptoms than those receiving placebo (8/104 *vs* 26/104, respectively) and supplementation appeared to abolish the seasonality of these infections [19]. The absolute number of infections recorded (34 over 3 years) was much lower than would be expected however. Secondary analysis of a larger UK trial of vitamin D supplementation for fracture prevention in older adults found that supplementation with 800 IU vitamin D3 per day did not have any significant effect on incidence of self-reported infection or antibiotic use [20]. However in a per-protocol analysis based on self-reported pill taking, the odds for infection and antibiotic usage in the D3 group were smaller, although this was of borderline statistical significance; hence poor compliance may have been an issue in this study.

In a follow-up to the study by Aloia *et al.*, Li-Ng conducted a 3 month placebo-controlled trial of vitamin D3 administered at a higher dose (2000 IU/day) in healthy adult volunteers recruited during winter and spring [22]. No difference in the frequency of self-reported URTI was seen in intervention *vs* control arms of this study. Mean baseline 25(OH)D levels were approximately 64 nmol/l in both groups and rose to 88.5 nmol/l in the intervention group. A longer follow-up period, or supplementation prior to winter might have yielded more significant findings, as with this daily dosing regimen repletion would only have been attained after 3 months. Laaksi *et al.* followed on from their observational study by conducting a trial of 400 IU/day vitamin D3 over a 6 month period in Finnish military recruits [21]. They found no significant difference in the number of days of absence from duty due to ARTI or frequency of self-reported ARTI symptoms between intervention and control groups. Their findings may reflect the very low dose of

Table 3. Interventional Trials Evaluating Vitamin D Supplementation for the Prevention of ARTI, URTI or ILI

Study, Country, Type	Study Population (N)	Main End Point [Case Definitions]	Intervention	Mean Baseline 25(OH)D Concentration (nmol/L)		Outcome
				Baseline	Follow-Up	
Aloia 2007 [19] USA Secondary analysis of adverse events reported during a randomised, double-blind, placebo-controlled trial of vitamin D supplementation for the prevention bone loss.	N=208 calcium replete post-menopausal African-American women. n1=104 received D3 n2=104 received placebo.	Self-reported cold or influenza in previous 6 months, recorded as part of 6-monthly adverse event reporting. Seasonality of cold/influenza symptoms.	800 IU/day D3 for 2 years followed by 2000 IU/day for 1 year, or placebo for 3 years.	n1=46.9 n2=43	n1=70.8 with 3 months of 800IU/d D3; and 86.9 after 3 months of higher dose. N2=no significant change.	1. A significantly greater proportion of participants reporting cold or flu symptoms were in the placebo group compared with the intervention group (26/34 *vs* 8/34; (p<0.002). 2. Placebo group had symptoms mainly during winter and intervention group had symptoms throughout the year. 3. Only one person reported symptoms whilst taking the higher D3 dose.
Avenell 2007 [20] UK Sub-study of a randomised, double-blind, placebo-controlled trial of vitamin D supplementation for the secondary prevention of osteoporotic fractures in older people (RECORD trial).	N=3444 adults aged 70y+ participating in RECORD trial who responded to a postal questionnaire in March 2002. n1=1740 receiving D3 with or without calcium. n2=1704 receiving calcium or placebo.	Self-reported infection or antibiotic use in the week prior to receiving the questionnaire.	800 IU/day D3, 1000mg calcium/day, 800 IU/day D3 + 1000mg calcium/day, or placebo for 24-62 months.	38 in a sample of 60 participants.	62 after 1 year of D3 in the same participants.	1. Adjusted odds of reported infection or antibiotic use in D3 group was not significantly lower than in placebo group (0.90 [95% CI 0.76-1.07]; p=0.23 and 0.84 [95% CI 0.64-1.09]; p=0.18 respectively). 2. In per protocol analyses based on self-reported pill taking, the odds for infection and antibiotic usage in D3 group were smaller but did not attain statistical significance (0.80, [95% CI 0.64 to 1.01]; p=0.06 and 0.74, [95% CI 0.52 to 1.06]; p=0.10 respectively).
Li-Ng 2009 [22] USA Randomised, Double-blind, placebo-controlled trial of vitamin D supplementation for the prevention of URTI.	N=162 healthy adult volunteers recruited between December '06 and March '07 n1=84 received D3 n=78 received placebo.	Self-reported URTI symptoms recorded in bi-weekly questionnaires. [URTI defined as presence of two or more of: fever, cough, productive sputum or change in sputum colour and quantity, muscle aches, nausea or vomiting in the absence of allergy symptoms].	2000 IU/day D3 for 3 months or placebo.	n1=64.3 n2=63.0	n1=88.5 n2=60.9	1. The frequency of reported URTI was slightly greater in the placebo compared to the D3 group, but did not reach statistical significance (50/363 [13.8%] *vs* 48/388 [12.4%] reports respectively, p=0.56). 2. There was no difference in severity or duration of URTIs between D3 and placebo groups.

(Table 3) contd…..

Study, Country, Type	Study Population (N)	Main End Point [Case Definitions]	Intervention	Mean Baseline 25(OH)D Concentration (nmol/L)		Outcome
				Baseline	Follow-Up	
Urashima 2010 [24] Japan Randomised, double-blind, placebo-controlled trial of vitamin D supplementation to prevent influenza A in school children.	N=430 children aged 6-15y recruited between November and December 2009, invited to participate by paediatricians at study sites (outpatient clinics). n1=217 received D3 n2=213 received placebo	Influenza A diagnosed by rapid test on nasopharyngeal swab. Secondary outcomes included physician-diagnosed asthma attacks, and non-specific febrile illness.	1200 IU/day D3 for 4 months or placebo.	Not measured	Not measured	1. The risk of influenza A was significantly less in the D3 compared to the placebo group (RR: 0.58; [95% CI 0.34-0.99]; p=0.04), and this was more prominent in children who had not been taking any other vitamin D supplements. 2. Influenza A occurred significantly less often in D3 compared to placebo group between days 31 and 60 of supplementation (RR:0.41; [95% CI 0.19-0.86], p=0.014). 3. In a subgroup analysis of children known to have asthma, significantly fewer children in the D3 group had asthma attacks compared with those in the placebo group (RR:0.17; [95% CI 0.04-0.73], p=0.006)
Laaksi 2010 [21] Finland Randomised, double-blind, placebo-controlled trial of vitamin D supplementation to prevent ARTI in young Finnish men.	N=164 Finnish military conscripts volunteering to participate from 400 entering the unit. n1=80 received D3 n2=84 received placebo.	Number of days absent from duty due to ARTI between October 2005 and March 2006, ascertained from documentation in medical records of diagnosis of ARTI. Secondary outcomes included self-reported ARTI symptoms (any of cough, runny nose, sore throat, fever, or common cold symptoms).	400 IU/day D3.	n1=78.7 n2=74.4	n1=71.6 n2=51.3	1. There was no difference in number of days absent from duty due to ARTI between intervention and control groups. 2. The frequency of self-reported ARTI symptoms did not differ between groups. 3. Cox regression analysis indicated the hazard radio for absence from duty due to ARTI was lower in the D3 group. 4. In the first 6 weeks of the study there was a trend towards less absence in the D3 compared to control groups, although this did not reach statistical significance (0.7 ± 2.1 days *vs* 1.4 ± 2.6 days respectively, p=0.06).

n1=Intervention arm; n2=control arm; URTI=upper respiratory tract infection; ARTI=acute respiratory tract infection.

vitamin D3 used, and the relatively high baseline vitamin D status of participants (mean serum 25(OH)D > 70 nmol/L). Follow up 25(OH)D levels were lower than at baseline in both intervention and placebo groups, although the difference was greater in the placebo group.

The strongest evidence to date that vitamin D supplementation might prevent URTI comes from a placebo-controlled trial of vitamin D supplementation conducted in Japanese school children for the prevention of influenza A [24]. Participants received 1200 IU/day of vitamin D3 or placebo and were followed up for 4 months. A rigorous case definition which required detection of influenza from nasopharyngeal swabs was used, but pre- and post-treatment 25(OH)D measurement was not available. The risk of influenza A was almost halved in children in the intervention arm, and this effect was greatest in the second month of supplementation; exacerbations of asthma were also reduced in the intervention arm of this study. No effect of vitamin D supplementation was seen on risk of influenza B however.

Hence on balance, although observational data supports an association between vitamin D insufficiency and susceptibility to acute upper or viral respiratory tract

infection, only two trials provides evidence that vitamin D supplementation prevents these infections [19, 24]. Aloia *et al.*'s findings although significant are limited by a case definition which uses 6-monthly self-reported symptoms, and the findings may not be generalisable as the study population were post-menopausal African-American women [19]. Further trials are needed in populations at high risk of ARTI, utilising bolus dosing regimens to rapidly correct vitamin D deficiency and case definitions which include detection of respiratory pathogen.

4. CONCLUSIONS

In developing countries where vitamin D deficiency is prevalent, high dose vitamin D supplementation in addition to routine pneumonia treatment significantly reduces the recurrence of disease in young children. Case-control studies in these settings demonstrate a strong association between vitamin D deficiency and ALRI, whilst similar studies in developed countries suggest that deficiency is associated only with more severe ALRI. Clinical trials using robust case definitions for ALRI are needed to evaluate the role of vitamin D supplementation in addition to other proven interventions in both children and pregnant women for the primary prevention of ALRI. Further trials of vitamin D supplementation for the prevention of URTI are also required: these should be conducted in populations where baseline vitamin D insufficiency is highly prevalent, and should utilise bolus dosing of vitamin D, which can be directly observed, and which results in rapid correction of vitamin D insufficiency [97].

REFERENCES

[1] BTS. The Burden of Lung Disease, 2nd Edition. London: British Thoracic Society; 2006.

[2] Falsey AR, McCann RM, Hall WJ, *et al.* The "common cold" in frail older persons: impact of rhinovirus and coronavirus in a senior daycare center. J Am Geriatr Soc 1997; 45(6): 706-11.

[3] Graat JM, Schouten EG, Heijnen ML, *et al.* A prospective, community-based study on virologic assessment among elderly people with and without symptoms of acute respiratory infection. J Clin Epidemiol 2003; 56(12): 1218-23.

[4] Nicholson KG, Kent J, Hammersley V, Cancio E. Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden. BMJ 1997; 315(7115): 1060-4.

[5] Bryce J, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. Lancet 2005; 365(9465): 1147-52.

[6] Wainwright C. Acute viral bronchiolitis in children- a very common condition with few therapeutic options. Paediatr Respir Rev 2010; 11(1): 39-45; quiz

[7] Fendrick AM, Monto AS, Nightengale B, Sarnes M. The economic burden of non-influenza-related viral respiratory tract infection in the United States. Arch Intern Med 2003; 163(4): 487-94.

[8] Ginde AA, Mansbach JM, Camargo CA, Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med 2009; 169(4): 384-90.

[9] Laaksi I, Ruohola JP, Tuohimaa P, *et al.* An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. Am J Clin Nutr 2007; 86(3): 714-7.

[10] Muhe L, Lulseged S, Mason KE, Simoes EA. Case-control study of the role of nutritional rickets in the risk of developing pneumonia in Ethiopian children. Lancet 1997; 349(9068): 1801-4.

[11] Najada AS, Habashneh MS, Khader M. The frequency of nutritional rickets among hospitalized infants and its relation to respiratory diseases. J Trop Pediatr 2004; 50(6): 364-8.

[12] Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA, Landry ML. Serum 25-hydroxyvitamin D and the incidence of acute viral respiratory tract infections in healthy adults. PLoSOne 2010; 5(6): e11088.

[13] Salimpour R. Rickets in Tehran. Study of 200 cases. Arch Dis Child 1975; 50(1): 63-6.

[14] Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357(3): 266-81.

[15] Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. J Immunol 2008; 181(10): 7090-9.

[16] Liu PT, Stenger S, Li H, *et al.* Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006; 311(5768): 1770-3.

[17] Wang TT, Nestel FP, Bourdeau V, *et al.* Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004; 173(5): 2909-12.

[18] Yim S, Dhawan P, Ragunath C, Christakos S, Diamond G. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3). J Cyst Fibros 2007; 6(6): 403-10.

[19] Aloia JF, Li-Ng M. Re: epidemic influenza and vitamin D. Epidemiol Infect 2007; 135(7): 1095-6; author reply 7-8.

[20] Avenell A, Cook JA, Maclennan GS, Macpherson GC. Vitamin D supplementation to prevent infections: a sub-study of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438). Age Ageing 2007; 36(5): 574-7.

[21] Laaksi I, Ruohola JP, Mattila V, Auvinen A, Ylikomi T, Pihlajamaki H. Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men. J Infect Dis 2010; 202(5): 809-14.

[22] Li-Ng M, Aloia JF, Pollack S, *et al.* A randomized controlled trial of vitamin D3 supplementation for the prevention of symptomatic upper respiratory tract infections. Epidemiol Infect 2009; 137(10): 1396-404.

[23] Manaseki-Holland S, Qader G, Isaq Masher M, *et al.* Effects of vitamin D supplementation to children diagnosed with pneumonia in Kabul: a randomised controlled trial. Trop Med Int Health 2010: 15(10): 1148-55.

[24] Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am J Clin Nutr 2010; 91(5): 1255-60.

[25] Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. Curr Opin Pharmacol 2010; 10(4): 482-96.

[26] Bellamy R, Ruwende C, Corrah T, *et al.* Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene. J Infect Dis 1999; 179(3): 721-4.

[27] Liu W, Cao WC, Zhang CY, *et al.* VDR and NRAMP1 gene polymorphisms in susceptibility to pulmonary tuberculosis among the Chinese Han population: a case-control study. Int J Tuberc Lung Dis 2004; 8(4): 428-34.

[28] Martineau AR, Leandro AC, Anderson ST, *et al.* Association between Gc genotype and susceptibility to TB is dependent on vitamin D status. Eur Respir J 2010; 35(5): 1106-12.

[29] Wilkinson RJ, Llewelyn M, Toossi Z, *et al.* Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. Lancet 2000; 355(9204): 618-21.

[30] Babb C, van der Merwe L, Beyers N, *et al.* Vitamin D receptor gene polymorphisms and sputum conversion time in pulmonary tuberculosis patients. Tuberculosis (Edinb) 2007; 87(4): 295-302.

[31] Roth DE, Soto G, Arenas F, *et al.* Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. J Infect Dis 2004; 190(5): 920-7.

[32] Leandro AC, Rocha MA, Cardoso CS, Bonecini-Almeida MG. Genetic polymorphisms in vitamin D receptor, vitamin D-binding protein, Toll-like receptor 2, nitric oxide synthase 2, and interferon-gamma genes and its association with susceptibility to tuberculosis. Braz J Med Biol Res 2009; 42(4): 312-22.

[33] Janssen R, Bont L, Siezen CL, *et al.* Genetic susceptibility to respiratory syncytial virus bronchiolitis is predominantly associated with innate immune genes. J Infect Dis 2007; 196(6): 826-34.

[34] Roth DE, Jones AB, Prosser C, Robinson JL, Vohra S. Vitamin D receptor polymorphisms and the risk of acute lower respiratory tract infection in early childhood. J Infect Dis 2008; 197(5): 676-80.

[35] Speeckaert M, Huang G, Delanghe JR, Taes YE. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin Chim Acta 2006; 372(1-2): 33-42.

[36] Arnaud J, Constans J. Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP). Hum Genet 1993; 92(2): 183-8.

[37] Lauridsen AL, Vestergaard P, Nexo E. Mean serum concentration of vitamin D-binding protein (Gc globulin) is related to the Gc phenotype in women. Clin Chem 2001; 47(4): 753-6.

[38] Fu L, Yun F, Oczak M, Wong BY, Vieth R, Cole DE. Common genetic variants of the vitamin D binding protein (DBP) predict differences in response of serum 25-hydroxyvitamin D [25(OH)D] to vitamin D supplementation. ClinBiochem 2009; 42(10-11): 1174-7.

[39] Lauridsen AL, Vestergaard P, Hermann AP, *et al*. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. Calcif Tissue Int 2005; 77(1): 15-22.

[40] Morrison NA, Qi JC, Tokita A, *et al*. Prediction of bone density from vitamin D receptor alleles. Nature 1994; 367(6460): 284-7.

[41] Ross J. Control of messenger RNA stability in higher eukaryotes. Trends Genet 1996; 12(5): 171-5.

[42] Durrin LK, Haile RW, Ingles SA, Coetzee GA. Vitamin D receptor 3'-untranslated region polymorphisms: lack of effect on mRNA stability. Biochim Biophys Acta 1999; 1453(3): 311-20.

[43] Verbeek W, Gombart AF, Shiohara M, Campbell M, Koeffler HP. Vitamin D receptor: no evidence for allele-specific mRNA stability in cells which are heterozygous for the Taq I restriction enzyme polymorphism. Biochem Biophys Res Commun 1997; 238(1): 77-80.

[44] van Etten E, Verlinden L, Giulietti A, *et al*. The vitamin D receptor gene FokI polymorphism: functional impact on the immune system. Eur J Immunol 2007; 37(2): 395-405.

[45] van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. J Steroid Biochem Mol Biol 2005; 97(1-2): 93-101.

[46] Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002; 415(6870): 389-95.

[47] Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J ClinNutr 2006; 83(4): 754-9.

[48] de Jong MD, Simmons CP, Thanh TT, *et al*. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat Med 2006; 12(10): 1203-7.

[49] Bennett BL, Garofalo RP, Cron SG, *et al*. Immunopathogenesis of respiratory syncytial virus bronchiolitis. J Infect Dis 2007; 195(10): 1532-40.

[50] Bont L, Heijnen CJ, Kavelaars A, *et al*. Local interferon-gamma levels during respiratory syncytial virus lower respiratory tract infection are associated with disease severity. J Infect Dis 2001; 184(3): 355-8.

[51] Herrmann M, Harwood T, Gaston-Parry O, *et al*. A new quantitative LC tandem mass spectrometry assay for serum 25-hydroxy vitamin D. Steroids 2010; 75(13-14): 1106-12.

[52] Maunsell Z, Wright DJ, Rainbow SJ. Routine isotope-dilution liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of the 25-hydroxy metabolites of vitamins D2 and D3. Clin Chem 2005; 51(9): 1683-90.

[53] Vogeser M. Quantification of circulating 25-hydroxyvitamin D by liquid chromatography-tandem mass spectrometry. J Steroid Biochem Mol Biol 2010; 121(3-5): 565-73.

[54] Binkley N, Krueger D, Cowgill CS, *et al*. Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. J Clin Endocrinol Metab 2004; 89(7): 3152-7.

[55] Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol 2009; 19(2): 73-8.

[56] Hypponen E, Turner S, Cumberland P, Power C, Gibb I. Serum 25-hydroxyvitamin D measurement in a large population survey with statistical harmonization of assay variation to an international standard. J Clin Endocrinol Metab 2007; 92(12): 4615-22.

[57] Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006; 84(1): 18-28.

[58] Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int 2005; 16(7): 713-6.

[59] Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006; 81(3): 353-73.

[60] 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(6): 626-32.

[61] Hypponen E, Power C. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. Am J Clin Nutr 2007; 85(3): 860-8.

[62] Cannell JJ, Vieth R, Umhau JC, *et al*. Epidemic influenza and vitamin D. Epidemiol Infect 2006; 134(6): 1129-40.

[63] Macfarlane J, Holmes W, Gard P, *et al*. Prospective study of the incidence, aetiology and outcome of adult lower respiratory tract illness in the community. Thorax 2001; 56(2): 109-14.

[64] Janssens W, Bouillon R, Claes B, *et al*. Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. Thorax 2010; 65(3): 215-20.

[65] Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DY. Vitamin D levels, lung function, and steroid response in adult asthma. Am J Respir Crit Care Med 2010; 181(7): 699-704.

[66] Jackson DJ, Johnston SL. The role of viruses in acute exacerbations of asthma. J Allergy Clin Immunol 2010; 125(6): 1178-87.

[67] Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. N Engl J Med 2008; 359(22): 2355-65.

[68] Hirani V, Primatesta P. Vitamin D concentrations among people aged 65 years and over living in private households and institutions in England: population survey. Age Ageing 2005; 34(5): 485-91.

[69] World Health Organization. International Statistical Classification of Diseases and Related Health Problems 10th Revision Version for 2007. World Health Organization; 2007. http://apps.who.int/classifications/apps/icd/icd10online/ [Accessed 30 September 2010].

[70] Fahey T, Stocks N, Thomas T. Systematic review of the treatment of upper respiratory tract infection. Arch Dis Child 1998; 79(3): 225-30.

[71] Niederman MS, Mandell LA, Anzueto A, *et al*. Guidelines for the management of adults with community-acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention. Am J Respir Crit Care Med 2001; 163(7): 1730-54.

[72] Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H. Epidemiology and etiology of childhood pneumonia. Bull World Health Organ 2008; 86(5): 408-16.

[73] Jackson GG, Dowling HF, Spiesman IG, Boand AV. Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity. AMA Arch Intern Med 1958; 101(2): 267-78.

[74] Payne L, Kuhlmann-Berenzon S, Ekdahl K, Giesecke J, Hogberg L, Penttinen P. 'Did you have flu last week?' A telephone survey to estimate a point prevalence of influenza in the Swedish population. Euro Surveill 2005; 10(12): 241-4.

[75] Roth DE, Caulfield LE, Ezzati M, Black RE. Acute lower respiratory infections in childhood: opportunities for reducing the global burden through nutritional interventions. Bull World Health Organ 2008 ; 86(5): 356-64.

[76] Fox JD. Nucleic acid amplification tests for detection of respiratory viruses. J ClinVirol 2007; 40(Suppl 1): S15-23.

[77] Lanata CF, Rudan I, Boschi-Pinto C, *et al*. Methodological and quality issues in epidemiological studies of acute lower respiratory infections in children in developing countries. Int J Epidemiol 2004; 33(6): 1362-72.

[78] World Health Organization. Integrated Management of Childhood Illness chart booklet. Geneva: World Health Organization, 2008. http://whqlibdoc.who.int/publications/2008/9789241597289_eng.pdf [Accessed 30 September 2010].

[79] WHO Department of Child and Adolescent Health and Development. Model chapter for textbooks: IMCI, Integrated Management of Childhood Illness. Geneva: World Health

Organization, 2001. http://whqlibdoc.who.int/hq/2001/WHO_FCH_CAH_01.01.pdf [Accessed 30 September 2010].

[80] Mulholland K. Childhood pneumonia mortality - a permanent global emergency. Lancet 2007; 370(9583): 285-9.

[81] Karatekin G, Kaya A, Salihoglu O, Balci H, Nuhoglu A. Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers. Eur J Clin Nutr 2009; 63(4): 473-7.

[82] McNally JD, Leis K, Matheson LA, Karuananyake C, Sankaran K, Rosenberg AM. Vitamin D deficiency in young children with severe acute lower respiratory infection. Pediatr Pulmonol 2009; 44(10): 981-8.

[83] Roth DE, Jones AB, Prosser C, Robinson JL, Vohra S. Vitamin D status is not associated with the risk of hospitalization for acute bronchiolitis in early childhood. Eur J Clin Nutr 2009; 63(2): 297-9.

[84] Roth DE, Shah R, Black RE, Baqui AH. Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh. Acta Paediatr 2010; 99(3): 389-93.

[85] Wayse V, Yousafzai A, Mogale K, Filteau S. Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y. Eur J Clin Nutr 2004; 58(4): 563-7.

[86] Rehman PK. Sub-clinical rickets and recurrent infection. J Trop Pediatr 1994; 40(1): 58.

[87] Smyth A, Broderick N. Rickets and childhood pneumonia. Lancet 1997; 350(9080): 811.

[88] Oginni LM, Worsfold M, Oyelami OA, Sharp CA, Powell DE, Davie MW. Etiology of rickets in Nigerian children. J Pediatr 1996; 128(5 Pt 1): 692-4.

[89] Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics 2008; 122(2): 398-417.

[90] Graham NM. The epidemiology of acute respiratory infections in children and adults: a global perspective. Epidemiol Rev 1990; 12: 149-78.

[91] Manaseki-Holland S, Zulf Mughal M, Bhutta Z, Qasem Shams M. Vitamin D status of socio-economically deprived children in Kabul, Afghanistan. Int J Vitam Nutr Res 2008; 78(1): 16-20.

[92] Monto AS, Ansaldi F, Aspinall R, *et al.* Influenza control in the 21st century: Optimizing protection of older adults. Vaccine 2009; 27(37): 5043-53.

[93] Cannell JJ, Zasloff M, Garland CF, Scragg R, Giovannucci E. On the epidemiology of influenza. Virol J 2008; 5: 29.

[94] Hope-Simpson RE. The spread of type A influenza. J R Coll Gen Pract 1985; 35(275): 267-9.

[95] Monto AS. The seasonality of rhinovirus infections and its implications for clinical recognition. ClinTher 2002; 24(12): 1987-97.

[96] Aloia JF, Talwar SA, Pollack S, Yeh J. A randomized controlled trial of vitamin D3 supplementation in African American women. Arch Intern Med 2005; 165(14): 1618-23.

[97] Ilahi M, Armas LA, Heaney RP. Pharmacokinetics of a single, large dose of cholecalciferol. Am J Clin Nutr 2008; 87(3): 688-91.

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Vitamin D and Tuberculosis

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Abstract: Tuberculosis is a major global health problem: the World Health Organisation estimates that there were 9.4 million incident cases and 1.8 million deaths from the disease in 2008. The development of new agents to prevent acquisition or reactivation of latent *Mycobacterium tuberculosis* infection and to allow shortening of antimicrobial therapy regimens for active tuberculosis without loss of efficacy is a research priority. In this article we describe the immunomodulatory actions of vitamin D metabolites in mycobacterial infection and review the growing body of evidence from observational and intervention studies suggesting that vitamin D might have a role in the prevention and treatment of TB.

Keywords: Tuberculosis, vitamin D, immunomodulation, clinical trials.

INTRODUCTION

Tuberculosis (TB) is a major public health problem. The global prevalence of latent *Mycobacterium tuberculosis* (MTB) infection has been estimated at 32% [1], and this carries a 5-20% lifetime risk of reactivation disease in people who are not infected with Human Immunodeficiency Virus (HIV) [2]; reactivation rates higher than 10% per annum have been reported in HIV-infected people [3]. The World Health Organisation estimates that in 2008 there were 9.4 million incident cases of active TB, 11.1 million prevalent cases of TB, 1.3 million deaths from TB in HIV-uninfected people and 0.52 million deaths from TB in HIV-infected people [4]. The development of new agents to prevent acquisition or reactivation of MTB infection and to allow shortening of antimicrobial therapy regimens for active TB without loss of efficacy is a research priority. In this article we will review the growing body of evidence from studies conducted both *in vitro* and *in vivo* suggesting that vitamin D might have a role in the prevention and treatment of TB.

IMMUNOMODULATORY ACTIONS OF VITAMIN D IN MYCOBACTERIAL INFECTION

With the exception of a single report [5], vitamin D and its metabolites have not been shown to possess antimycobacterial activity in the absence of cells. However, the active metabolite of vitamin D, 1,25-dihydoxyvitamin D ($1,25(OH)_2D$), has long been recognised to induce antimycobacterial activity *in vitro* in mononuclear phagocytes, the cells which control growth of MTB [6]. Ligation of macrophage toll-like receptor 2/1 heterodimers by mycobacterial antigens induces expression of the vitamin D receptor (VDR) and the 1-alpha hydroxylase enzyme CYP27B1 [7, 8] which synthesises $1,25(OH)_2D$ from the principal circulating vitamin D metabolite 25-hydroxy-vitamin D (25[OH]D). Because extra-renal 1-alpha hydroxy-lase follows first order kinetics, the rate at which it synthesizes $1,25(OH)_2D$ depends on availability of 25(OH)D substrate [9]. Orally ingested vitamin D is freely converted to 25(OH)D, and this provides the rationale for administering 'parent' vitamin D to induce antimycobacterial responses at the site of disease.

$1,25(OH)_2D$ modulates immune responses by ligating membrane VDR to induce rapid effects (within minutes), or nuclear VDR to induce genomic effects (within hours) [10]. Experiments using selective agonists and antagonists of these two receptors indicate that ligation of nuclear VDR is both necessary and sufficient for induction of antimycobacterial responses by $1,25(OH)_2D$ *in vitro* [11]. $1,25(OH)_2D$ modulates the host response to mycobacterial infection by pleiotropic mechanisms including the induction of reactive nitrogen and oxygen intermediates [12, 13], down-regulation of the gene encoding tryptophan-aspartate containing coat protein [14], promotion of phagolysosome fusion [15], suppression of matrix metalloproteinase enzymes implicated in the pathogenesis of pulmonary cavitation [16] and induction of antimicrobial peptides including cathelicidin LL-37 [7, 11] and human beta defensin 2 [17]. Cathelicidin LL-37 possesses antimycobacterial activity [7, 18] and also induces autophagy [19]; $1,25(OH)_2D_3$-induced antimycobacterial activity has been reported to be dependent on expression of the gene encoding cathelicidin LL-37 [20].

VITAMIN D AND TUBERCULOSIS: HISTORICAL ASPECTS

In 1849, Chapman reported results of administering cod liver oil to patients with 'consumption', and made the following observation: 'the beneficial effects of the oil were manifested most speedily and most decisively, in the improvement of the appetite, aspect of the countenance, strength and spirits'. He concluded that cod liver oil was 'probably the only remedial agent by which the vital powers may be enabled to struggle successfully against that malady'

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[21]. This report represents the first circumstantial evidence that administration of a preparation containing vitamin D improved clinical outcome in patients with TB, although it should be noted that no control group was studied, and that any beneficial effects of cod liver oil may have been attributable to its content of vitamin A rather than vitamin D [22]. The first TB sanatorium was opened in Gorbersdorf, Germany (today Sokolowsko, Poland) in 1859, and heliotherapy (exposure of TB patients to sunlight, which induces cutaneous vitamin D synthesis) subsequently became common practice, and was credited with improvements in clinical outcome in many cases [23]. In 1903, Niels Finsen was awarded the Nobel Prize in Physiology or Medicine for his discovery that shortwave ultra-violet light was effective in the treatment of cutaneous TB [24]. Vitamin D_2 was purified and crystallised in 1931 [25] and Charpy subsequently pioneered the use of pharmacologic doses (≥1.25 mg daily) of vitamin D_2 to treat cutaneous TB [26]. Vitamin D_2 was also used to treat pulmonary TB, both as a single agent and, following the introduction of effective anti-tuberculous chemotherapy, as an adjunct to antibiotic treatment [27].

ASSOCIATION BETWEEN VITAMIN D DEFICIENCY AND SUSCEPTIBILITY TO TUBERCULOSIS

In 1985, Davies observed that people migrating to the United Kingdom from countries with a high incidence of latent MTB infection experienced rates of active TB that exceeded rates in their countries of origin, and that this increased risk coincided with the development of vitamin D deficiency, probably arising as a result of decreased sun exposure [28]. He suggested that vitamin D deficiency may predispose to reactivation of latent MTB infection in this setting, a hypothesis supported by his observation that vitamin D deficiency associated with susceptibility to active tuberculosis [29]. Since then, a further 11 case control studies investigating the association between vitamin D status and susceptibility to active TB have been published. Of these, 7 have reported a statistically significant association between vitamin D deficiency and susceptibility to active TB [30-36], 3 have reported a non-statistically significant trend towards such an association [37-39] and one [40] has reported that active TB was associated with both 'high' and 'low' serum 25(OH)D concentrations (>140 nmol/L and < 75 nmol/L, respectively). Potential explanations for an association between vitamin D deficiency and active TB include both causality (i.e. vitamin D deficiency impairs host immune response to MTB and causes susceptibility) and reverse causality (i.e. active TB causes vitamin D deficiency, due to anorexia, decreased exposure to sunlight in debilitated patients, or MTB-induced dysregulation of vitamin D metabolism [34]).

ASSOCIATION BETWEEN SUSCEPTIBILITY TO TUBERCULOSIS AND POLYMORPHISMS IN THE VITAMIN D RECEPTOR AND VITAMIN D BINDING PROTEIN

Human VDR is encoded by the *VDR* gene located on chromosome 12q. This gene is polymorphic, and numerous single nucleotide polymorphisms have been described. The hypothesis that VDR variants might associate with susceptibility to active TB was first investigated by Bellamy and colleagues, who reported an association between carriage of the T allele of the *TaqI* VDR polymorphism and susceptibility to active TB in a case control study conducted in Gambian adults [41]. Wilkinson and colleagues subsequently reported that associations between susceptibility to TB and carriage of the T allele of the *TaqI* VDR polymorphism and the ff genotype of the *FokI* VDR polymorphism in Gujarati Asians living in London were restricted to vitamin D-deficient individuals [32]; this study is the first to report that gene: environment interactions may operate to influence susceptibility to active TB. Numerous case control studies investigating the association between VDR variants and susceptibility to active TB have since been published; a recent meta-analysis of 23 such studies reported that in Asian populations, the *FokI ff* genotype associated with susceptibility to active TB (pooled OR 2.0, 95% CI 1.3 to 3.2), and the BsmI *bb* genotype (defined by the presence of two restriction sites for the *Bsm*1 endonuclease) was associated with protection against active TB (pooled OR 0.5, 95% CI 0.4 to 0.8); no associations were seen in African or Latin American populations however [42].

Further case control studies have investigated associations between polymorphisms in the vitamin D binding protein (DBP) and susceptibility to active TB. DBP is a highly expressed multifunctional 58 kDa serum glycoprotein encoded on chromosome 4. Two common polymorphisms at codons 416 and 420 of exon 11 of the *DBP* gene give rise to the three major electrophoretic variants of DBP, termed group-specific component 1 fast (Gc1F), Gc1 slow (Gc1S) and Gc2. These variants differ in their functional characteristics: the Gc1F and Gc1S variants have been reported to have greater affinity for 25-hydroxyvitamin D (25[OH]D) than the Gc2 variant [43], potentially leading to more efficient delivery of 25(OH)D to the target tissues, while the Gc2 variant is associated with decreased circulating concentrations of 25(OH)D, $1,25[OH]_2D$ and DBP [44, 45]. Case control studies conducted in India, Russia and Kuwait have not reported any association between DBP genotype and susceptibility to TB [46-48], but a more recent study reported an association between the Gc2 allele of vitamin D binding protein and susceptibility to active TB among Gujarati Asians living in London. This association was preserved if serum 25(OH)D concentration was <20 nmol/L, but not if serum 25(OH)D was ≥20 nmol/L, suggesting that profound vitamin D deficiency and Gc2 genotype may interact to increase susceptibility to TB [39].

PROSPECTIVE OBSERVATIONAL STUDIES

In contrast to the numbers of published cross-sectional studies, relatively few cohort studies investigating associations between vitamin D status/VDR genotype and TB have been conducted. Two studies have examined the influence of VDR genotype on response to antimicrobial therapy: Roth and colleagues reported that the *FF* genotype of the *FokI* VDR polymorphism and the *Tt* genotype of the *TaqI* VDR polymorphism associated with faster sputum culture conversion in a cohort of pulmonary TB patients in Peru [49], while Babb and colleagues reported no difference in time to sputum culture conversion according to *TaqI* or

FokI VDR genotype among South African TB patients [50]. Recently, a cohort study conducted in Pakistan [51] reported that profound vitamin D deficiency among healthy household TB contacts at baseline associated with increased risk of development of active TB over the subsequent 4 years: 7/30 contacts with baseline plasma 25(OH)D < 17.5 nmol/L developed active TB during follow-up, compared with 1/32 with plasma 25(OH)D 17.5 – 33.5 nmol/L and 0/30 with plasma 25(OH)D > 33.5 nmol/L. This association retained significance after adjustment for age and sex, although other potential confounders were not taken into account in the analysis. The observation that increased risk of TB reactivation was almost exclusively confined to individuals with profound vitamin D deficiency is interesting, particularly when taken together with reports from case control studies that profound vitamin D defiency is most strongly associated with susceptibility to TB [32]: the implication is that, if vitamin D deficiency does indeed predispose to active TB, then relatively modest elevations of serum 25(OH)D might be effective for prevention of active disease.

INTERVENTION STUDIES

Despite the compelling results from the laboratory and observational studies reviewed above, no randomised controlled trials of vitamin D supplementation for the prevention of active TB have been published to date. The absence of such studies reflects the very considerable methodological and logistic challenges of conducting them. Because the annual risk of reactivation of latent TB is low in immunocompetent individuals (<1% even in individuals with a strongly positive and newly-converted tuberculin skin test [2]), very large sample sizes and prolonged follow-up will be needed to detect all but the largest effects of vitamin D supplementation on TB incidence in such populations. Our own group has attempted to circumvent this problem by investigating the effect of vitamin D supplementation on a surrogate outcome measure of antimycobacterial response – the BCG-lux assay, which measures the ability of whole blood to restrict bioluminescence of a reporter mycobacterium [52]. We found that a single dose of 2.5 mg vitamin D enhanced the ability of TB contacts' whole blood to restrict mycobacterial bioluminescence at 24 hours post-inoculation [53], providing further evidence that trials of vitamin D supplementation for prevention of TB are justified.

In contrast to prevention studies, randomised controlled trials to determine whether adjunctive vitamin D enhances response to antimicrobial therapy can be powered with more modest numbers, because the majority of TB patients respond rapidly to antimicrobial therapy. Seven such studies have been published to date (summarised in Table **1**). Three of these trials had biochemical primary outcomes: two reported no hypercalcaemia in tuberculosis patients receiving either 125 mcg vitamin D daily [54] or a single oral dose of 2.5 mg vitamin D [55], and one reported hypercalcaemia occurring in 19/30 tuberculosis patients receiving daily doses of 10 – 95 mcg vitamin D daily [56]. However, this third study, by Narang and colleagues, also reported that a daily dose of 60 mcg vitamin D elevated mean serum calcium in healthy controls – a finding which contrasts with other studies which demonstrate that identical [57] or considerably higher [58] doses of vitamin D do not induce hypercalcemia in healthy people. It is possible, therefore, that the actual doses of vitamin D administered in Narang's study were considerably higher than reported. The remaining four clinical trials listed in the table had clinical primary outcomes. Morcos and colleagues investigated the effects of 25 mcg vitamin D daily in a 24 children in Egypt receiving antimicrobial therapy for tuberculosis, and showed no effect on body weight or resolution of symptoms [59]. Nursyam and colleagues subsequently conducted a trial of a daily dose of 250 mcg vitamin D in 67 pulmonary TB patients in Indonesia [60]. In this study, adjunctive vitamin D enhanced sputum smear conversion at 6 weeks after initiation of antimicrobial therapy (34/34 *vs* 25/33 smear-converted in intervention *vs* control arm at 6 weeks, p=0.002); no effect of the intervention was seen at 8 weeks. The vitamin D status of participants was not assessed at either baseline or follow-up in this study, and details of safety monitoring, including monitoring of serum calcium concentrations, were not reported. In the largest treatment trial to date, Wejse and colleagues randomised 365 adult TB patients in Guinea-Bissau to receive three doses of 2.5 mg vitamin D3 or placebo at initiation of antimicrobial therapy, and again at 5 and 8 months [61]. The intervention had no effect on the primary outcome measure (a specially designed TB score) or on serum 25(OH)D concentration. Mean serum 25(OH)D concentrations at baseline were 78 nmol/L *vs* 79 nmol/L in intervention *vs* control groups. Most recently, our own group investigated the effect of a 2-weekly dose of 2.5 mg vitamin D on time to sputum culture conversion in 146 patients with smear-positive pulmonary TB in the UK [62]. A 79 nmol/L increase in serum 25(OH)D was seen among participants in the intervention arm of the study, which was associated with a non-statistically significant trend towards faster sputum culture conversion (p=0.14). A pre-planned sub-group analysis revealed that adjunctive vitamin D significantly hastened sputum culture conversion by more than 17 days in participants with the *tt* genotype of the *Taq*I vitamin D receptor polymorphism (hazard ratio 8.09, 95% CI 1.36-48.01; p=0.02).

CONCLUSIONS

Much remains to be done to evaluate whether vitamin D might have a role in the prevention or treatment of TB. A key research priority is to establish randomised controlled trials of vitamin D supplementation for the prevention of tuberculosis in individuals with latent MTB infection. Although some question the need for such studies to be conducted on the grounds that the methodological challenges they pose are too great [63], we remain convinced that these trials are necessary, fundable and feasible. Equivalent trials have been conducted to establish the role of chemoprophylaxis for tuberculosis prevention [64], and investigation of the role of vitamin D supplementation in this regard should be no less of a research priority, given the safety and low cost of this intervention. Investigations of the potential role of vitamin D as an adjunct to antimicrobial therapy are more advanced, but results from clinical trials published to date have shown little if any benefit in drug-sensitive disease. This is not the end of the road for this line of enquiry however. First, five other similar trials are

Table 1. Summary of Randomised Controlled Trials Investigating Effects of Adjunctive Vitamin D in Patients with Tuberculosis

First Author, Year, Reference	Sample Size, Setting	Vitamin D Dose Administered	Effect on Serum 25-Hydroxyvitamin D Concentration	Primary Outcome
Gwinup 1981 [54]	23 adults, USA	125 mcg daily	Not reported	Serum calcium: no change
Narang 1984 [56]	30 adults, India	10-95 mcg daily	Not reported	Serum calcium: hypercalcaemia in 63%
Morcos 1998 [59]	24 children, Egypt	25 mcg daily	Not reported	Body weight/symptoms: no change
Nursyam 2006 [60]	67 adults, Indonesia	250 mcg daily	Not reported	Smear conversion: increased rate at 6 wks
Martineau 2009 [55]	25 adults, UK	1 x 2.5 mg D2 @ 0 months	22 nmol/L increase in active arm	Serum 25(OH)D: small increase at 8 wks
Wejse 2009 [61]	365 adults, Guinea Bissau	3 x 2.5 mg D3 @ 0/5/8 months	25 nmol/L increase both arms	TB score: no effect
Martineau 2011 [62]	146 adults, UK	4 x 2.5 mg D3 @ 0/2/4/6 weeks	79 nmol/L increase in active arm	Culture conversion: no effect in study population as a whole, but effect seen in subgroup with tt genotype of the *TaqI* VDR polymorphism

ongoing [63]; a meta-analysis of the results of these studies may reveal a benefit which existing studies have not been powered to demonstrate. Second, the doses of vitamin D administered in trials conducted to date are considerably lower than those reported to be effective historically [27]; the effects of pharmacological dosing regimens are worthy of investigation. Finally, on-going investigations from our recently completed trial [62] reveal that administration of adjunctive vitamin D is associated with favourable immunomodulatory activity; this observation raises the possibility that individuals with multi-drug resistant TB, in whom antimicrobial therapy is less effective, might derive a clinical benefit from enhancement of their antimycobacterial immune response using adjunctive vitamin D therapy.

REFERENCES

[1] Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. JAMA 1999; 282(7): 677-86.

[2] Horsburgh CR, Jr. Priorities for the treatment of latent tuberculosis infection in the United States. N Engl J Med 2004; 350(20): 2060-7.

[3] Wood R, Maartens G, Lombard CJ. Risk factors for developing tuberculosis in HIV-1-infected adults from communities with a low or very high incidence of tuberculosis. J Acquir Immune Defic Syndr 2000; 23(1): 75-80.

[4] WHO. Global tuberculosis control: a short update to the 2009 report. Geneva; 2009.

[5] Raab W. Vitamin D - its bactericidal action. Chest 1946; 12: 409-15.

[6] Rook GA, Steele J, Fraher L, *et al*. Vitamin D3, gamma interferon, and control of proliferation of Mycobacterium tuberculosis by human monocytes. Immunology 1986; 57(1): 159-63.

[7] Liu PT, Stenger S, Li H, *et al*. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006; 311(5768): 1770-3.

[8] Krutzik SR, Hewison M, Liu PT, *et al*. IL-15 links TLR2/1-induced macrophage differentiation to the vitamin D-dependent antimicrobial pathway. J Immunol 2008; 181(10): 7115-20.

[9] Vieth R, McCarten K, Norwich KH. Role of 25-hydroxyvitamin D3 dose in determining rat 1,25-dihydroxyvitamin D3 production. Am J Physiol 1990; 258(5 Pt 1): E780-9.

[10] Norman AW, Mizwicki MT, Norman DP. Steroid-hormone rapid actions, membrane receptors and a conformational ensemble model. Nat Rev Drug Discov 2004; 3(1): 27-41.

[11] Martineau AR, Wilkinson KA, Newton SM, *et al*. IFN-{gamma}- and TNF-Independent Vitamin D-Inducible Human Suppression of Mycobacteria: The Role of Cathelicidin LL-37. J Immunol 2007; 178(11): 7190-8.

[12] Rockett KA, Brookes R, Udalova I, Vidal V, Hill AV, Kwiatkowski D. 1,25-Dihydroxyvitamin D3 induces nitric oxide synthase and suppresses growth of Mycobacterium tuberculosis in a human macrophage-like cell line. Infect Immun 1998; 66(11): 5314-21.

[13] Sly LM, Lopez M, Nauseef WM, Reiner NE. 1alpha,25-Dihydroxyvitamin D3-induced monocyte antimycobacterial activity is regulated by phosphatidylinositol 3-kinase and mediated by the NADPH-dependent phagocyte oxidase. J Biol Chem 2001; 276(38): 35482-93.

[14] Anand PK, Kaul D. Vitamin D3-dependent pathway regulates TACO gene transcription. Biochem Biophys Res Commun 2003; 310(3): 876-7.

[15] Hmama Z, Sendide K, Talal A, Garcia R, Dobos K, Reiner NE. Quantitative analysis of phagolysosome fusion in intact cells: inhibition by mycobacterial lipoarabinomannan and rescue by an 1alpha,25-dihydroxyvitamin D3-phosphoinositide 3-kinase pathway. J Cell Sci 2004; 117(10): 2131-40.

[16] Coussens A, Timms PM, Boucher BJ, *et al*. 1alpha,25-dihydroxyvitamin D inhibits matrix metalloproteinases induced by Mycobacterium tuberculosis infection. Immunology 2009; 127(4): 539-48.

[17] Liu PT, Schenk M, Walker VP, *et al*. Convergence of IL-1beta and VDR activation pathways in human TLR2/1-induced antimicrobial responses. PLoS One 2009; 4(6): e5810.

[18] Martineau AR, Newton SM, Wilkinson KA, *et al*. Neutrophil-mediated innate immune resistance to mycobacteria. J Clin Invest 2007; 117(7): 1988-94.

[19] Yuk JM, Shin DM, Lee HM, *et al*. Vitamin D3 induces autophagy in human monocytes/macrophages *via* cathelicidin. Cell host & microbe 2009; 6(3): 231-43.

[20] Liu PT, Stenger S, Tang DH, Modlin RL. Cutting edge: vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. J Immunol 2007; 179(4): 2060-3.

[21] Chapman HT. On the use of cod-liver oil in diseases of the bones and joints, in consumption and in other maladies attended by great emaciation. Phamaceutical Transactions 1849: 1-16.

[22] Karyadi E, West CE, Schultink W, *et al*. A double-blind, placebo-controlled study of vitamin A and zinc supplementation in persons with tuberculosis in Indonesia: effects on clinical response and nutritional status. Am J Clin Nutr 2002; 75(4): 720-7.

[23] Mayer E. Heliotherapy of tuberculosis. Ann Int Med 1938; 11(10): 1856-60.

[24] Roelandts R. A new light on Niels Finsen, a century after his Nobel Prize. Photodermatol Photoimmunol Photomed 2005; 21(3): 115-7.

[25] Askew FA, Bruce HM, Callow RK, Philpot JS, Webster TA. Crystalline vitamin D. Nature 1931; 128: 758.

[26] Charpy J. Quelques traitments vitaminés ou par substances fonctionelles en dermatologie. Bull Méd 1950; 24: 505.

[27] Martineau AR, Honecker FU, Wilkinson RJ, Griffiths CJ. Vitamin D in the treatment of pulmonary tuberculosis. J Steroid Biochem Mol Biol 2007; 103: 793-8.

[28] Davies PD. A possible link between vitamin D deficiency and impaired host defence to Mycobacterium tuberculosis. Tubercle 1985; 66(4): 301-6.

[29] Davies PD, Brown RC, Woodhead JS. Serum concentrations of vitamin D metabolites in untreated tuberculosis. Thorax 1985; 40(3): 187-90.

[30] Davies PD, Church HA, Brown RC, Woodhead JS. Raised serum calcium in tuberculosis patients in Africa. Eur J Respir Dis 1987; 71(5): 341-4.

[31] Davies PD, Church HA, Bovornkitti S, Charumilind A, Byrachandra S. Altered vitamin D homeostasis in tuberculosis. Int Med Thailand 1988; 4: 45-7.

[32] Wilkinson RJ, Llewelyn M, Toossi Z, *et al*. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. Lancet 2000; 355(9204): 618-21.

[33] Sasidharan PK, Rajeev E, Vijayakumari V. Tuberculosis and vitamin D deficiency. J Assoc Physicians India 2002; 50: 554-8.

[34] Sita-Lumsden A, Lapthorn G, Swaminathan R, Milburn HJ. Reactivation of tuberculosis and vitamin D deficiency: the contribution of diet and exposure to sunlight. Thorax 2007; 62(11): 1003-7.

[35] Gibney KB, MacGregor L, Leder K, *et al*. Vitamin D deficiency is associated with tuberculosis and latent tuberculosis infection in immigrants from sub-Saharan Africa. Clin Infect Dis 2008; 46(3): 443-6.

[36] Ho-Pham LT, Nguyen ND, Nguyen TT, *et al*. Association between vitamin D insufficiency and tuberculosis in a Vietnamese population. BMC Infect Dis 2010; 10: 306.

[37] Grange JM, Davies PD, Brown RC, Woodhead JS, Kardjito T. A study of vitamin D levels in Indonesian patients with untreated pulmonary tuberculosis. Tubercle 1985; 66(3): 187-91.

[38] Chan TY, Poon P, Pang J, *et al*. A study of calcium and vitamin D metabolism in Chinese patients with pulmonary tuberculosis. The Journal of tropical medicine and hygiene. 1994; 97(1): 26-30.

[39] Martineau AR, Leandro AC, Anderson ST, *et al*. Association between Gc genotype and susceptibility to TB is dependent on vitamin D status. Eur Respir J 2010; 35(5): 1106-12.

[40] Nielsen NO, Skifte T, Andersson M, *et al*. Both high and low serum vitamin D concentrations are associated with tuberculosis: a case-control study in Greenland. Br J Nutr 2010: 104(10): 1487-91.

[41] Bellamy R, Ruwende C, Corrah T, *et al*. Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene. J Infect Dis 1999; 179(3): 721-4.

[42] Gao L, Tao Y, Zhang L, Jin Q. Vitamin D receptor genetic polymorphisms and tuberculosis: updated systematic review and meta-analysis. Int J Tuberc Lung Dis 2010; 14(1): 15-23.

[43] Arnaud J, Constans J. Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP). Hum Genet 1993; 92(2): 183-8.

[44] Lauridsen AL, Vestergaard P, Hermann AP, *et al*. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. Calcif Tissue Int 2005; 77(1): 15-22.

[45] Abbas S, Linseisen J, Slanger T, *et al*. The Gc2 allele of the vitamin D binding protein is associated with a decreased postmenopausal breast cancer risk, independent of the vitamin d status. Cancer Epidemiol Biomarkers Prev 2008; 17(6): 1339-43.

[46] Papiha SS, Agarwal SS, White I. Association between phosphoglucomutase (PGM1) and group-specific component (Gc) subtypes and tuberculosis. J Med Genet 1983; 20(3): 220-2.

[47] Spitsyn VA, Titenko NV. [Subtypes of serum group specific component (Gc) in normal conditions and in pathology]. Genetika 1990; 26(4): 749-59.

[48] Bahr GM, Eales LJ, Nye KE, *et al*. An association between Gc (vitamin D-binding protein) alleles and susceptibility to rheumatic fever. Immunology 1989; 67(1): 126-8.

[49] Roth DE, Soto G, Arenas F, *et al*. Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. J Infect Dis 2004; 190(5): 920-7.

[50] Babb C, van der Merwe L, Beyers N, *et al*. Vitamin D receptor gene polymorphisms and sputum conversion time in pulmonary tuberculosis patients. Tuberculosis (Edinb) 2007; 87(4): 295-302.

[51] Talat N, Perry S, Parsonnet J, Dawood G, Hussain R. Vitamin D deficiency and tuberculosis progression. Emerg Infect Dis 2010; 16(5): 853-5.

[52] Kampmann B, Gaora PO, Snewin VA, Gares MP, Young DB, Levin M. Evaluation of human antimycobacterial immunity using recombinant reporter mycobacteria. J Infect Dis 2000; 182(3): 895-901.

[53] Martineau AR, Wilkinson RJ, Wilkinson KA, *et al*. A single dose of vitamin D enhances immunity to mycobacteria. Am J Respir Crit Care Med 2007; 176(2): 208-13.

[54] Gwinup G, Randazzo G, Elias A. The influence of vitamin D intake on serum calcium in tuberculosis. Acta Endocrinol (Copenh) 1981; 97(1): 114-7.

[55] Martineau AR, Nanzer AM, Satkunam KR, *et al*. Influence of a single oral dose of vitamin D(2) on serum 25-hydroxyvitamin D concentrations in tuberculosis patients. Int J Tuberc Lung Dis 2009; 13(1): 119-25.

[56] Narang NK, Gupta RC, Jain MK. Role of vitamin D in pulmonary tuberculosis. J Assoc Physicians India 1984; 32(2): 185-8.

[57] Tjellesen L, Hummer L, Christiansen C, Rodbro P. Serum concentration of vitamin D metabolites during treatment with vitamin D2 and D3 in normal premenopausal women. Bone Miner 1986; 1(5): 407-13.

[58] Stern PH, Taylor AB, Bell NH, Epstein S. Demonstration that circulating 1 alpha, 25-dihydroxyvitamin D is loosely regulated in normal children. J Clin Invest 1981; 68(5): 1374-7.

[59] Morcos MM, Gabr AA, Samuel S, *et al*. Vitamin D administration to tuberculous children and its value. Boll Chim Farm 1998; 137(5): 157-64.

[60] Nursyam EW, Amin Z, Rumende CM. The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. Acta Med Indones 2006; 38(1): 3-5.

[61] Wejse C, Gomes VF, Rabna P, *et al*. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. Am J Respir Crit Care Med 2009; 179(9): 843-50.

[62] Martineau AR, Timms PM, Bothamley GH, *et al*. High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet 2011; 377(9761): 242-50.

[63] Vieth R. Vitamin D nutrient to treat TB begs the prevention question. Lancet 2011; 377(9761): 189-90.

[64] Ferebee SH. Controlled chemoprophylaxis trials in tuberculosis. A general review. Bibl Tuberc 1970; 26: 28-106.

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Vitamin D and Lung Cancer

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Abstract: Lung cancer is one of the most common cancers but treatment has limited success and survival rates remain low. The active metabolite of vitamin D, 1,25-dihydroxyvitamin D, has anti-proliferative and pro-apoptotic actions in lung carcinoma cell lines *in vitro*. Vitamin D deficiency is associated with poor prognosis in lung cancer, and lung cancer survival varies according to vitamin D receptor genotype. Randomized controlled trials should be performed to determine whether vitamin D supplementation might have a role as an adjunct to conventional treatments for lung cancer.

Keywords: Vitamin D, lung cancer, incidence, mortality.

INTRODUCTION

Lung cancer is the leading cause of cancer death among both men and women [1], responsible for 1.3 million deaths per year worldwide [2]. The two major forms are non-small-cell lung cancer (NSCLC), representing 85% of all lung cancers, and small-cell lung cancer (SCC), accounting for 15%. Treatments include surgery, chemotherapy, radiotherapy or a combination of the above. Overall 5-year survival remains low at 14% [3].

The most common cause of lung cancer is long-term exposure to tobacco smoke, but a growing body of literature suggests that vitamin D deficiency may also be independently associated with increased lung cancer incidence and decreased lung cancer survival in some groups. Moreover, some lung cancer cell lines have been reported to express both vitamin D receptor (VDR) and the enzyme capable of synthesising the active metabolite of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), which exerts anti-proliferative and pro-apoptotic effects *in vitro*. In this article, I will describe the *in vitro* actions of vitamin D metabolites in lung cancer cell lines, review the epidemiological literature relating to the association between vitamin D deficiency and lung cancer incidence and mortality and highlight the need for randomised controlled trials of vitamin D supplementation in patients with lung cancer.

MECHANISMS OF ACTION OF VITAMIN D IN CANCER CELLS

Vitamin D, obtained through cutaneous synthesis on exposure to ultraviolet B (UVB) light or diet, is transported in the circulation bound to vitamin D binding protein, and is hydroxylated in the liver by the mitochondrial cytochrome P450 enzyme 25-hydroxylase (CYP27A1) to form 25-hydroxyvitamin D (25[OH]D), the major circulating metabolite of vitamin D. The production of 25(OH)D is not tightly regulated and correlates with substrate availability rather than with physiological requirement [4, 5]. Determination of circulating 25(OH)D concentrations thus provides a useful indicator of vitamin D status that is widely applied in clinical practice [6]. 25(OH)D is metabolised by another mitochondrial cytochrome P450 enzyme, 1 alpha-hydroxylase (CYP27B1) into the hormonally active form $1,25(OH)_2D$; CYP27B1 is expressed in the kidney and in extra-renal tissues including NSCLC cells [7]. Synthesis of $1,25(OH)_2D$ is tightly regulated, both by $1,25(OH)_2D$ itself and by parathyroid hormone, fetal growth factor 23 and serum concentrations of calcium and phosphate [4], which influence expression of the major vitamin D catabolic enzyme, 24-hydroxylase (CYP24). The gene encoding this enzyme has been identified as a candidate oncogene in a number of cancer cell lines, and its expression is increased in NSCLC cells [8].

$1,25(OH)_2D$ exerts its biological effects by binding VDR within the nuclei of cells in target organs. VDR is expressed in at least thirty-six tissues of the body [4], and its presence has been detected in approximately 65% of primary non-small cell lung cancers [8]. VDR regulates the expression of as many as 500 genes of the ≈ 20,488 genes in the human genome [9]; many of these are involved in the control of apoptosis, cell division and adhesion, raising the possibility that $1,25(OH)_2D$ may influence the growth of malignant cells [10].

In vitro, $1,25(OH)_2D$ has been reported to possess anti-proliferative, anti-angiogenic and pro-apoptotic activity in a wide variety of cancer cell lines including leukaemia cells and malignant cells from skin, breast, colon and prostate [11-23]. $1,25(OH)_2D$ has also been reported to inhibit proliferation of two SCLC cell lines (NCI-H82 and NCI-H209) [24] and one out of five squamous cell carcinoma cell lines investigated (EBC-1) [25]; in the latter study, exertion of anti-proliferative action by $1,25(OH)_2D$ was found to be dependent on expression of VDR. *In vivo*, both $1,25(OH)_2D$ and a low-calcaemic analogue, 22-oxa-1α,25-dihydroxy-vitamin D_3, reduced the number of metastases and tumour burden in mice injected with a lung carcinoma cell line [26].

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VITAMIN D AND LUNG CANCER INCIDENCE

The overwhelming contribution of smoking as a cause of lung cancer dominates amongst the causes, and this may obscure associations between vitamin D status and susceptibility to lung cancer, particularly because smokers have been reported to have lower serum 25(OH)D concentrations than non-smokers [27]. Nevertheless, there is some evidence that greater UVB exposure or supplemental vitamin D may be independently associated with reduced incidence of the disease. Grant has recently summarised the evidence for increased solar UVB exposure and higher vitamin D status as factors for the prevention of cancer using Hill's criteria for causality [28]. Vitamin D status/UVB exposure satisfied the causality criteria most convincingly for breast and colorectal cancer, he found evidence that they also fulfilled the causality criteria for 14 other cancers – one of them being cancer of the lung.

In an ecological study, Mohr *et al.* examined incidence rates of lung cancer in 111 countries according to the UVB irradiance while controlling for cigarette smoking, cloud cover and anthropogenic aerosols. Lower UVB irradiance was associated with higher incidence rates of lung cancer. Latitude was positively related to incidence rates in both men (r^2=0.55, $p<0.01$) and women (r^2=0.36, $p<0.01$) [29]. In a prospective study, Kilkkinen *et al.* followed up 6937 patients in Finland over 24 years and 122 lung cancers were identified. After adjustment for potential confounders, serum 25(OH)D concentration was inversely associated with lung cancer incidence for women (RR, 0.16; 95% CI, 0.04-0.59; P < 0.001) and younger participants (RR, 0.34; 95% CI, 0.13-0.90; P = 0.04) but not for men (RR, 1.03; 95% CI, 0.59-1.82; P = 0.81) or older individuals (RR, 0.92; 95% CI, 0.50-1.70; P = 0.79) [30].

Clinical trials of vitamin D supplementation specifically for the prevention of lung cancer have not been performed, but secondary analysis of trials of vitamin D supplementation related to bone health may yield pertinent data. In one such randomised controlled trial, 1179 post menopausal women were randomised to receive 1100 IU vitamin D + calcium *vs* calcium alone *vs* placebo. A 77% reduction of all cancer risk, with 35% of the cancer risk reduction attributed to the vitamin D, was reported [31]. In this trial there was one lung cancer in the vitamin D + calcium group after four years, but three in the placebo group. The reason why such a large treatment success was observed after the study's relatively short duration of four years might be because vitamin D is thought to be effective in preventing progress of early cancers, consistent with the 15 - 20 year latency period before clinical detection [32]. Two previous negative studies analysed data from the women's health initiative and found no effect of vitamin D supplementation on incidence of cancer of the colon [33] or the breast [34]; however these studies have been criticised for the low dosing regimen (400 IU vitamin D daily) used in the intervention arm, and for having had poor compliance [35, 36].

VITAMIN D AND LUNG CANCER MORTALITY

The hypothesis that solar UVB radiation and cancer mortality were inversely related was first proposed by Apperly in 1941 [37]. Grant, who carried out an ecological study in the US, was the first to identify an inverse association between UVB irradiance/vitamin D status and lung cancer mortality rates [32]. Another ecological study has since reported an inverse association between UVB irradiance and lung cancer mortality rates in male, but not female, black Americans [38]. Zhou *et al*. investigated the effects of seasonal influences and vitamin D intake on survival of early stage NSCLC and found that both higher UVB exposure (patients who had surgery in the summer) and higher vitamin D intake (diet and supplement, assessed by a validated food frequency questionnaire) was associated with improved lung cancer survival [39]. They observed a 26% improved survival in those with serum 25(OH)D concentration >50 nmol/L *vs* those with serum 25(OH)D concentration <25 nmol/L. Chen *et al.* recently reported an inverse relation between ambient UBV and mortality rates for all cancers in a large study conducted in China, with lung cancer showing the strongest inverse relation, even if adjusted for smoking [40]. However, cross-sectional analysis of a very large data set from the US National Health and Nutrition Examination Survey III did not demonstrate an association between serum 25(OH)D concentration and lung cancer mortality [41]. A large prospective study has estimated that a 25 nmol/L difference in predicted 25(OH)D concentration was associated with a 20% reduction in lung cancer mortality risk in men, although this trend did not attain statistical significance [42].

A number of studies have investigated whether VDR genotype is associated with lung cancer survival. Zhou *et al.* conducted a cohort study of 373 patients with early stage NSCLC has demonstrated an association between the G/A+A/A genotype group of the Cdx-2 VDR polymorphisms and improved survival among patients with squamous cell carcinoma (SCC) but not among those with adenocarcinoma; 5-year survival rates were 41% for the G/G and 55% for the G/A+A/A genotypes. Concerning the joint effects of the three relevant VDR polymorphisms, Cdx-2 G>A, FokI C>T, and BsmI C>T, SCC patients with two or more "protective" alleles had better overall survival, with adjusted hazard ratios of 0.20 (95% CI, 0.09-0.48), 0.40 (95% CI, 0.19-0.87) and 0.43 (95% CI, 0.19-0.97) for subjects with two, three and four or more "protective" alleles, respectively, when compared with subjects with zero or one "protective" allele [43]. The same research group has also reported that the C/C genotype of the FokI VDR polymorphism was associated with better survival in a cohort of 294 patients with advanced stage NSCLC (21.4 months for C/C versus 12.1 months for C/T and 15.6 months for T/T). The G-T-C (Cdx-2-FokI-BsmI) haplotype was associated with worse survival compared with the most common haplotype of G-C-T (adjusted hazard ratio, 1.61; 95% CI, 1.21 to 2.14; P = .001) [44]. To date, no randomised controlled trials of vitamin D supplementation have been performed in patients with lung cancer. However, Cannell and Hollis have proposed that patients with cancer require significantly higher doses of vitamin D and should maintain serum 25(OH)D concentrations >137 nmol/L [45]. It is reasonable to hypothesise that vitamin D might produce maximal clinical benefit in the early stages of tumour development, when malignant cells are more likely to retain both the VDR and the enzymes needed to activate vitamin D

[9]. Two observations support this hypothesis: firstly the finding of an association between improved survival for cancers diagnosed in the summer (when serum 25(OH)D concentrations are higher) *vs* the winter [39], and secondly, the higher metabolic clearance of 25(OH)D once the cancer has developed [45]. If supplementation in early cancer is considered, frequent monitoring of serum 25(OH)D and calcium concentrations should guide dosing [45, 46].

CONCLUSIONS

Randomised controlled trials are needed to test the hypothesis that increases in circulating 25(OH)D concentration are effective in reducing cancer risk before any public health recommendations can be made for vitamin D supplementation on a population level. Considering the widespread vitamin D insufficiency and deficiency which has in particular been observed in cancer and lung cancer patients, it might be prudent to consider treating vitamin D deficiency and insufficiency in those diagnosed with cancer. Further information from trials is needed to determine whether vitamin D supplementation might prolong survival in lung cancer. However, sufficient observational, ecological and epidemiological evidence is now available to argue the case to conduct clinical trials of vitamin D supplementation in this patient group.

REFERENCES

[1] Herbst RS, Heymach JV, Lippman SM. Lung Cancer. Review Article. N Engl J Med 2008; 359: 1367-80.

[2] WHO. Fact Sheet No 297. February 2009.

[3] Minna JD, Schiller JH. Harrison's Principles of Internal Medicine (17th ed.). McGraw-Hill 2008; 551-62.

[4] Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr 2008; 88(suppl): 491S-9S.

[5] Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357: 266-81.

[6] Hansen CM, Binderup L, Hamberg KJ, Carlberg C. Vitamin D and cancer: effects of 1,25(OH)2D3 and its analogs on growth control and tumorigenesis. Front Biosci 2001; 6: d820-48.

[7] Jones G, Ramshaw H, Zhang A, *et al*. Expression and activity of vitamin D-metabolizing cytochrome P450s (CYP1a and CYP24) in human non-small cell lung carcinomas. Endocrinology 1999; 140: 3303-10.

[8] Parise RA, Egorin MJ, Kanterewicz B, *et al*. CYP24, the enzyme that catabolizes the antiproliferative agent vitamin D, is increased in lung cancer. Int J Cancer 2006; 119: 1819-28.

[9] Fleet JC. Molecular actions of vitamin D contributing to cancer prevention. Mol Aspects Med 2008; 29(6): 388-96.

[10] Luong K, Nguyen LT. The beneficial role of vitamin D and its analogs in cancer treatment and prevention. Crit Rev Oncol Hematol 2010; 73(3): 192-201.

[11] Miyaura C, Abe E, Kuribayashi T, Tanaka H, Konno K, Nishii Y. 1 alpha, 25-Dihydroxyvitamin D3 induces differentiation of human myeloid leukemia cells. Biochem Biophys Res Commun 1981; 102: 937-43.

[12] Chen TC, Persons K, Liu WW, Chen ML, Holick MF. The antiproliferative and differentiative activities of 1,25-dihydroxyvitamin D3 are potentiated by epidermal growth factor and attenuated by insulin in cultured human keratinocytes. J Invest Dermatol 1995; 104: 113-7.

[13] Thomas MG, Tebbutt S, Williamson RC. Vitamin D and its metabolites inhibit cell proliferation in human rectal mucosa and a colon cancer cell line. Gut 1992; 33: 1660-3.

[14] Tanaka Y, Bush K, Klauck T, Higgins P. Enhancement of butyrate-induced differentiation of HT-29 human colon carcinoma cells by 1,25-dihydroxyvitamin D. Biochem Pharmacol 1998; 38: 3859-65.

[15] Frappart L, Falette N, Lefebre M, Bremond A, Vauzelle J, Saez S. *In vitro* study of the effects of 1,25 dihydroxyvitamin D3 on the morphology of human breast cancer cell line BT-20. Differentiation 1989; 40: 63-9.

[16] Colston KW, Berger U, Coombes RC. Possible role for vitamin D in controlling breast cancer cell proliferation, Lancet 1989; 1: 188-91.

[17] Mangelsdorf DJ, Koeffler HP, Donaldson CA, Pike JW, Haussler MR. 1,25-Dihydroxyvitamin D3-induced differentiation in a human promyelocytic leukemia cell line (HL-60): receptor-mediated maturation to macrophage-like cells. J Cell Biol 1984; 98: 391-8.

[18] Abe E, Miyaura C, Sakagami H, Takeda M, Konno K, Yamazaki T. Differentiation of mouse myeloid leukemia cells induced by 1 alpha, 25-dihydroxyvitamin D3. Proc Natl Acad Sci USA 1981; 78: 4990-4.

[19] Lointier P, Wargovich MJ, Saez S, *et al*. The role of vitamin D3 in the proliferation of a human colon cancer cell line *in vitro*. Anticancer Res 1987; 7: 817-21.

[20] Gross M, Kost SB, Ennis B, Stumpf W, Kumar R. Effect of 1,25-dihydroxyvitamin D3 on mouse mammary tumor (GR) cells: evidence for receptors, cellular uptake, inhibition of growth and alteration in morphology at physiologic concentrations of hormone. J Bone Miner Res 1986; 1(5): 457-67.

[21] Skowronski RJ, Peehl DM, Feldman D. Vitamin D and prostate cancer: 1,25 dihydroxyvitamin D3 receptors and actions in human prostate cancer cell lines. Endocrinology 1993; 132: 1952-60.

[22] Diaz GD, Paraskeva C, Thomas MG, Binderup L, Hague A. Apoptosis is induced by the active metabolite of vitamin D3 and its analogue EB1089 in colorectal adenoma and carcinoma cells: possible implications for prevention and therapy. Cancer Res 2000; 60: 2304-12.

[23] Welsh J, VanWeelden K, Flanagan L, Byrne I, Nolan E, Narvaez CJ. The role of vitamin D3 and antiestrogens in modulating apoptosis of breast cancer cells and tumors. Subcell Biochem 1998; 30: 245-70.

[24] Guzey M, Sattler C, DeLuca HF. Combinational effects of vitamin D3 and retinoic acid (all trans and 9 cis) on proliferation, differentiation, and programmed cell death in two small cell lung carcinoma cell lines. Biochem Biophys Res Commun 1998; 249: 735-44.

[25] Higashimoto Y, Ohata M, Nishio K, *et al*. 1 alpha, 25-dihydroxyvitamin D3 and all-trans-retinoic acid inhibit the growth of a lung cancer cell line. Anticancer Res 1996; 16: 2653-9.

[26] Nakagawa K, Kawaura A, Kato S, *et al*. 1 alpha,25-Dihydroxyvitamin D(3) is a preventive factor in the metastasis of lung cancer. Carcinogenesis 2005; 26: 429-40.

[27] Brot C, Jorgensen NR, Sorensen OH. The influence of smoking on vitamin D status and calcium metabolism. Eur J Clin Nutr 1999; 53: 920-6.

[28] Grant WB. How strong is the evidence that solar ultraviolet B and vitamin D reduce the risk of cancer? An examination using Hill's criteria for causality. Dermato-Endocrinology 2009; 1: 17-24.

[29] Mohr SB, Garland CF, Gorham ED, Grant WB, Garland FC. Research report. Could ultraviolet B irradiance and vitamin D be associated with lower incidence rates of lung cancer? Epidemiol Community Health 2008; 62: 69-74.

[30] Kilkkinen A, Knekt P, Heliövaara M, *et al*. Vitamin D status and the risk of lung cancer: a cohort study in Finland. Cancer Epidemiol Biomarkers Prev 2008; 17(11): 3274-8.

[31] Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr 2007; 85(6): 1586-91.

[32] Grant WB. An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. Cancer 2002; 94: 1867-75.

[33] Wactawski-Wende J, Kotchen JM, Anderson GL, *et al*. for the Women's Health Initiative Investigators. Calcium plus vitamin D supplementation and the risk of colorectal cancer. N Engl J Med 2006; 354: 684-96.

[34] Chlebowski R, Johnson K, Kooperberg C, *et al*. for the Women's Health Initiative Investigators. Calcium plus vitamin D supplementation and the risk of breast cancer. J Natl Cancer Inst 2008; 100: 1581-91.

[35] Garland CF, Gorham ED, Mohr SB, Garland FC. Vitamin D for Cancer Prevention: Global Perspective. Review Article. Ann Epidemiol 2009; 19(7): 468-83.

[36] Hollis BW, Wagner CL. Nutritional vitamin D status during pregnancy: reasons for concern. CMAJ 2006; 174(9): 1287-90.
[37] Apperly FL. The relation of solar radiation to cancer mortality in North American. Cancer Res 1941; 1: 191-5.
[38] Grant WB. Lower vitamin-D production from solar ultraviolet-B irradiance may explain some differences in cancer survival rates. J Natl Med Assoc 2006; 98: 357-64.
[39] Zhou W, Heist RS, Liu G, *et al*. Circulating 25-hydroxyvitamin D levels predict survival in early-stage non-small-cell lung cancer patients. J Clin Oncol 2007; 25: 479-85.
[40] Chen W, Clements M, Rahman B, Zhang S, Qiao Y, Armstrong BK. Relationship between cancer mortality/incidence and ambient ultraviolet B irradiance in China. Cancer Causes Control 2010; 21(10): 1701-9.
[41] Freedman DM, Looker AC, Chang SC, Graubard BI. Prospective study of serum vitamin D and cancer mortality in the United States. J Natl Cancer Inst 2007; 99: 1594-602.
[42] Giovannucci E, Liu Y, Rimm EB, *et al*. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. J Natl Cancer Inst 2006; 98: 451-9.
[43] Zhou W, Heist RS, Liu G, *et al*. Polymorphisms of vitamin D receptor and survival in early-stage non-small cell lung cancer patients. Cancer Epidemiol Biomarkers Prev 2006; 15: 2239-45.
[44] Heist RS, Zhou W, Wang Z, *et al*. Circulating 25-hydroxyvitamin D, VDR polymorphisms, and survival in advanced non-small-cell lung cancer. J Clin Oncol 2008; 26(34): 5596-602.
[45] Cannell JJ, Hollis BW. Use of vitamin D in clinical practice. Altern Med Rev 2008; 13(1): 6-20.
[46] Goodwin PJ. Vitamin D in Cancer Patients: Above All, Do No Harm. Editorial. J Clin Oncol 2009; 27(13): 2117-9.

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Immunomodulatory Actions of Vitamin D Metabolites and their Potential Relevance to Human Lung Disease

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Abstract: The non-classical role for vitamin D in maintaining immune homeostasis has been recognised for 30 years. A definitive link between vitamin D status and the immune response has now been established by a multitude of association studies which link both vitamin D deficiency and genetic polymorphisms in vitamin D-related genes to susceptibility to respiratory diseases including tuberculosis, upper respiratory tract infection, chronic obstructive pulmonary disease (COPD), asthma and lung cancer. This review considers the mechanisms by which immune cells and lung epithelial cells respond to infection or injury by inducing intracellular metabolism of vitamin D. The effects of vitamin D metabolites on induction of phagocyte antimicrobial responses, modulation of DC maturation and T cell priming, skewing of the cytokine milieu towards a type 2 inflammatory response and promotion of regulatory T (Treg) cell development will also be described.

Keywords: Immunomodulation, 1,25-dihydroxyvitamin D, cytokine, macrophage, T-cell, dendritic cell, T-reg, autoimmunity, infection.

INTRODUCTION

Over the past three decades our understanding of the role vitamin D plays in human physiology has changed dramatically. Once considered primarily as a regulator of calcium and bone homoeostasis, vitamin D is now recognised to have a diverse range of physiological functions. One reason for its pleiotropic actions may be the fact that vitamin D differs from other vitamins in that its primary active metabolite, 1alpha,25-dihydroxyvitamin D ($1,25(OH)_2D$), is a steroid hormone. Moreover, unlike many other vitamins which act as antioxidants or enzyme co-factors, vitamin D metabolites complex with their receptor to form a ligand-dependant transcription factor complex that regulates the expression of more than 900 genes [1].

The classic model of vitamin D metabolism involves its initial 25-hydroxylation in the liver to produce 25-hydroxyvitamin D (25(OH)D), the major circulating vitamin D metabolite and accepted measure of vitamin D status. Once 25(OH)D reaches the kidney it is 1α-hydroxylated by the CYP27B1 enzyme to form $1,25(OH)_2D$. This metabolite acts on the kidney, the gastro-intestinal tract and bone to regulate serum calcium concentration as well as its own catabolism, *via* induction of the 24-hydroxylase enzyme CYP24A1 (Fig. **1**). In 1981 it was discovered that $1,25(OH)_2D$ can also be produced in extra-renal tissues, including macrophages [2]. Subsequent *in vitro* studies have demonstrated that during certain infections, microbial binding to pattern recognition receptors (PRRs) on leucocytes and epithelial cells can upregulate expression of CYP27B1 in these cells to induce local synthesis of $1,25(OH)_2D$ [3, 4]. These observations suggest that during an immune response cells require high levels of $1,25(OH)_2D$ for optimum functionality. It is now known that many cell types upregulate CYP27B1 to induce synthesis of $1,25(OH)_2D$ in response to a range of stimuli. Consequently, vitamin D is now recognised to regulate numerous aspects of cellular physiology, including cell proliferation, differentiation, activation and death.

Vitamin D metabolites modulate both innate and adaptive immune responses (summarised in Fig. **2**). The wide-ranging clinical implications of this immunomodulatory activity are reflected by the steady increase in the number of diseases being associated with vitamin D deficiency and the large number of clinical trials of vitamin D for the prevention and treatment of respiratory infections, cancer, diabetes mellitus, cardiovascular disease and multiple sclerosis [5-12]. Moreover, with the worldwide prevalence of vitamin D deficiency on the increase [13, 14], two internationally recognised bodies have released new supplementation guidelines [15, 16] within the last year.

The aim of this review is to discuss the emerging immunomodulatory roles of $1,25(OH)_2D$ and its precursors, catabolites and analogues in controlling the development and function of monocytes (MN), macrophages, dendritic cells (DC), T cells, B cells, mast cells and airway epithelial cells. Particular attention will be paid to the mechanisms by which vitamin D metabolites modulate the immune response to respiratory pathogens.

VITAMIN D STATUS AND DISEASE ASSOCIATION

Vitamin D exists in two forms: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) (Fig. **1**). Vitamin D_3 is the predominant form in humans and is primarily obtained through skin exposure to UVB irradiation. This cleaves 7-dehydrocholestrol, stored in the epidermis, into pre-vitamin D_3 which spontaneously isomerises to form pro-vitamin D_3. Vitamin D_3 is also obtained from dietary sources such as oily fish and their derived products like cod liver oil, while vitamin D_2 is

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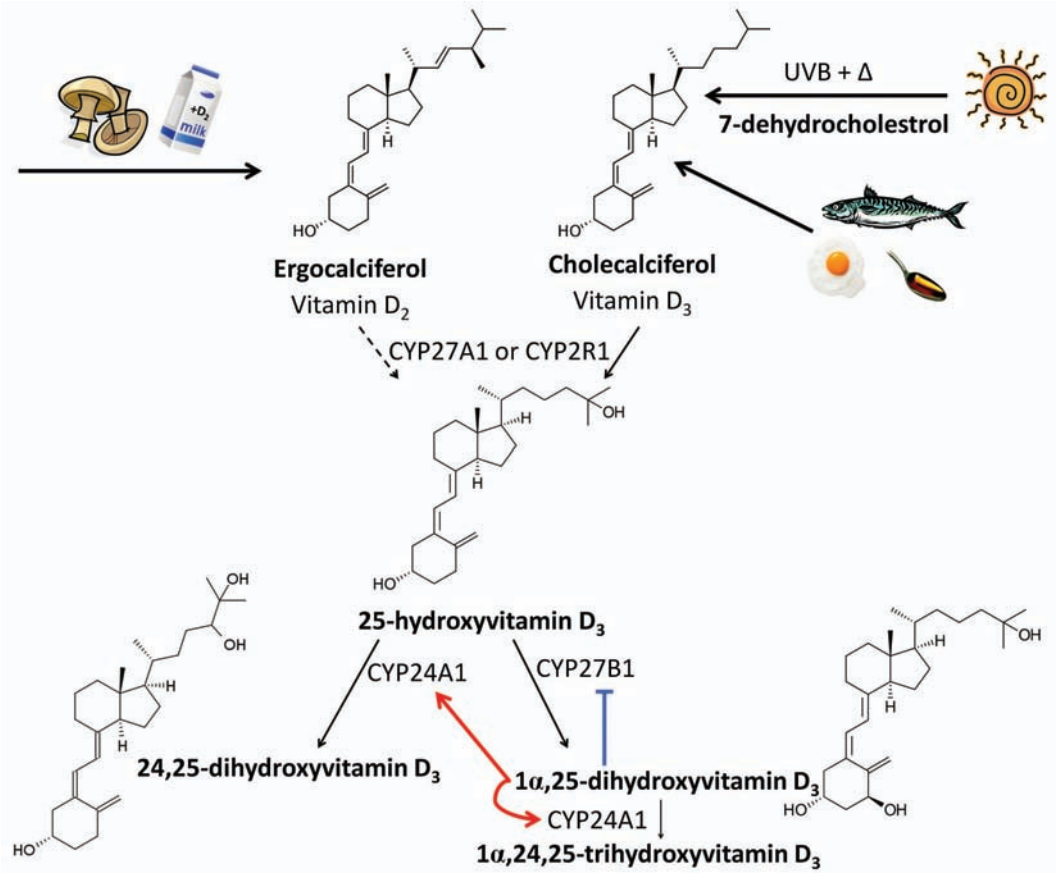

Fig. (1). Vitamin D metabolism pathway. The primary form of vitamin D in humans, D_3, is obtained by ultra violet B irradiation and photolytic conversion of 7-dehydrocholesterol stored in the epidermis or directly from animal products such as oily fish and egg yolk. Vitamin D_2 is obtained nutritionally from fungus or dairy products supplemented with D_2. Cytochrome p450 (CYP) enzymes are responsible for vitamin D metabolism, CYP27A1 and CYP2R1 are the primary 25-hydroxylases found in the liver. 25-hydroxyvitamin D (25(OH)D) is the major circulatory form of vitamin D and is 1 alpha-hydroxylated to produce the primary active metabolite 1α,25-dihydroxyvitamin D ($1,25(OH)_2D$) by CYP27B1, either in the kidney or in a tissue-specific manner. $1,25(OH)_2D$ controls its own levels by inhibiting CYP27B1 and inducing its own catabolising enzyme CYP24A1, which can also 24-hydroxylate 25(OH)D into 24,25-dihydroxyvitamin D.

obtained from plant sources, primarily fungus and yeast, and from some nutritional supplements. Some countries also fortify dairy products, margarine and other foodstuffs with vitamin D to prevent profound vitamin D deficiency arising as a consequence of limited sunlight exposure and poor dietary vitamin D intake.

The sum of $25(OH)D_2$ and $25(OH)D_3$ concentrations in serum is used as a measure of vitamin D status; the half-life of 25(OH)D is 15-20 days [17, 18] compared to that of $1,25(OH)_2D$, which is 4-8 hours [19]. A serum concentration of total 25(OH)D <25 nmol/L was the traditional clinical cut-off for deficiency due to its association with susceptibility to rickets. However, recent studies suggest that circulating 25(OH)D concentrations lower than 75-80 nmol/L may be insufficient for optimal physiological function. Recent studies and meta-analyses have identified significant associations between lower serum 25(OH)D concentrations and impaired lung function [20], decreased survival from lung cancer [21, 22], and increased rates of severe asthma exacerbation [23], tuberculosis (TB) [24], acute respiratory tract infections [25] and chronic obstructive pulmonary disease COPD [26]. Consequently, new supplementation guidelines released by the Institute of Medicine (IOM) in November 2010 defined serum 25(OH)D concentrations <50 nmol/L as deficient and increased the recommended daily intake (RDI) from 400 IU to 600 IU [15]. However, many scientists within the field believe that concentrations of 50-75 nmol/L are insufficient for optimal nonskeletal health benefits of vitamin D [27]. Therefore, in opposition to the IOM report, the Endocrine Society released its own guidelines in July 2011 recommending an RDI of at least 600 IU is needed to prevent deficiency but 1500–2000 IU/day is needed to elevate serum 25(OH)D above 75 nmol/L [16].

Both vitamin D_2 and vitamin D_3 may be administered to correct vitamin D deficiency. However, controversy exists over whether vitamin D_2 elicits the same physiological responses as vitamin D_3. Some investigators have reported that $25(OH)D_2$ has a shorter half-life than $25(OH)D_3$ and have questioned whether vitamin D_2 is an appropriate supplement to correct vitamin D deficiency [28]. However, others have reported that vitamin D_2 and vitamin D_3 can induce equivalent increases in serum 25(OH)D concentration [29]. Studies comparing the effect of $25(OH)D_2$ and $25(OH)D_3$ on immune cell function are needed to determine

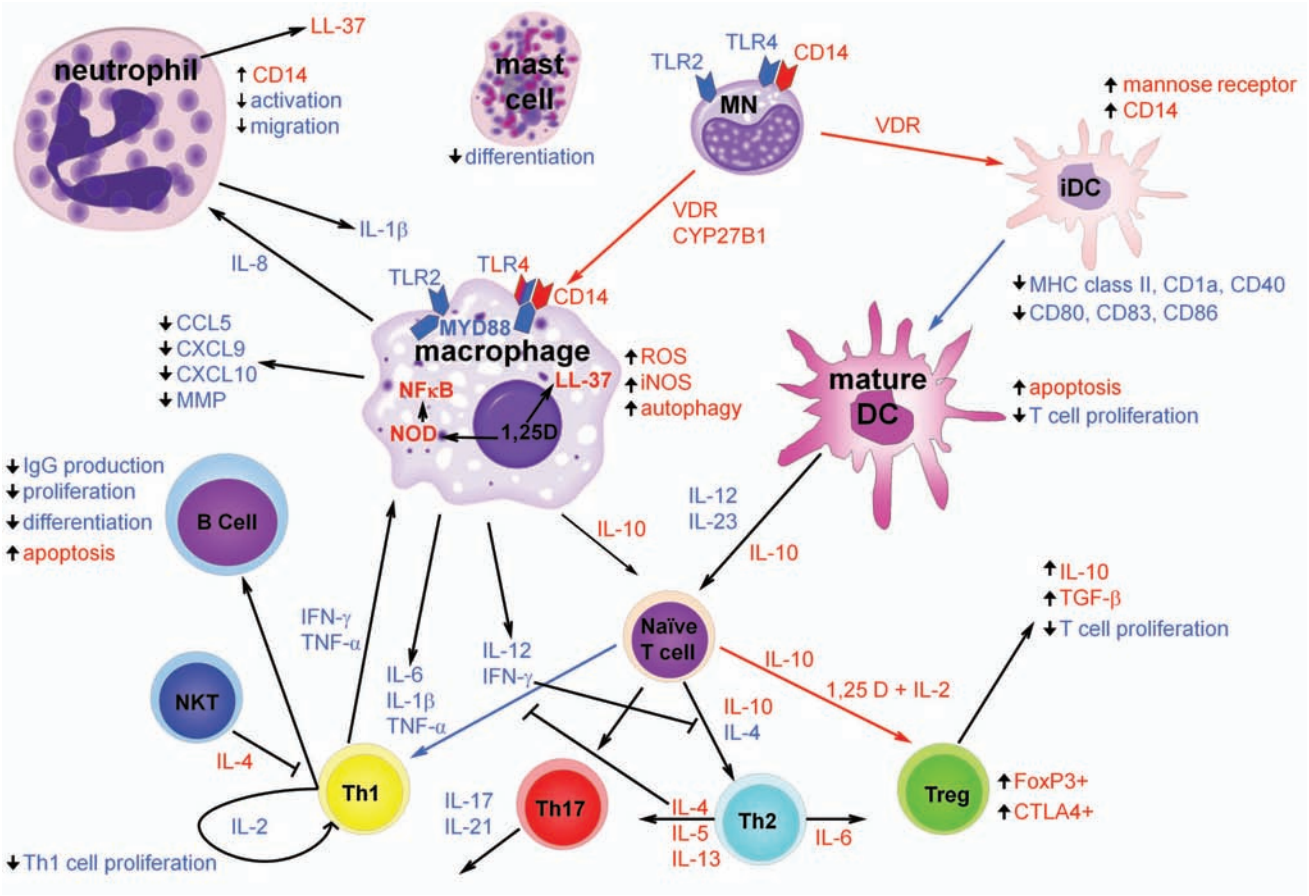

Fig. (2). Vitamin D has a wide range of actions on all immune cells. Vitamin D can induce (red) monocyte (MN) differentiation into macrophages and immature dendritic cells (iDC). Vitamin D inhibits (blue) iDC maturation towards mature DC. Vitamin D also inhibits type 1 cytokine production from mature DC and enhances the anti-inflammatory cytokines IL-10 which primes naïve T cells to differentiate into Th2 cells, producing type 2 cytokines and along with IL-2 vitamin D (1,25D) can induce regulatory T (Treg) cell development. Vitamin D also inhibits TLR2 and MYD88 (the toll-like receptor (TLR) adapter molecule), but upregulates CD14 and in some cases TLR4. Vitamin D also upregulates the antimicrobial peptide cathelicidin (LL-37) which has direct antimicrobial properties and induces autophagy. The intracellular pattern recognition receptor NOD is also upregulated by vitamin D and this induces NFκB. However, vitamin D also inhibits pro-inflammatory cytokine production by macrophages and Th1 cells and the interferon-gamma (IFN-γ) inducible chemokines CXCL9, CXCL10 and CCL5 and matrix metalloproteinases (MMP). Less information is known regarding its effects on B cells, mast cells and neutrophils, but vitamin D has a general inhibitory effect on either their differentiation, activation, proliferation or antibody (IgG) production. In general, vitamin D enhances direct anti-microbial properties of antigen presenting cells, it inhibits pro-inflammatory cytokine and promotes a type 2 inflammatory response.

whether or not these metabolites possess equivalent immunomodulatory activity.

Environmental and dietary intakes of vitamin D are not the only determinant of vitamin D status. Circulating levels of 25(OH)D also depend on levels of vitamin D binding protein (DBP), and on the activity of CYP450 enzymes which regulate vitamin D metabolism and catabolism. Moreover, genetic variation in the genes encoding these proteins also influences serum 25(OH)D concentrations [30]. DBP (also known as group-specific component, GC) contains 3 major haplotypes, due to two polymorphisms: GC-1F, GC-1S (D416E amino acid change) and GC-2 (T420K amino acid change). The GC-1F gene product has the highest affinity for 25(OH)D, while the GC-1S gene product has 2-fold lower affinity and the GC-2 gene product has 4-fold lower affinity [31]. Consequently, individuals possessing the GC-2 allele have lower circulating levels of 25(OH)D than non-carriers [32, 33]. Gujarati Asians who carry the GC-2 allele and who are vitamin D deficient have greater susceptibility to active TB, raising the possibility that low 25(OH)D levels and impaired ability to traffic 25(OH)D to the site of disease may have a combined detrimental effect on antimycobacterial immunity [34]. Conversely, GC-1F has been identified as a risk factor for COPD in a Chinese Han population, and carriage of the GC-2 allele has been suggested to have a protective effect on pathogenesis of COPD [35]. CYP24A1 and CYP27B1 polymorphisms are associated with circulating 25(OH)D levels [36] and CYP24B1 polymorphisms are associated with increased risk of asthma [33].

An effective response to vitamin D is dependent on a functional vitamin D receptor (VDR). There are a number of single nucleotide polymorphisms (SNPs) in the VDR which affect its function and abundance, including a start codon

SNP identified by *FokI* restriction enzyme digestion and multiple SNPs in the 3' untranslated region (UTR) identified by digestion with *ApaI*, *BsmI* and *TaqI* restriction enzymes. *TaqI TT* and *FokI FF* genotypes have been associated with high VDR mRNA levels, and the *TT* genotype of the *TaqI* polymorphism has been associated with high VDR protein levels [37]. Six CpG islands (associated with DNA methylation) have recently been identified in VDR; the promoter region encompassing the above described SNPs falls within the CpG island with the highest variability in methylation status [38]. Furthermore, the *TaqI* genotype showed an interaction with regional methylation status, ethnicity and disease susceptibility, indicating that epigenetic factors may influence relationships between VDR genotype and susceptibility to disease. In the context of respiratory disease, VDR polymorphisms have been associated with susceptibility to asthma [39], lung cancer [40], acute lower respiratory tract infection [41], COPD [42, 43] and TB [38, 44-46]. VDR polymorphisms have also been associated with enhanced response to antimicrobial therapy for active TB; however, the two studies to present such data had conflicting observations. One found that patients with *FokI ff* and *TaqI Tt* genotypes had faster time to sputum culture conversion [47], while the other found that patients with *ApaI AA* and *TaqI TT* genotypes had faster smear conversion [48]. These studies used different measures of treatment outcome and were performed in small populations without measuring baseline serum 25(OH)D concentrations. Recently a clinical trial has reported that high dose adjunctive vitamin D therapy accelerates sputum culture conversion in TB patients with the *tt* genotype of the *TaqI* VDR polymorphism, but not in those with *TT* or *Tt* genotypes [11]. More studies in larger population groups from various ethnogeographic backgrounds are needed to confirm these results.

TISSUE-SPECIFIC VITAMIN D UPTAKE AND METABOLISM

The greatest leap forward in elucidating non-classical roles of vitamin D occurred in 1981 with the discovery of extra-renal 1α-hydroxylation of 25(OH)D [2]. Shortly afterwards it was reported that $1,25(OH)_2D_3$ induces differentiation of monocytes into macrophage-like cells [49] and that both these cell types express VDR [50]. In 1983, it was shown that macrophages isolated from sarcoid granulomas could convert $25(OH)D_3$ into $1,25(OH)_2D_3$; they lacked 24-hydroxylase activity however [51, 52]. These observations also provided an explanation for the high serum $1,25(OH)_2D$ levels and low serum 25(OH)D levels that are sometimes seen in chronic granulomatous diseases such as sarcoidosis, TB and lymphoma [53]. Interferon-gamma (IFN-γ) was also found to enhance macrophage levels of $1,25(OH)_2D_3$ by antagonising the ability of $1,25(OH)_2D_3$ to induce CYP24A1 and suppress CYP27B1 [54, 55]. In 2006 it was discovered that stimulation of the macrophage PRR Toll-like receptor 2 (TLR2) with *M. tuberculosis* (MTB)-derived TLR2/1 ligand induces intracellular expression of CYP27B1 and VDR to enhance action of endogenously synthesised $1,25(OH)_2D_3$ [4]. Treatment of TLR2/1 stimulated macrophages with $25(OH)D_3$ also led to upregulation of CYP24A1, suggesting the presence of its inducer, $1,25(OH)_2D_3$, and thus intracellular conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$. Intracellular $1,25(OH)_2D_3$ production has subsequently been confirmed in monocyte-derived macrophages (MDM) and immature DC (iDC) using radio-labelled $25(OH)D_3$ [56].

Since the discovery that MN and MDM express a repertoire of vitamin D-regulated genes, further studies have made similar observations in a range of other cell types. Activated T and B cells, DC and lung epithelial cells all express VDR, CYP27B1 and CYP24A1, indicating that they possess the machinery to utilise and generate $1,25(OH)_2D_3$ from $25(OH)D_3$ [57-60]. Mast cells have also been shown to express VDR and respond to $1,25(OH)_2D_3$, but not 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) [61]. Furthermore, MDM have been shown to have high expression of the 25-hydroxylase enzyme CYP27A1, whereas MN and DC have only low expression. Accordingly, MDM were also found to be able to convert vitamin D_3 to $1,25(OH)_2D_3$ with greater efficiency than DC [62]. It could be argued that lymphocytes activated *in vitro* do not represent the same population of cells which infiltrate the lung during pathological processes. However, the fact that they express both VDR and the enzymatic machinery capable of producing intracellular $1,25(OH)_2D_3$ indicates that they have the capacity to utilise circulating 25(OH)D when stimulated to do so and to respond to locally produced $1,25(OH)_2D_3$. *In vivo* work is needed to characterise the response of these cells to vitamin D supplementation in patients with respiratory disease.

In order to produce intracellular $1,25(OH)_2D_3$ cells also need a mechanism to internalise its precursor compounds. Megalin and cublin, two proteins involved in active transport of the 25(OH)D-DBP complex in the renal proximal tubule have also been shown to be expressed by non-renal cells, including lung epithelium [63]. Interestingly, macrophages do not express these proteins but can still obtain and utilise $25(OH)D_3$. *In vitro*, human MN cultured in serum from individuals carrying the DBP GC-2 allele have an enhanced transcriptional response to $25(OH)D_3$ treatment [64]. It has been suggested that this is due to less $25(OH)D_3$ being bound to DBP, making more free $25(OH)D_3$ available for cellular uptake *via* diffusion. Similar experiments using serum from DBP knockout (KO) mice during *in vitro* culture of mouse osteoblastic cells showed that co-culture with DBP KO serum resulted in greater sensitivity to $1,25(OH)_2D_3$ treatment [65]. Moreover, DBP KO mice had higher tissue concentrations of $1,25(OH)_2D_3$ than wild-type mice despite having lower circulating concentrations of $25(OH)D_3$. Thus, it appears that the concentration of 'free' circulating 25(OH)D may be a more important determinant of cellular response to vitamin D than the total circulating 25(OH)D concentration. This genotypic effect may therefore be an important point to take into consideration when prescribing vitamin D therapy.

Apart from transporting 25(OH)D, DBP also plays a role in scavenging extracellular globular(G)-actin [66], activation of macrophages [67] and enhancement of the chemotactic activity of C5a for neutrophils and monocytes [68, 69]. G-actin is released by necrotic cells and is thus a major by-product of inflammation; DBP binding facilitates clearance of G-actin, preventing harmful effects of filamentous (F)-actin formation during inflammation [70]. Furthermore, F-actin can bind the antimicrobial peptide cathelicidin, inhibiting its function. Dissociation of these F-actin bundles

restores cathelicidin's antimicrobial activity [71]. DBP variants with different binding affinities may therefore affect the role of DBP in resolution of inflammation [72] and indirectly control antimicrobial peptide activity.

The peptide encoded by the GC-2 allele is also the only DBP variant which cannot be converted to the potent macrophage activation factor GC-MAF, as it lacks a threonine residue that is targeted for deglycosylation by T- and B-cell glycosidases during formation of the sialic acid-free variant of DBP [73]. Thus, the lower binding capacity for $25(OH)D_3$ and the inability to form GC-MAF makes carriage of the GC-2 variant potentially detrimental for a vitamin D deficient individual. This observation is consistent with the finding that vitamin D deficient carriers of the GC-2 allele are more susceptible to TB [34]. Polymorphisms which affect the binding efficiency of DBP may therefore associate with variation in responses to vitamin D deficiency.

VDR-LIGAND INTERACTION AND RECEPTOR SIGNALLING

The VDR can function at both the cell membrane and as a nuclear receptor. Formation of an active receptor in the nucleus requires 1:1 heterodimerisation with the vitamin A receptor, retinoid X receptor (RXR). Binding of $1,25(OH)_2D$ to the nuclear receptor complex results in nuclear translocation and a conformational change which exposes the VDR DNA binding site and thus activates the transcription factor complex. This complex binds repeats of PuG(G/T)TCA, known as vitamin D response elements (VDREs), in the promoter of more than 900 genes to regulate their expression [1].

$1,25(OH)_2D_3$ is the most potent ligand for this receptor complex as it has the strongest affinity for the VDR-RXR ligand binding domain of all natural vitamin D metabolites (K_d-value 0.1nM - 50x stronger than for $25(OH)D_3$) [74, 75]. $1,25(OH)_2D_3$ is therefore recognised as the primary active metabolite of vitamin D_3. However, circulating $25(OH)D_3$ and its 24-hydroxyled catabolite, $24,25(OH)_2D_3$, can also activate VDR ligand-dependant transcription [76, 77], although higher concentrations are required to achieve the same effect. Moreover, the natural 26-hydroxylated forms of $25(OH)D_3$ and $1,25(OH)_2D_3$ (25,26-dihydroxyvitamin D_3 and 1,25,26-trihydroxyvitamin D_3, respectively) induce similar restriction of *M. tuberculosis* growth in MDM as $1,25(OH)_2D_3$ when they are used at the supra-physiological concentration of 10 μmol/L [78]. However, $1,25(OH)_2D_3$ can achieve the same degree of restriction as $25(OH)D_3$ at much lower concentrations (1 nmol/L), while $1,24,25(OH)_3D_3$ can achieve this at 100 nmol/L [79]. Numerous synthetic analogues of vitamin D that can transactivate VDR without inducing hypercalcemia (a detrimental effect of excessive vitamin D supplementation) have also been produced [80]. 22-oxa-1,25-dihydroxyvitamin D_3 is one such analogue that has been reported to have the same potential as $1,25(OH)_2D_3$ to inhibit functional differentiation of DC, cytotoxic T cells and helper T cells [81].

Vitamin D metabolites are not the sole activators of VDR-RXR. Retinoic acid (RA) in combination with $1,25(OH)_2D_3$ (both at 1 μmol/L) has been shown to down-regulate expression of coronin-1 (also known as Tryptophan Aspartate containing Coat protein, TACO), an endogenous macrophage actin-binding protein that inhibits phagosome maturation during infection [82]. Pre-treatment with RA/$1,25(OH)_2D_3$ also inhibited macrophage phagocytosis of MTB [83]. Taken together, this suggests that maintaining physiological levels of both vitamin D and vitamin A may be important for correct functioning of the VDR-RXR complex.

Immunomodulatory actions of $1,25(OH)_2D_3$ may also be mediated by activation of membrane VDR and phosphatidylinositol 3-kinase (PI3K) signal induction. PI3K signalling has been linked to induction of monocyte-macrophage differentiation [84], generation of reactive oxygen species (ROS) [85], induction of nitric oxide (NO) [86] and phagosome/lysosome fusion during MTB-MDM infection [87]. However, experiments using selective agonists and antagonists of membrane and nuclear VDR indicate that $1,25(OH)_2D_3$–induced restriction of mycobacterial growth during *in vitro* peripheral blood mononuclear cell (PMBC) culture was predominantly due to nuclear VDR signalling [88]. Moreover, the capacity for all immune cells to generate intracellular $1,25(OH)_2D$ suggests that the main action of vitamin D during an immune response is *via* nuclear VDR. This is further supported by recent work showing that T cell receptor signalling in naïve T cells induces nuclear VDR expression and VDR-mediated transcription of phospholipase C-γ1 (PLC-γ1), which controls the Ras-mitogen-activated protein kinase (MAPK) cascade during T cell activation [89].

INDUCTION OF ANTI-MICROBIAL RESPONSES BY VITAMIN D

The ability to mount an immune response to microbial infection is reliant on the expression of PRR by myeloid cells. $1,25(OH)_2D_3$ stimulates the expression of CD14 (which forms a functional heterodimer with TLR4) in MN and MDM, but it inhibits TLR4 and TLR2 expression during $1,25(OH)_2D_3$-induced MDM differentiation [90]. In the HL-60 macrophage cell line, however, $1,25(OH)_2D_3$ does not inhibit TLR4 expression [91]. These results indicate that the effect of $1,25(OH)_2D_3$ on TLR4 expression depends on macrophage phenotype, raising the possibility that, *in vivo*, macrophages in different tissues may have different responses to $1,25(OH)_2D_3$. In the context of lung disease, more work therefore needs to be performed using alveolar macrophages (AM) as well as MDM.

Along with experiments showing that $25(OH)D_3$ treatment of TLR2/1-stimulated MDM induces $1,25(OH)_2D_3$ production, Liu and colleagues have also reported that $25(OH)D_3$ treatment induces expression of the gene which encodes the antimicrobial peptide cathelicidin (*hCAP18*) which is proteolytically cleaved to produce the active antimicrobial peptide LL-37 [92]. Co-treatment with itraconazole, a non-specific CYP450 enzyme antagonist, inhibited cathelicidin expression in these experiments, suggesting that $25(OH)D_3$ metabolism was required for *hCAP18* induction. It was subsequently reported that *in vitro* treatment of MTB-infected PBMC with $1,25(OH)_2D_3$ induces *hCAP18* mRNA expression and that LL-37 restricts growth of MTB in liquid broth culture [88]. Further studies have reported that epithelial cells in the upper and lower respiratory tract also secrete cathelicidin and that viruses can also induce cathelicidin expression when sufficient 25(OH)D is available [93, 94]. Furthermore, neutrophils, T cells, B cell, NK cells, DC and mast

cells also express *hCAP18* [95, 96], suggesting that $1,25(OH)_2D_3$ could induce cathelicidin expression in diverse cell populations that infiltrate the lung in response to a range of bacterial and viral infections. The finding that MTB induces greater cathelicidin expression in AM isolated from healthy controls, than in MDM differentiated *in vitro*, suggests that models using MDM may be underestimating the *in vivo* cathelicidin-mediated immune response [97]. However, interestingly, granuloma tissue from TB patients show very weak LL-37 staining, whereas tissue sections from patients with acute pneumonia show intense LL-37 staining, particularly in activated macrophages [97]. The lack of LL-37 in TB patients may be due to the common occurrence of vitamin D deficiency in these patients and highlights one of the potential benefits of vitamin D supplementation in these patients. Although there has been a number of recent vitamin D supplementation trials in TB patients [10-11, 98], obtaining clinical samples from the lower airway of these patients is methodologically problematic. Furthermore, the presence of three VDREs in the cathelicidin gene promoter (consequently making it one of the most highly induced genes by vitamin D) appears to be a recent evolutionary addition to the innate immune response, which only exists in humans and non-human primates [99]. Thus, non-primate animal models are not an appropriate model in which to study the effect of vitamin D on LL-37 distribution *in vivo*, when the aim is to translate findings to humans.

The bactericidal activity of cathelicidin was first shown to be mediated by its ability to bind and disrupt bacterial cell wall phosphatidylglycerol monolayers [100]. However, recent work has shown that the cathelicidin peptide also induces 2 genes (*Beclin-1* and *Atg5*) involved in autophagy *via* activation of p38 MAPK and extracellular signal-regulated kinase (ERK) 1/2 signalling, but not *via* activation of nuclear factor (NF)-κB. Autophagy is an intracellular process inducing autophagosome/lysosome fusion and subsequent lysosome-mediated degradation of phagosome contents [101]. This finding is key to tuberculosis pathogenesis as MTB is known to subvert the immune response by inhibiting phagosome/lysosome fusion [102]. Thus, cathelicidin appears to have direct and indirect antimicrobial properties.

Since it was discovered that TLR2/1 stimulation induced VDR expression in MN, it has also been shown that $1,25(OH)_2D_3$ induces expression of another PRR, the intracellular nucleotide-binding oligomerization domain containing 2 (NOD2) receptor in MN and intestinal epithelial cells [103]. NOD2 signalling activates the NFκB transcription factor, which together with the VDR transcription factor complex induces expression of the antimicrobial peptide beta-defensin 2 (hBD-2 or DEFB4) by binding the one VDRE and two NFκB sites in its promoter. By contrast, the cathelicidin promotor has 3 VDREs but no NFκB sites [104]. The observation that cathelicidin activates MAPK and ERK signalling, but not NFκB, during $1,25(OH)_2D_3$-induced autophagy [101] indicates that $1,25(OH)_2D_3$ regulates the immune response *via* two separate mechanisms: one *via* NFκB signalling and one *via* cathelicidin-induced MAPK signalling (Fig. **2**).

VITAMIN D INHIBITS A TYPE 1 AND PROMOTES A TYPE 2 INFLAMMATORY RESPONSE

Paradoxically, despite its ability to induce NFκB *via* NOD2 activation [103], $1,25(OH)_2D_3$ inhibits NFκB-induced type-1 pro-inflammatory cytokines TNFα, IFN-γ, IL-12p40, IL-6, IL-8, IL-1β and IL-23 and induces the anti-inflammatory cytokine IL-10 in MN, MDM and DC [88, 105-107]. Pre-treatment of human MN with $1,25(OH)_2D_3$ induces maturation and augments TNFα production following stimulation with lipopolysaccharides (LPS, a TLR4 ligand found in the cell wall component of gram negative pulmonary pathogens such as *Hemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila* and *Pseudomonas aeruginosa*) [108]. However, combined pre-treatment of mouse bone marrow-derived macrophages (BMDM) with $1,25(OH)_2D_3$ and IFN-γ decreased the bactericidal activity of BMDM by inhibiting the phagocyte oxidase-mediated oxidative burst and suppressing IFN-γ induction of chemokines CCL5 (RANTES), CXCL10 (IP10) and CXCL9 (MIG) and PRRs TLR2 and Fcγ receptor 1 and 2 [109]. Thus, it is possible that $1,25(OH)_2D_3$ controls a negative feedback loop, limiting the inflammatory immune response at the same time as inducing a direct anti-microbial response.

T cells also have the capacity to generate endogenous $1,25(OH)_2D_3$, and this has been shown to have direct effects on different T cell populations. $1,25(OH)_2D_3$ treatment of human $CD8^+$ and $CD4^+$ PBMC promotes a type 2 phenotype, reducing the number of IL-2 secreting T cells and completely blocking IL-12-induced IFN-γ production by CD4+ and CD8+ subsets [110] and IL-17 and IL-21 production by $CD4^+CD25^-$ T cells [111]. Conversely, $1,25(OH)_2D_3$ has also been shown to increase IL-4 and IL-13 positivity in $CD4^+$ and $CD8^+$ subsets, along with IL-6 positive $CD4^+$ T cells. In combination with IL-4, $1,25(OH)_2D_3$ enhances IL-6 induction in $CD4^+$ and $CD8^+$ cells [110]. Furthermore, in the presence of IL-2, $1,25(OH)_2D_3$ induces expression of FoxP3, the marker of adaptive regulatory T (Treg) cells that suppress T cell proliferation. This *in vitro* observation is validated by *in vivo* work showing that serum 25(OH)D levels are positively correlated with Treg function [112]. Moreover, IL-10 producing Tregs have been shown to be protective in the mouse asthma model [113], suggesting that vitamin D treatment of asthma patients may limit its severity. In contrast to its actions in activated CD4+ and CD8+ T cells, $1,25(OH)_2D_3$ has been reported to suppress IL-4 production by naïve CD62 $ligand^+$ $CD4^+$ T cells during their *in vitro* polarization. This effect was greatest if $1,25(OH)_2D_3$ was given before initiation of differentiation and it was significantly diminished if it was added after polarization [114]. Taken together, the studies above provide further evidence that the immunomodulatory effects of $1,25(OH)_2D_3$ are dependent on the activity and environment of its target cells at the time of exposure. The relevance of these *in vitro* findings to the clinical situation may also be limited, and while there are some *in vivo* findings in animal models, more findings from clinical trials are needed to characterise the effect of vitamin D supplementation on human T cell responses.

VITAMIN D REGULATES DC MATURATION AND DC-MEDIATED T-CELL ACTIVATION

DC play a fundamental role bridging innate and adaptive immunity, due their ability to present antigen and activate T helper cells. DC are hypothesized to be responsible both for

extra-pulmonary dissemination of MTB during chronic infection [115] and for delayed lymph-node trafficking of MTB, resulting in the delayed induction of the adaptive immune response following infection [116]. $1,25(OH)_2D_3$ inhibits the maturation of immature DC, blocking CD14 down-regulation and inhibiting cell surface expression of MHC-class II molecules, CD40, CD80, CD83 and CD86. Functionally, this results in inhibition of chemokine secretion, inhibition of Th1 and Th17 cytokine production and induction of mature DC apoptosis [105, 106, 117]. Consequently, $1,25(OH)_2D_3$-treated DC have a reduced capacity to activate Th1 T cell proliferation and to induce CD4+ cells to secrete IFN-γ [106, 118]. Moreover, $1,25(OH)_2D_3$ switches DC phenotype towards production of type 2 cytokines, with upregulation of IL-10 and promotion of differentiation of naïve T cells into Tregs, independently of its effect on DC maturation [119]. This effect of $1,25(OH)_2D_3$ on DC-mediated Treg induction has been reported in a mouse model of asthma: pre-treatment of DC with $1,25(OH)_2D_3$ and dexamethasone induced IL-10 production, generated DC which stimulated CD4+ naïve T cells to differentiate into Tregs, and ultimately resulted in reduced airway hyper-responsiveness [120].

VITAMIN D AND DESTRUCTION OF PULMONARY EXTRACELLULAR MATRIX

Matrix metalloproteinases (MMP) are a family of enzymes capable of degrading all components of pulmonary extracellular matrix. They are generally upregulated in activated cells where their primary role is to facilitate tissue remodelling and repair. MMP also regulate the innate immune response by controlling cytokine and chemokine processing, apoptosis and antimicrobial peptide cleavage and activation [121]. However, excess MMP activity can lead to excess matrix degradation and ultimately lung tissue destruction in chronic inflammatory conditions. MN, MDM and PBMCs infected with MTB induce expression and secretion of MMP-1, MMP-7 and MMP-10 [122, 123]. Increased expression of *MMP-1, MMP-7* and *MMP-9* has been demonstrated in cells isolated from the lungs of TB patients, with MMP-1 and MMP-7 co-localising to macrophages around the central area of necrosis in tuberculous granulomata [123, 124]. Circulating concentrations of MMP-9 have also been shown to correlate with the severity of pulmonary TB [125]; in a zebra fish model of TB, disruption of MMP-9 function attenuates granuloma formation and bacterial growth [126]. TNFα up-regulates MMP-9 expression and activity in alveolar macrophages from COPD patients, and elevated MMP-9 levels have been detected in the sputum of COPD patients [127, 128]. Airway smooth muscle cells from asthma patients have increased MMP-9 expression [39], raising the possibility that chronic asthma-associated lung destruction is also linked to MMP-9.

$1,25(OH)_2D_3$ has also been reported to inhibit the expression, secretion and activity of a number of MMP during *in vitro* MTB infection [122, 129]. There is limited *in vivo* data, however, to support a role for vitamin D in limiting lung destruction through inhibiting MMP. However, inhibition of MMP activity has the potential to limit AMP activity. Thus, the net effect of vitamin D supplementation on MMP function, if any, needs to be investigated in clinical trials.

CONCLUSION

There is increasing evidence that $1,25(OH)_2D_3$ and other vitamin D metabolites regulate innate and adaptive immune responses to pulmonary pathogens, and elicit direct antimicrobial activity in cells which populate the lung. Vitamin D metabolites also inhibit pro-inflammatory cytokine production, enhance Treg proliferation, induce type 2 cytokine production and inhibit MMP expression and secretion. Taken together, these observations indicate that vitamin D has the potential to boost innate antimicrobial responses and limit adaptive inflammatory responses to prevent the excessive tissue destruction which often accompanies chronic lung disease. *In vitro* observations now need to be taken forward into clinical trials to characterise the immunomodulatory actions of vitamin D supplementation in patients with infectious and immune-mediated lung disease.

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REFERENCES

[1] Wang TT, Tavera-Mendoza LE, Laperriere D, *et al.* Large-scale in silico and microarray-based identification of direct 1,25-dihydroxyvitamin D3 target genes. Mol Endocrinol 2005; 19(11): 2685-95.

[2] Barbour GL, Coburn JW, Slatopolsky E, Norman AW, Horst RL. Hypercalcemia in an anephric patient with sarcoidosis: evidence for extrarenal generation of 1,25-dihydroxyvitamin D. N Engl J Med 1981; 305(8): 440-3.

[3] Schauber J, Dorschner RA, Coda AB, *et al.* Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism. J Clin Invest 2007; 117(3): 803-11.

[4] Liu PT, Stenger S, Li H, *et al.* Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006; 311(5768): 1770-3.

[5] Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr 2007; 85(6): 1586-91.

[6] Kimball SM, Ursell MR, O'Connor P, Vieth R. Safety of vitamin D3 in adults with multiple sclerosis. Am J Clin Nutr 2007; 86(3): 645-51.

[7] Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am J Clin Nutr 2010; 91(5): 1255-60.

[8] Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr 2006; 83(4): 754-9.

[9] Avenell A, Cook JA, Maclennan GS, Macpherson GC. Vitamin D supplementation to prevent infections: a sub-study of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438). Age Ageing 2007.

[10] Wejse C, Gomes VF, Rabna P, *et al.* Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. Am J Respir Crit Care Med 2009; 179(9): 843-50.

[11] Martineau AR, Timms PM, Bothamley GH, *et al.* High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet 2011; 377(9761): 242-50.

[12] Wicklow BA, Taback SP. Feasibility of a type 1 diabetes primary prevention trial using 2000 IU vitamin D3 in infants from the

general population with increased HLA-associated risk. Ann N Y Acad Sci 2006; 1079: 310-2.
[13] 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(6): 626-32.
[14] Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357(3): 266-81.
[15] Ross AC, Manson JE, Abrams SA, *et al.* The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011; 96(1): 53-8.
[16] Holick MF, Binkley NC, Bischoff-Ferrari HA, *et al*. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96(7): 1911-30.
[17] Smith JE, Goodman DS. The turnover and transport of vitamin D and of a polar metabolite with the properties of 25-hydroxycholecalciferol in human plasma. J Clin Invest 1971; 50(10): 2159-67.
[18] Jones G. Pharmacokinetics of vitamin D toxicity. Am J Clin Nutr 2008; 88(2): 582S-6S.
[19] Levine BS, Singer FR, Bryce GF, Mallon JP, Miller ON, Coburn JW. Pharmacokinetics and biologic effects of calcitriol in normal humans. J Lab Clin Med 1985; 105(2): 239-46.
[20] Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin D and pulmonary function in the third national health and nutrition examination survey. Chest 2005; 128(6): 3792-8.
[21] Porojnicu AC, Robsahm TE, Dahlback A, *et al.* Seasonal and geographical variations in lung cancer prognosis in Norway. Does Vitamin D from the sun play a role? Lung Cancer 2007; 55(3): 263-70.
[22] Pilz S, Tomaschitz A, Obermayer-Pietsch B, Dobnig H, Pieber TR. Epidemiology of vitamin D insufficiency and cancer mortality. Anticancer Res 2009; 29(9): 3699-704.
[23] Brehm JM, Schuemann B, Fuhlbrigge AL, *et al.* Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. J Allergy Clin Immunol 2010.
[24] Nnoaham KE, Clarke A. Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. Int J Epidemiol 2008; 37(1): 113-9.
[25] Laaksi I, Ruohola JP, Tuohimaa P, *et al*. An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. Am J Clin Nutr 2007; 86(3): 714-7.
[26] Hughes DA, Norton R. Vitamin D and respiratory health. Clin Exp Immunol 2009; 158(1): 20-5.
[27] Zittermann A. Vitamin D in preventive medicine: are we ignoring the evidence? Br J Nutr 2003; 89(5): 552-72.
[28] Houghton LA, Vieth R. The case against ergocalciferol (vitamin D2) as a vitamin supplement. Am J Clin Nutr 2006; 84(4): 694-7.
[29] Holick MF, Biancuzzo RM, Chen TC, *et al.* Vitamin D2 Is as Effective as Vitamin D3 in Maintaining Circulating Concentrations of 25-Hydroxyvitamin D. J Clin Endocrinol Metab 2008; 93(3): 677-81.
[30] Wang TJ, Zhang F, Richards JB, *et al.* Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 2010; 376(9736): 180-8.
[31] Arnaud J, Constans J. Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP). Hum Genet 1993; 92(2): 183-8.
[32] Lauridsen AL, Vestergaard P, Hermann AP, *et al*. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. Calcif Tissue Int 2005; 77(1): 15-22.
[33] Wjst M, Altmuller J, Faus-Kessler T, Braig C, Bahnweg M, Andre E. Asthma families show transmission disequilibrium of gene variants in the vitamin D metabolism and signalling pathway. Respir Res 2006; 7: 60.
[34] Martineau AR, Leandro AC, Anderson ST, *et al.* Association between Gc genotype and susceptibility to TB is dependent on vitamin D status. Eur Respir J 2010; 35(5): 1106-12.
[35] Lu M, Yang B, Cai YY. [The relationship between vitamin D binding protein gene polymorphism and chronic obstructive pulmonary disease]. Zhonghua Nei Ke Za Zhi 2004; 43(2): 117-20.
[36] Orton SM, Morris AP, Herrera BM, *et al.* Evidence for genetic regulation of vitamin D status in twins with multiple sclerosis. Am J Clin Nutr 2008; 88(2): 441-7.
[37] Ogunkolade BW, Boucher BJ, Prahl JM, *et al*. Vitamin D receptor (VDR) mRNA and VDR protein levels in relation to vitamin D status, insulin secretory capacity, and VDR genotype in Bangladeshi Asians. Diabetes 2002; 51(7): 2294-300.
[38] Andraos C, Koorsen G, Knight JC, Bornman L. Vitamin D receptor gene methylation is associated with ethnicity, tuberculosis, and TaqI polymorphism. Hum Immunol 2011; 72(3): 262-8.
[39] Song Y, Qi H, Wu C. Effect of 1,25-(OH)2D3 (a vitamin D analogue) on passively sensitized human airway smooth muscle cells. Respirology 2007; 12(4): 486-94.
[40] Dogan I, Onen HI, Yurdakul AS, *et al.* Polymorphisms in the vitamin D receptor gene and risk of lung cancer. Med Sci Monit 2009; 15(8): BR232-42.
[41] Roth DE, Jones AB, Prosser C, Robinson JL, Vohra S. Vitamin D receptor polymorphisms and the risk of acute lower respiratory tract infection in early childhood. J Infect Dis 2008; 197(5): 676-80.
[42] Hopkinson NS, Li KW, Kehoe A, *et al.* Vitamin D receptor genotypes influence quadriceps strength in chronic obstructive pulmonary disease. Am J Clin Nutr 2008; 87(2): 385-90.
[43] Ito I, Nagai S, Hoshino Y, *et al.* Risk and severity of COPD is associated with the group-specific component of serum globulin 1F allele. Chest 2004; 125(1): 63-70.
[44] Wilkinson RJ, Llewelyn M, Toossi Z, *et al.* Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. Lancet 2000; 355(9204): 618-21.
[45] Liu W, Cao WC, Zhang CY, *et al*. VDR and NRAMP1 gene polymorphisms in susceptibility to pulmonary tuberculosis among the Chinese Han population: a case-control study. Int J Tuberc Lung Dis 2004; 8(4): 428-34.
[46] Selvaraj P, Chandra G, Jawahar MS, Rani MV, Rajeshwari DN, Narayanan PR. Regulatory role of vitamin D receptor gene variants of Bsm I, Apa I, Taq I, and Fok I polymorphisms on macrophage phagocytosis and lymphoproliferative response to mycobacterium tuberculosis antigen in pulmonary tuberculosis. J Clin Immunol 2004; 24(5): 523-32.
[47] Roth DE, Soto G, Arenas F, *et al.* Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. J Infect Dis 2004; 190(5): 920-7.
[48] Babb C, van der Merwe L, Beyers N, *et al.* Vitamin D receptor gene polymorphisms and sputum conversion time in pulmonary tuberculosis patients. Tuberculosis (Edinb) 2007; 87(4): 295-302.
[49] Abe E, Miyaura C, Sakagami H, *et al.* Differentiation of mouse myeloid leukemia cells induced by 1 alpha,25-dihydroxyvitamin D3. Proc Natl Acad Sci USA 1981; 78(8): 4990-4.
[50] Mangelsdorf DJ, Koeffler HP, Donaldson CA, Pike JW, Haussler MR. 1,25-Dihydroxyvitamin D3-induced differentiation in a human promyelocytic leukemia cell line (HL-60): receptor-mediated maturation to macrophage-like cells. J Cell Biol 1984; 98(2): 391-8.
[51] Adams JS, Gacad MA. Characterization of 1 alpha-hydroxylation of vitamin D3 sterols by cultured alveolar macrophages from patients with sarcoidosis. J Exp Med 1985; 161(4): 755-65.
[52] Adams JS, Sharma OP, Gacad MA, Singer FR. Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis. J Clin Invest 1983; 72(5): 1856-60.
[53] Fuss M, Pepersack T, Gillet C, Karmali R, Corvilain J. Calcium and vitamin D metabolism in granulomatous diseases. Clin Rheumatol 1992; 11(1): 28-36.
[54] Dusso AS, Kamimura S, Gallieni M, *et al*. gamma-Interferon-induced resistance to 1,25-(OH)2 D3 in human monocytes and macrophages: a mechanism for the hypercalcemia of various granulomatoses. J Clin Endocrinol Metab 1997; 82(7): 2222-32.
[55] Vidal M, Ramana CV, Dusso AS. Stat1-vitamin D receptor interactions antagonize 1,25-dihydroxyvitamin D transcriptional activity and enhance stat1-mediated transcription. Mol Cell Biol 2002; 22(8): 2777-87.
[56] Krutzik SR, Hewison M, Liu PT, *et al*. IL-15 links TLR2/1-induced macrophage differentiation to the vitamin D-dependent antimicrobial pathway. J Immunol 2008; 181(10): 7115-20.

[57] Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. J Immunol 2007; 179(3): 1634-47.

[58] Sigmundsdottir H, Pan J, Debes GF, *et al.* DCs metabolize sunlight-induced vitamin D3 to 'program' T cell attraction to the epidermal chemokine CCL27. Nat Immunol 2007; 8(3): 285-93.

[59] Fritsche J, Mondal K, Ehrnsperger A, Andreesen R, Kreutz M. Regulation of 25-hydroxyvitamin D3-1 alpha-hydroxylase and production of 1 alpha,25-dihydroxyvitamin D3 by human dendritic cells. Blood 2003; 102(9): 3314-6.

[60] Ramirez AM, Wongtrakool C, Welch T, Steinmeyer A, Zugel U, Roman J. Vitamin D inhibition of pro-fibrotic effects of transforming growth factor beta1 in lung fibroblasts and epithelial cells. J Steroid Biochem Mol Biol 2010; 118(3): 142-50.

[61] Shalita-Chesner M, Koren R, Mekori YA, *et al.* 1,25-Dihydroxyvitamin D3 enhances degranulation of mast cells. Mol Cell Endocrinol 1998; 142(1-2): 49-55.

[62] Gottfried E, Rehli M, Hahn J, Holler E, Andreesen R, Kreutz M. Monocyte-derived cells express CYP27A1 and convert vitamin D3 into its active metabolite. Biochem Biophys Res Commun 2006; 349(1): 209-13.

[63] Lundgren S, Carling T, Hjalm G, *et al.* Tissue distribution of human gp330/megalin, a putative Ca(2+)-sensing protein. J Histochem Cytochem 1997; 45(3): 383-92.

[64] Chun RF, Lauridsen AL, Suon L, *et al.* Vitamin D-Binding Protein Directs Monocyte Responses to 25-Hydroxy- and 1,25-Dihydroxyvitamin D. J Clin Endocrinol Metab 2010; 95(7): 3368-76.

[65] Zella LA, Shevde NK, Hollis BW, Cooke NE, Pike JW. Vitamin D-binding protein influences total circulating levels of 1,25-dihydroxyvitamin D3 but does not directly modulate the bioactive levels of the hormone *in vivo*. Endocrinology 2008; 149(7): 3656-67.

[66] Van Baelen H, Bouillon R, De Moor P. Vitamin D-binding protein (Gc-globulin) binds actin. J Biol Chem 1980; 255(6): 2270-2.

[67] Yamamoto N, Homma S, Millman I. Identification of the serum factor required for *in vitro* activation of macrophages. Role of vitamin D3-binding protein (group specific component, Gc) in lysophospholipid activation of mouse peritoneal macrophages. J Immunol 1991; 147(1): 273-80.

[68] Kew RR, Webster RO. Gc-globulin (vitamin D-binding protein) enhances the neutrophil chemotactic activity of C5a and C5a des Arg. J Clin Invest 1988; 82(1): 364-9.

[69] Piquette CA, Robinson-Hill R, Webster RO. Human monocyte chemotaxis to complement-derived chemotaxins is enhanced by Gc-globulin. J Leukoc Biol 1994; 55(3): 349-54.

[70] Dahl B, Schiodt FV, Kiaer T, Ott P, Bondesen S, Tygstrup N. Serum Gc-globulin in the early course of multiple trauma. Crit Care Med 1998; 26(2): 285-9.

[71] Weiner DJ, Bucki R, Janmey PA. The antimicrobial activity of the cathelicidin LL37 is inhibited by F-actin bundles and restored by gelsolin. Am J Respir Cell Mol Biol 2003; 28(6): 738-45.

[72] Speeckaert M, Huang G, Delanghe JR, Taes YE. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin Chim Acta 2006; 372(1-2): 33-42.

[73] Kanan RM, Cook DB, Datta HK. Lectin immunoassay for macrophage-activating factor (Gc-MAF) produced by deglycosylation of Gc-globulin: evidence for noninducible generation of Gc-MAF. Clin Chem 2000; 46(3): 412-4.

[74] Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. Nuclear receptors and lipid physiology: opening the X-files. Science 2001; 294(5548): 1866-70.

[75] Bouillon R, Okamura WH, Norman AW. Structure-function relationships in the vitamin D endocrine system. Endocr Rev 1995; 16(2): 200-57.

[76] Uchida M, Ozono K, Pike JW. Activation of the human osteocalcin gene by 24R,25-dihydroxyvitamin D3 occurs through the vitamin D receptor and the vitamin D-responsive element. J Bone Miner Res 1994; 9(12): 1981-7.

[77] Lou YR, Molnar F, Perakyla M, *et al.* 25-Hydroxyvitamin D(3) is an agonistic vitamin D receptor ligand. J Steroid Biochem Mol Biol; 118(3): 162-70.

[78] Crowle AJ, Ross EJ. Comparative abilities of various metabolites of vitamin D to protect cultured human macrophages against tubercle bacilli. J Leukoc Biol 1990; 47(6): 545-50.

[79] Rook GA, Steele J, Fraher L, *et al*. Vitamin D3, gamma interferon, and control of proliferation of Mycobacterium tuberculosis by human monocytes. Immunology 1986; 57(1): 159-63.

[80] Mathieu C, Adorini L. The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. Trends Mol Med 2002; 8(4): 174-9.

[81] Imazeki I, Matsuzaki J, Tsuji K, Nishimura T. Immunomodulating effect of vitamin D3 derivatives on type-1 cellular immunity. Biomed Res 2006; 27(1): 1-9.

[82] Anand PK, Kaul D. Vitamin D3-dependent pathway regulates TACO gene transcription. Biochem Biophys Res Commun 2003; 310(3): 876-7.

[83] Anand PK, Kaul D. Downregulation of TACO gene transcription restricts mycobacterial entry/survival within human macrophages. FEMS Microbiol Lett 2005; 250(1): 137-44.

[84] Hmama Z, Nandan D, Sly L, Knutson KL, Herrera-Velit P, Reiner NE. 1alpha,25-dihydroxyvitamin D(3)-induced myeloid cell differentiation is regulated by a vitamin D receptor-phosphatidylinositol 3-kinase signaling complex. J Exp Med 1999; 190(11): 1583-94.

[85] Sly LM, Lopez M, Nauseef WM, Reiner NE. 1alpha,25-Dihydroxyvitamin D3-induced monocyte antimycobacterial activity is regulated by phosphatidylinositol 3-kinase and mediated by the NADPH-dependent phagocyte oxidase. J Biol Chem 2001; 276(38): 35482-93.

[86] Rockett KA, Brookes R, Udalova I, Vidal V, Hill AV, Kwiatkowski D. 1,25-Dihydroxyvitamin D3 induces nitric oxide synthase and suppresses growth of Mycobacterium tuberculosis in a human macrophage-like cell line. Infect Immun 1998; 66(11): 5314-21.

[87] Fratti RA, Backer JM, Gruenberg J, Corvera S, Deretic V. Role of phosphatidylinositol 3-kinase and Rab5 effectors in phagosomal biogenesis and mycobacterial phagosome maturation arrest. J Cell Biol 2001; 154(3): 631-44.

[88] Martineau AR, Wilkinson KA, Newton SM, *et al.* IFN-{gamma}- and TNF-Independent Vitamin D-Inducible Human Suppression of Mycobacteria: The Role of Cathelicidin LL-37. J Immunol 2007; 178(11): 7190-8.

[89] von Essen MR, Kongsbak M, Schjerling P, Olgaard K, Odum N, Geisler C. Vitamin D controls T cell antigen receptor signaling and activation of human T cells. Nat Immunol 2010; 11(4): 344-9.

[90] Sadeghi K, Wessner B, Laggner U, *et al.* Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns. Eur J Immunol 2006; 36(2): 361-70.

[91] Li C, Wang Y, Gao L, *et al*. Expression of toll-like receptors 2 and 4 and CD14 during differentiation of HL-60 cells induced by phorbol 12-myristate 13-acetate and 1 alpha, 25-dihydroxy-vitamin D(3). Cell Growth Differ 2002; 13(1): 27-38.

[92] Sorensen OE, Follin P, Johnsen AH, *et al.* Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. Blood 2001; 97(12): 3951-9.

[93] Cannell JJ, Vieth R, Umhau JC, *et al.* Epidemic influenza and vitamin D. Epidemiol Infect 2006; 134(6): 1129-40.

[94] Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. J Immunol 2008; 181(10): 7090-9.

[95] Di Nardo A, Vitiello A, Gallo RL. Cutting edge: mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide. J Immunol 2003; 170(5): 2274-8.

[96] Agerberth B, Charo J, Werr J, *et al.* The human antimicrobial and chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations. Blood 2000; 96(9): 3086-93.

[97] Rivas-Santiago B, Hernandez-Pando R, Carranza C, *et al.* Expression of cathelicidin LL-37 during Mycobacterium tuberculosis infection in human alveolar macrophages, monocytes, neutrophils, and epithelial cells. Infect Immun 2008; 76(3): 935-41.

[98] Nursyam EW, Amin Z, Rumende CM. The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. Acta Med Indones 2006; 38(1): 3-5.

[99] Gombart AF, Saito T, Koeffler HP. Exaptation of an ancient Alu short interspersed element provides a highly conserved vitamin D-

mediated innate immune response in humans and primates. BMC Genomics 2009; 10: 321.

[100] Neville F, Cahuzac M, Konovalov O, *et al.* Lipid headgroup discrimination by antimicrobial peptide LL-37: insight into mechanism of action. Biophys J 2006; 90(4): 1275-87.

[101] Yuk JM, Shin DM, Lee HM, *et al*. Vitamin D3 induces autophagy in human monocytes/macrophages *via* cathelicidin. Cell Host Microbe 2009; 6(3): 231-43.

[102] Hart PD, Young MR, Gordon AH, Sullivan KH. Inhibition of phagosome-lysosome fusion in macrophages by certain mycobacteria can be explained by inhibition of lysosomal movements observed after phagocytosis. J Exp Med 1987; 166(4): 933-46.

[103] Wang TT, Dabbas B, Laperriere D, *et al.* Direct and indirect induction by 1,25-dihydroxyvitamin D3 of the NOD2/CARD15-defensin beta2 innate immune pathway defective in Crohn disease. J Biol Chem 2010; 285(4): 2227-31.

[104] Liu PT, Schenk M, Walker VP, *et al.* Convergence of IL-1beta and VDR activation pathways in human TLR2/1-induced antimicrobial responses. PLoS One 2009; 4(6): e5810.

[105] Gauzzi MC, Purificato C, Donato K, *et al.* Suppressive effect of 1alpha,25-dihydroxyvitamin D3 on type I IFN-mediated monocyte differentiation into dendritic cells: impairment of functional activities and chemotaxis. J Immunol 2005; 174(1): 270-6.

[106] 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(5): 2405-11.

[107] Giulietti A, van Etten E, Overbergh L, Stoffels K, Bouillon R, Mathieu C. Monocytes from type 2 diabetic patients have a pro-inflammatory profile. 1,25-Dihydroxyvitamin D(3) works as anti-inflammatory. Diabetes Res Clin Pract 2007; 77(1): 47-57.

[108] Rook GA, Taverne J, Leveton C, Steele J. The role of gamma-interferon, vitamin D3 metabolites and tumour necrosis factor in the pathogenesis of tuberculosis. Immunology 1987; 62(2): 229-34.

[109] Helming L, Bose J, Ehrchen J, *et al*. 1alpha,25-Dihydroxyvitamin D3 is a potent suppressor of interferon gamma-mediated macrophage activation. Blood 2005; 106(13): 4351-8.

[110] Thien R, Baier K, Pietschmann P, Peterlik M, Willheim M. Interactions of 1 alpha,25-dihydroxyvitamin D3 with IL-12 and IL-4 on cytokine expression of human T lymphocytes. J Allergy Clin Immunol 2005; 116(3): 683-9.

[111] Jeffery LE, Burke F, Mura M, *et al*. 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. J Immunol 2009; 183(9): 5458-67.

[112] Smolders J, Menheere P, Thewissen M, *et al.* Regulatory T cell function correlates with serum 25-hydroxyvitamin D, but not with 1,25-dihydroxyvitamin D, parathyroid hormone and calcium levels in patients with relapsing remitting multiple sclerosis. J Steroid Biochem Mol Biol 2010; 121(1-2): 243-6.

[113] Akbari O, Stock P, DeKruyff RH, Umetsu DT. Role of regulatory T cells in allergy and asthma. Curr Opin Immunol 2003; 15(6): 627-33.

[114] Staeva-Vieira TP, Freedman LP. 1,25-dihydroxyvitamin D3 inhibits IFN-gamma and IL-4 levels during *in vitro* polarization of primary murine CD4+ T cells. J Immunol 2002; 168(3): 1181-9.

[115] Wolf AJ, Linas B, Trevejo-Nunez GJ, *et al.* Mycobacterium tuberculosis infects dendritic cells with high frequency and impairs their function *in vivo*. J Immunol 2007; 179(4): 2509-19.

[116] Wolf AJ, Desvignes L, Linas B, *et al*. Initiation of the adaptive immune response to Mycobacterium tuberculosis depends on antigen production in the local lymph node, not the lungs. J Exp Med 2008; 205(1): 105-15.

[117] Pedersen AW, Holmstrom K, Jensen SS, *et al.* Phenotypic and functional markers for 1alpha,25-dihydroxyvitamin D(3)-modified regulatory dendritic cells. Clin Exp Immunol 2009; 157(1): 48-59.

[118] Matsuzaki J, Tsuji T, Zhang Y, *et al.* 1alpha,25-Dihydroxyvitamin D3 downmodulates the functional differentiation of Th1 cytokine-conditioned bone marrow-derived dendritic cells beneficial for cytotoxic T lymphocyte generation. Cancer Sci 2006; 97(2): 139-47.

[119] Szeles L, Keresztes G, Torocsik D, *et al.* 1,25-dihydroxyvitamin D3 is an autonomous regulator of the transcriptional changes leading to a tolerogenic dendritic cell phenotype. J Immunol 2009; 182(4): 2074-83.

[120] Van Overtvelt L, Lombardi V, Razafindratsita A, *et al.* IL-10-inducing adjuvants enhance sublingual immunotherapy efficacy in a murine asthma model. Int Arch Allergy Immunol 2008; 145(2): 152-62.

[121] Wilson CL, Ouellette AJ, Satchell DP, *et al.* Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. Science 1999; 286(5437): 113-7.

[122] Coussens A, Timms PM, Boucher BJ, *et al.* 1alpha,25-dihydroxyvitamin D inhibits matrix metalloproteinases induced by Mycobacterium tuberculosis infection. Immunology 2009; 127(4): 539-48.

[123] Elkington PT, Nuttall RK, Boyle JJ, *et al.* Mycobacterium tuberculosis, but not vaccine BCG, specifically upregulates matrix metalloproteinase-1. Am J Respir Crit Care Med 2005; 172(12): 1596-604.

[124] Chang JC, Wysocki A, Tchou-Wong KM, Moskowitz N, Zhang Y, Rom WN. Effect of Mycobacterium tuberculosis and its components on macrophages and the release of matrix metalloproteinases. Thorax 1996; 51(3): 306-11.

[125] Hrabec E, Strek M, Zieba M, Kwiatkowska S, Hrabec Z. Circulation level of matrix metalloproteinase-9 is correlated with disease severity in tuberculosis patients. Int J Tuberc Lung Dis 2002; 6(8): 713-9.

[126] Volkman HE, Pozos TC, Zheng J, Davis JM, Rawls JF, Ramakrishnan L. Tuberculous granuloma induction *via* interaction of a bacterial secreted protein with host epithelium. Science 2010; 327(5964): 466-9.

[127] Li Y, Zhang Z, Xu Y, Xiong S, Ni W, Chen S. TNF-alpha up-regulates matrix metalloproteinase-9 expression and activity in alveolar macrophages from patients with chronic obstructive pulmonary disease. J Huazhong Univ Sci Technolog Med Sci 2006; 26(6): 647-50.

[128] Mercer PF, Shute JK, Bhowmik A, Donaldson GC, Wedzicha JA, Warner JA. MMP-9, TIMP-1 and inflammatory cells in sputum from COPD patients during exacerbation. Respir Res 2005; 6: 151.

[129] Anand SP, Selvaraj P. Effect of 1, 25 dihydroxyvitamin D(3) on matrix metalloproteinases MMP-7, MMP-9 and the inhibitor TIMP-1 in pulmonary tuberculosis. Clin Immunol 2009; 133(1): 126-31.

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