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Innate immune modulation in COPD: moving closer towards vitamin D therapy

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BAL, bronchoalveolar lavage; COPD; chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; GC, glucocorticosteroid; GR, glucocorticosteroid receptor; HDAC2, histone deacetylase 2; IKK; IκB kinase; MAPK, mitogen-activated protein kinase; MKP-1, MAPK phosphatase-1; NF-κB; nuclear factor-kappa B; Nrf2, nuclear factor erythroid 2-related factor 2; TGF-β, transforming growth factor-beta; VDR, vitamin D receptor; VDRE, vitamin D response element;

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Abstract

Chronic obstructive pulmonary disease (COPD) is one of the most common respiratory diseases and a major cause of morbidity and mortality worldwide. Disturbed innate immune processes characterize the pathogenesis of COPD. Vitamin D deficiency is very common in COPD patients and has been associated with disease severity. Interestingly, a number of mechanistic evidence from animal and *in vitro* studies has demonstrated important innate immunomodulatory functions of vitamin D, including anti-inflammatory, anti-oxidative and antimicrobial functions. In this review, we will discuss in detail how these innate immunomodulatory functions of vitamin D could provide therapeutic potential in COPD patients. Finally, the remaining challenges associated with vitamin D therapy in COPD patients will be discussed.

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic disease characterized by progressive expiratory airflow limitation, which is not fully reversible (Decramer, et al., 2012). Cigarette smoking is the major risk factor for COPD. Currently, COPD is the fourth leading cause of death worldwide, while prevalence and mortality rates are still increasing. With progression of the disease, COPD patients become more susceptible to exacerbations, which are primarily caused by respiratory bacterial and viral infections (Wedzicha and Seemungal, 2007). COPD exacerbations are an important cause of hospitalization, reduced quality of life and mortality. When presenting with an acute exacerbation, COPD patients are often treated with inhaled corticosteroids. However, the majority of COPD patients appear to be resistant to the anti-inflammatory effects of corticosteroids (Barnes, 2013;Culpitt, et al., 2003).

The pathophysiology of COPD includes small airway disease (or chronic bronchiolitis), characterized by thickening and fibrosis of small airways, and emphysema, which both contribute to airflow limitation. In COPD patients, the airflow limitation is associated with an abnormal inflammatory response in the airways and lung parenchyma. The inflammatory response involves both innate immune cells (neutrophils, macrophages) and adaptive immune cells (T and B lymphocytes) but also involves the activation of structural cells, including airway epithelial cells (Barnes, 2014). Inhaled cigarette smoke induces pulmonary inflammation through activation of pro-inflammatory pathways, including the nuclear factor-kappa B (NF- κ B) pathway and the p38 mitogen-activated protein kinase (MAPK) pathway. The oxidative stress resulting from cigarette smoke and inflammatory cells further amplifies the inflammation in COPD (Kirkham and Barnes, 2013). In addition to enhanced pulmonary inflammation and oxidative stress, COPD is characterized by an impaired host defense in the

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lungs (Berenson, et al., 2006;Taylor, et al., 2010). This potentially leads to increased bacterial colonization of the lower airways, which may in turn predispose COPD patients to acute exacerbations.

With the discovery of important immunomodulatory properties of vitamin D, the interest for a potential role for vitamin D in COPD has increased over the years. Vitamin D deficiency (defined as serum 25-hydroxyvitamin D < 20 ng/ml) has shown to be highly prevalent in COPD patients and furthermore correlates with disease severity, as assessed by forced expiratory volume in 1 second (FEV1) (Berg, et al., 2013;Janssens, et al., 2010;Persson, et al., 2012;Romme, et al., 2013). Low serum levels of vitamin D have furthermore been associated with characteristic disease features of COPD, including reduced and faster lung function decline (Afzal, et al., 2014;Berg, et al., 2013;Black and Scragg, 2005;Janssens, et al., 2010;Lange, et al., 2012), severity of CT-defined emphysema (Berg, et al., 2013) and increased risk at upper respiratory infections (Cannell, et al., 2006;Ginde, et al., 2009) and COPD exacerbations (Heulens, et al., 2013;Lehouck, et al., 2012). Although an intervention trial with vitamin D supplementation in COPD patients did not show an overall effect on exacerbation rates, a post-hoc analysis did reveal that vitamin D supplementation may significantly reduce the number of exacerbations in COPD patients with severe vitamin D deficiency (25-hydroxyvitamin D < 10 ng/ml) at baseline (Lehouck, et al., 2012). The observed benefit of vitamin D supplementation in COPD patients with established vitamin D deficiency has recently been confirmed by the ViDiCo trial, demonstrating a clinically and statistically significant reduction of moderate to severe exacerbations (Martineau, et al., 2014).

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Abundant mechanistic *in vitro* as well as *in vivo* animal studies suggest a potential involvement of vitamin D in various pathogenic processes in COPD, as shown in Fig. 1. In this review, we will discuss how vitamin D could be a therapeutic agent in COPD through its effect on inflammation, antimicrobial responses, lung remodeling, oxidative stress and the anti-inflammatory effects of corticosteroids. Although vitamin D has been shown to play an important role in adaptive immunity, emphasis will be put on the effect of vitamin D on pulmonary innate immunity (alveolar macrophages, neutrophils and airway epithelial cells), which constitutes the driving force of perpetuating inflammation in COPD. Finally, we will discuss the challenges associated with the use of vitamin D therapy as an anti-inflammatory agent in COPD patients.

Effect of vitamin D on important pathogenic processes in COPD

Local vitamin D metabolism in the innate immune system

Vitamin D is obtained primarily through conversion of 7-dehydrocholesterol in the skin by UV light, and to a lesser extent from the diet. Activation of vitamin D requires two hydroxylations. The first hydroxylation occurs in the liver and leads to the production of 25-hydroxyvitamin D, the main circulating form of vitamin D and determinant of vitamin D status. Subsequently, 25-hydroxyvitamin D is converted by CYP27B1 (1 α -hydroxylase) into the biologically active 1,25-dihydroxyvitamin D, which occurs mainly in the kidneys. 1,25-dihydroxyvitamin D exerts its biological functions by binding to the vitamin D receptor (VDR). After ligand binding, a heterodimer is formed with the retinoid X receptor (RXR) and this VDR-RXR complex binds to specific genomic sequences (vitamin D response elements, VDRE) in the promoter region of target genes, whereby gene expression is regulated. In addition to this well-known genomic signaling, 1,25-dihydroxyvitamin D can also exert non-genomic actions by interacting with signaling pathways outside the nucleus (Losel and Wehling, 2003). In the kidneys, expression of CYP27B1 and thus 1,25-dihydroxyvitamin D production is strictly regulated by calcium, phosphorus and their regulating hormones (including parathyroid hormone), thereby maintaining calcium and bone metabolism. Negative feedback is provided by 1,25-dihydroxyvitamin D itself through downregulation of CYP27B1 and upregulation of CYP24A1 (24-hydroxylase) (which catalyzes the first step in vitamin D catabolism), thereby preventing the excessive production of 1,25-dihydroxyvitamin D.

Expression of both CYP27B1 and VDR has been found in various cell types of the lung innate immune system, including airway epithelial cells, macrophages and neutrophils

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(Hansdottir, et al., 2008; Monkawa, et al., 2000; Overbergh, et al., 2000; Provvedini, et al., 1983; Takahashi, et al., 2002). Consequently, conversion of 25-hydroxyvitamin D into the biologically active 1,25-dihydroxyvitamin D can occur locally in the lungs and in this way act in an autocrine or paracrine fashion to exert its immunomodulatory functions. In sharp contrast to the renal regulation of CYP27B1, the expression of CYP27B1 in immune cells is controlled by immune signals. For example, in monocytes/macrophages, CYP27B1 expression is upregulated by IFN- γ , LPS and TLR2/1 ligands (Esteban, et al., 2004; Liu, et al., 2006; Overbergh, et al., 2000). In further contrast with the renal control of 1,25-dihydroxyvitamin D production, negative feedback control by 1,25-dihydroxyvitamin itself is lacking in immune cells. In this regard, expression of CYP27B1 in immune cells is not suppressed by 1,25-dihydroxyvitamin D (Overbergh, et al., 2000). Finally, it has been shown that macrophages express a truncated, catabolically inactive form of CYP24A1, thereby preventing catabolism of 1,25-dihydroxyvitamin D (Ren, et al., 2005). The latter suggests that macrophages are able to maintain an over-production of 1,25-dihydroxyvitamin D, which could promote innate effector functions. Besides the vast amount of studies in myeloid-derived cells, little is known about the mechanisms regulating vitamin D metabolism in airway epithelial cells.

How could vitamin D influence lung inflammation in COPD?

Recognition of particles from cigarette smoke by airway epithelial cells and alveolar macrophages results in the activation of intracellular signaling pathways, including the NF- κ B and p38 MAPK pathways. The activity of both NF- κ B and p38 MAPK is enhanced in epithelial cells and alveolar macrophages of COPD patients (Caramori, et al., 2003; Di Stefano, et al., 2002; Rajendrasozhan, et al., 2008; Renda, et al., 2008). These pathways are pivotal in driving the inflammatory response in COPD patients and, once activated, lead to the

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enhanced secretion of a number of inflammatory cytokines and chemokines, including IL-1 β , IL-6, IL-8, TNF- α and MCP-1. In response to these inflammatory signals, neutrophils, monocytes and T lymphocytes rapidly migrate into the lungs and further promote the inflammatory response.

Interestingly, the active form of vitamin D has been shown to exert anti-inflammatory effects by inhibiting NF- κ B and MAPK activity (Hansdottir, et al., 2010;Takahashi, et al., 2002;Zhang, et al., 2012) (Fig. 1). In the inactive state, NF- κ B is sequestered in the cytoplasm by interaction with I κ B. Upon cell activation by proinflammatory stimuli, I κ B is phosphorylated by I κ B kinase (IKK), leading to the degradation of I κ B by the proteasome. Subsequently, free NF- κ B can translocate to the nucleus where it activates transcription of several proinflammatory cytokines (e.g. IL-6, IL-8, TNF- α). Several mechanisms have been proposed explaining how vitamin D could counteract the activity of NF- κ B. For example, *in vitro* 1,25-dihydroxyvitamin D inhibited the translocation of the NF- κ B complex to the nucleus in several cell types including macrophages and airway epithelial cells, either by upregulation of I κ B expression (Cohen-Lahav, et al., 2006;Hansdottir, et al., 2010;Riis, et al., 2004) or by direct interaction of 1,25-dihydroxyvitamin D-VDR with IKK, thereby blocking IKK activity (Chen, et al., 2013). However, vitamin D could also inhibit the binding of NF- κ B to DNA and in this way may interfere in the transcriptional activity of NF- κ B (D'Ambrosio, et al., 1998;Harant, et al., 1998). Another central transducer of inflammatory signals leading to the activation of several transcription factors is the p38 MAPK pathway. In this regard, inhibition of p38 MAPK activity in monocytes by 1,25-dihydroxyvitamin D was linked to the induction of MAPK phosphatase-1 (MKP-1), which dephosphorylates p38 and inhibits its subsequent activation (Zhang, et al., 2012). A similar mechanism was found in

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prostate cells where induction of MKP-5 by 1,25-dihydroxyvitamin D was responsible for inhibition of p38 MAPK activity (Nonn, et al., 2006).

The inhibitory effect of 1,25-dihydroxyvitamin D signaling on major inflammatory pathways results in the decreased expression of pro-inflammatory cytokines and chemokines in macrophages, dendritic cells, neutrophils and airway epithelial cells (Ferreira, et al., 2014; Hansdottir, et al., 2010; Korf, et al., 2012; Takahashi, et al., 2002; Zhang, et al., 2012) (Fig. 1). Therefore, vitamin D signaling could contribute to the decreased influx of inflammatory cells in COPD and consequently dampen the inflammatory response. In an animal model of acute lung injury, it has already been shown that peroral or intratracheal administration of 1,25-dihydroxyvitamin D inhibits the recruitment of neutrophils after LPS inhalation (Takano, et al., 2011). In mice, vitamin D deficiency furthermore exacerbated neutrophil infiltration into the lungs after challenge with *Aspergillus fumigatus* (Li, et al., 2014), and also in a model of allergic airway disease, neutrophil influx was increased in vitamin D deficient mice (Gorman, et al., 2013). Moreover, knockout of the VDR in mice increased neutrophilic infiltration in the lungs, which was accompanied by enhanced activity of NF- κ B and elevated levels of MCP-1 and the neutrophil chemoattractant KC in the lungs (Sundar, et al., 2011).

How could vitamin D influence antimicrobial defense in COPD?

Alveolar macrophages and neutrophils are responsible for a broad set of host defense functions including recognition and phagocytosis of pathogens. However, in COPD, a growing body of evidence suggests that alveolar macrophages and neutrophils are defective in their antimicrobial functions. In this regard, alveolar macrophages from COPD patients show reduced phagocytic uptake of bacteria commonly found during exacerbations, such as *S.*

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pneumoniae and nontypeable *H. influenzae* (Berenson, et al., 2006; Taylor, et al., 2010). *In vitro*, it was shown that cigarette smoke impairs both the phagocytic and respiratory burst function, necessary for the effective killing of the phagocytized material, of neutrophils (Green and Carolin, 1967; Stringer, et al., 2007). *In vivo* studies in mice furthermore confirm that cigarette smoke exposure impairs the phagocytic uptake of bacteria by alveolar macrophages, resulting in an increased bacterial load in cigarette smoke-exposed animals (Harvey, et al., 2011; Ortega, et al., 1994; Phipps, et al., 2010). This reduced phagocytic capacity of alveolar macrophages and neutrophils in COPD may lead to chronic colonization of the lower airways and consequently to acute exacerbations. Macrophages from COPD patients are not only defective in the uptake of microbes, but also in the phagocytosis of apoptotic cells ('efferocytosis') (Hodge, et al., 2003; Hodge, et al., 2007; Kirkham, et al., 2004). Impaired phagocytosis of apoptotic neutrophils leads to decreased neutrophil clearance and thereby increased secondary necrosis and release of toxic cell contents, perpetuating inflammation in COPD (Vandivier, et al., 2006).

In vitro studies have shown that 1,25-dihydroxyvitamin D can enhance the phagocytic capacity as well as the respiratory burst functions of monocytes (Goldman, 1984; Sly, et al., 2001; Tokuda and Levy, 1996; Xu, et al., 1993). The effect of vitamin D on monocyte phagocytosis is most likely explained by the pro-differentiating effects of vitamin D on these monocytes, which adopt a more mature phenotype (Goldman, 1984). Whether vitamin D can enhance the phagocytic uptake of microbes or apoptotic cells by mature alveolar macrophages, which has shown to be defective in COPD, has yet to be investigated. In VDR knockout mice, no defects were observed in the *ex vivo* phagocytic and respiratory burst capacities of peritoneal macrophages (Mathieu, et al., 2001). Whereas the effect of vitamin D on alveolar macrophage phagocytosis is still debatable, vitamin D

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may certainly enhance pulmonary antimicrobial defense by stimulating the production of antimicrobial peptides (Korf, et al., 2014) (Fig. 1). Antimicrobial peptides, such as cathelicidin and defensin- β 2, are produced in the lungs by several cell types, including airway epithelial cells, macrophages and neutrophils, and are very effective in killing a number of Gram-positive and Gram-negative antibiotic-resistant strains, such as *Pseudomonas* and *Staphylococcus aureus* and different viruses (Tjabringa, et al., 2006). VDREs were identified in the promoters of the genes for both cathelicidin and defensin- β 2, indicating that 1,25-dihydroxyvitamin D-VDR signaling can stimulate the transcription of these genes (Wang, et al., 2004). Indeed, in several cell types including monocytes and neutrophils, 1,25-dihydroxyvitamin D was shown to upregulate the expression of cathelicidin and to a lesser extent defensin- β 2, showing the potential of vitamin D as an antimicrobial agent in COPD (Gombart, et al., 2005; Wang, et al., 2004).

Several human intervention studies have demonstrated that vitamin D supplementation reduced the risk for respiratory tract infections (Bergman, et al., 2012; Gunville, et al., 2013; Laaksi, et al., 2010; Majak, et al., 2011; Manaseki-Holland, et al., 2012). However, for COPD in particular, limited data are available. Although COPD exacerbations are often caused by respiratory infections, the association between vitamin D levels and risk at COPD exacerbations is debatable, as several studies show negative results (Kunisaki, et al., 2012; Puhan, et al., 2014; Quint, et al., 2012). However, preliminary results of Bellocchia and colleagues have shown that severe vitamin D deficiency increases the risk at frequent exacerbations and hospitalizations in COPD (Bellocchia, et al., 2013). Moreover, in an intervention trial with vitamin D supplementation in COPD patients, vitamin D supplemented patients with severe vitamin D deficiency at baseline showed a reduced number of exacerbations compared to the placebo group, although no overall effect of vitamin D

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supplementation on exacerbations were observed. In this intervention trial, the *ex vivo* phagocytosis of *E. coli* by peripheral blood monocytes was found to be improved in COPD patients which had received vitamin D supplementation compared to placebo and these differences were even more pronounced in the subgroup of subjects with severe vitamin D deficiency at baseline (Lehouck, et al., 2012).

How could vitamin D influence lung remodeling in COPD?

The chronic airflow limitation characteristic of COPD is a result of parenchymal destruction (emphysema) and/or small airway disease (obstructive bronchiolitis). In COPD, there is a disruption of the balance between proteases and anti-proteases, resulting in increased proteolytic activity in the lungs of COPD patients. This imbalance results in the destruction of parenchyma and thus development of emphysema. Epithelial cells, macrophages and neutrophils release a variety of proteases, including neutrophil elastase, cathepsins and matrix metalloproteinases. Increases in matrix metalloproteinases MMP-1, MMP-2, MMP-8, MMP-9 and MMP-12 have been demonstrated in the lungs of COPD patients (Finlay, et al., 1997; Molet, et al., 2005; Segura-Valdez, et al., 2000).

Small airway disease in COPD includes goblet cell hyperplasia, increased airway smooth muscle cells as well as fibrosis in the airway wall (Hogg, 2004). Small airway fibrosis contributes to increased thickness of the airway wall and thereby contributes to airflow limitation. Transforming growth factor-beta (TGF- β) plays an important role in the development of small airway fibrosis in COPD and expression of TGF- β has been shown to be increased in the airway epithelium of COPD patients (de Boer, et al., 1998; Takizawa, et al., 2001).

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Mechanistic *in vitro* studies suggest that vitamin D may influence both emphysema development and small airway fibrosis. 1,25-dihydroxyvitamin D has been shown to inhibit the expression of several matrix metalloproteinases in different cell types, including monocytes and alveolar macrophages (Bahar-Shany, et al., 2010; Coussens, et al., 2009; Lacraz, et al., 1994) (Fig. 1). In an animal model, knockout of VDR led to the development of early-onset emphysema, which was associated with increased activity of MMP-2, MMP-9 and MMP-12 (Sundar, et al., 2011). The increased extracellular matrix formation (including collagen) by fibroblasts in response to TGF- β takes part in the airway remodeling characteristic of COPD. 1,25-dihydroxyvitamin D has been shown to inhibit the proliferation and activation of murine fibroblasts treated with TGF- β 1 and to reduce the expression of extracellular matrix proteins by these fibroblasts (Ramirez, et al., 2010) (Fig. 1). Furthermore, 1,25-dihydroxyvitamin D has also been shown to prevent intestinal and renal fibrosis through inhibition of TGF- β signaling (Ito, et al., 2013; Tao, et al., 2014).

Although fibrotic remodeling in human airways has never been linked to vitamin D deficiency, indirect evidence may come from epidemiological studies showing positive associations between serum 25-hydroxyvitamin D levels, lung function (FEV1, FVC), both in the general population and COPD patients, and lung function decline (Afzal, et al., 2014; Black and Scragg, 2005; Janssens, et al., 2010; Lange, et al., 2012). Low serum 25-hydroxyvitamin D levels have furthermore been associated with the severity of CT-defined emphysema (Berg, et al., 2013).

How could vitamin D influence oxidative stress in COPD?

Oxidative stress occurs when reactive oxygen species (ROS) are produced in excess of the antioxidant defense mechanisms. In COPD, oxidative stress results from high concentrations

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of oxidants present in cigarette smoke, but is also generated endogenously from the activation of inflammatory cells in the lungs, such as macrophages and neutrophils. Oxidative stress is an important amplifying mechanism in COPD pathogenesis by activating NF- κ B and p38 MAPK pathways, by inhibiting antiprotease activity, by impairing antimicrobial defenses and by increased release of TGF- β (Kirkham and Barnes, 2013). Moreover, oxidative stress has been shown to contribute to the defective ability of corticosteroids to repress inflammation in COPD patients (Barnes, 2013).

As oxidative stress is a main player in various pathogenic processes in COPD, therapies targeting to reduce oxidative stress may be of crucial importance in COPD patients. A major contributing factor to the oxidative stress in COPD is the reduced expression of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2), which is activated by oxidative stress and which is the transcriptional regulator of most antioxidant genes. In COPD, the expression and activity of Nrf2 has been shown to be decreased despite high levels of oxidative stress in the lungs (Goven, et al., 2008;Suzuki, et al., 2008). Several studies suggest that vitamin D or vitamin D analog therapy may suppress oxidative stress in several disease models (Noyan, et al., 2005;Tanaka, et al., 2011;Husain, et al., 2009;Fedirko, et al., 2010;Hamden, et al., 2009), and this could be related to the effect of vitamin D on Nrf2 (Fig. 1). Vitamin D analogs have been shown to activate Nrf2 in squamous carcinoma cells and myeloid leukemia cells (Lin, et al., 2002;Bobilev, et al., 2011). In the study of Nakai and colleagues, treatment of diabetic rats with maxacalcitol, a vitamin D analog, resulted in the reduced expression of Nrf2 and increased expression of its negative regulator Keap1 (Kelch-like erythroid cell-derived protein with CNC homology-associated protein 1), together with suppression of NF- κ B (Nakai, et al., 2014). These effects of vitamin D on Nrf2 might also have implications for the phagocytic capacity of alveolar macrophages, as it has been

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demonstrated that restoration of Nrf2 activity results in improved phagocytosis of *H. influenzae* and *P. aeruginosa* by alveolar macrophages from COPD patients (Harvey, et al., 2011).

Effect of vitamin D on the anti-inflammatory action of glucocorticosteroids in COPD

Glucocorticosteroids (GCs) are well-known for their anti-inflammatory actions. However, the inflammation in COPD has appeared to be unresponsive to the effects of GCs (Culpitt, et al., 2003). This resistance to the anti-inflammatory effects of GCs is a major barrier to the effective treatment of COPD.

GCs can suppress inflammation via different mechanisms. Binding of GCs to glucocorticoid receptors (GR) can result in the activation of anti-inflammatory genes, including MKP-1 and IL-10. However, the major anti-inflammatory action of GCs is to switch off the expression of several pro-inflammatory genes. In this case, GC-GR complexes translocate to the nucleus and recruit histone deacetylase 2 (HDAC2). This results in histone deacetylation, making the DNA inaccessible for pro-inflammatory transcription factors, including NF- κ B, and consequently suppresses the expression of inflammatory genes. One of most characterized molecular mechanisms contributing to GC resistance in COPD is the decreased expression and activity of HDAC2 in alveolar macrophages, airways and peripheral lung in COPD patients (Ito, et al., 2004).

Interestingly, vitamin D has been shown to enhance GC action. Zhang *et al.* (2012) demonstrated that vitamin D enhances responses to GC in human monocytes and murine bone marrow-derived macrophages by stimulating GC-induced upregulation of MKP-1, leading to a decrease in the production of proinflammatory mediators regulated by p38 MAPK (Zhang,

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et al., 2012). Similar results were obtained by Searing and colleagues (2010), who found that levels of MKP-1 and IL-10 induced by vitamin D plus dexamethasone (a GC) in PBMCs from asthmatic children were greater than those induced by dexamethasone alone (Searing, et al., 2010). Sutherland *et al.* (2010) demonstrated that reduced serum 25-hydroxyvitamin D levels in asthmatic patients are associated with reduced *ex vivo* responses of peripheral blood mononuclear cells to GCs, as shown by a reduction in GC-induced expression of MKP-1 (Sutherland, et al., 2010). Furthermore, treatment of CD4⁺ regulatory T cells from subjects with GC-resistant asthma with 1,25-dihydroxyvitamin D in combination with dexamethasone restored the ability of these cells to release IL-10 up to levels similar to those in cells from GC-sensitive asthma patients (Xystrakis, et al., 2006). In the same study, it was shown that oral administration of vitamin D (0,5 µg/day) for 7 days to patients with GC-resistant asthma enhanced *ex vivo* T cell responses to dexamethasone. A small clinical trial furthermore demonstrated that (a 4-week) treatment with 1,25-dihydroxyvitamin D may improve the clinical responsiveness of GC-resistant asthma patients, as assessed by changes in FEV1 (Nanzer, et al., 2014). This suggests that vitamin D therapy could possibly potentiate the therapeutic response to GCs in patients with GC-resistant asthma. Similar principles could apply in GC-resistant COPD patients, but relevant research is limited. To our knowledge, only one small sample size study has investigated the association between serum 25-hydroxyvitamin D levels and responsiveness to inhaled CS treatment, as assessed by changes in FEV1 (Kunisaki and Rector, 2011). In this study, no correlation between baseline serum 25-hydroxyvitamin D levels and changes in FEV1 after a short-term treatment (4 weeks) with inhaled CS was observed.

Therapeutic perspectives

Due to its anti-inflammatory and antimicrobial functions (Fig. 1), vitamin D supplementation may have a beneficial therapeutic impact in COPD patients, in whom prevalence of vitamin D deficiency is high. However, different uncertainties remain to be addressed, as discussed below.

Evidence from clinical trials

Until now, two double-blind randomized placebo-controlled trials have investigated the effect of oral vitamin D supplementation on the incidence of exacerbations in COPD patients. In the single-center trial of Lehouck *et al.* (2012), 182 COPD patients with moderate to very severe COPD and a history of recent exacerbations were randomized to receive a monthly oral dose of 100,000 IU vitamin D₃ or placebo for 1 year (Lehouck, et al., 2012). No overall effect of high-dose bolus vitamin D supplementation on exacerbation rate or time to first exacerbation was observed in the population as a whole. However, a post-hoc subgroup analysis in a limited amount of patients (n=30) with severe vitamin D deficiency (25-hydroxyvitamin D < 10 ng/ml) at baseline showed a significant reduction in exacerbations in the vitamin D supplemented patients compared to placebo (rate ratio=0.57, p=0.042). These findings were recently confirmed by the ViDiCo trial (Martineau, et al., 2014). In this multi-center trial, 240 COPD patients, recruited from both hospital and community settings, were randomly allocated to receive a 2-monthly oral dose of 120,000 IU vitamin D₃ or placebo for 1 year. Vitamin D supplementation did not affect time to first moderate or severe exacerbation in the whole population. However, in accordance with the trial of Lehouck *et al.*, a pre-specified subgroup analysis (n=148) revealed that time to first moderate or severe exacerbation was significantly reduced in vitamin D supplemented patients with vitamin D deficiency (25-hydroxyvitamin D < 20 ng/ml) at baseline (hazard ratio=0.57, p=0.021) compared to placebo.

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The results from these two trials suggest that benefits of vitamin D therapy may only be seen in vitamin D deficient COPD patients whose 25-hydroxyvitamin D levels are elevated over a certain threshold level by supplementation, rather than a continual improvement with any increase in vitamin D levels. This could also explain the lack of an overall effect in both trials. Randomized-controlled trials in COPD patients with baseline 25-hydroxyvitamin D levels below 20 ng/ml are therefore needed to confirm these findings. Alternatively, the overall null result in both trials may be related to inefficient vitamin D treatment, which could in turn be related to an ineffective dosing regimen (intermittent vs daily dosing) or dosage form, or an inadequate route of administration, as discussed below. Finally, the absence of therapeutic effect of vitamin D in the whole study population in both trials may be explained by COPD disease heterogeneity with regards to pathology, immunology and genetics, which could modify the response of COPD patients to vitamin D supplementation (Gold and Manson, 2012).

Furthermore, preventive trials assessing the effect of vitamin D supplementation on the risk for developing COPD will give better insights into the role of vitamin D in COPD pathogenesis. In the United States, a large randomized clinical trial among more than 20,000 participants aged 50 years or older (lungVITAL study) is currently ongoing and will assess the effect of daily oral vitamin D supplementation (2000 IU) with or without fish oil (rich in omega-3 fatty acids) on pulmonary outcomes, including new-onset COPD, decline in lung function and COPD exacerbations (ClinicalTrials.gov Identifier: NCT01728571).

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Remaining challenges

Although results from observational studies as well as clinical trials show therapeutic potential for vitamin D in COPD patients, several challenges associated with vitamin D therapy in COPD patients remain to be addressed.

Vitamin D supplementation is mainly provided via oral supplements. However, the optimal dosing regimen for oral vitamin D supplementation, either intermittent bolus dosing or daily dosing, remains a matter of debate. In clinical trials, intermittent bolus doses of vitamin D are often used to maximize patient compliance to the treatment. With bolus dosing, sporadic high doses of vitamin D result in a sharp rise in 25-hydroxyvitamin D to often supraphysiological concentrations at the time of administration (Ilahi, et al., 2008). However, very high levels of serum 25-hydroxyvitamin D have been associated with impaired immune responses, hypercalcemia and even mortality (Durup, et al., 2012;Martineau, 2012;Melamed, et al., 2008;Nielsen, et al., 2010). Theoretically, the high transient peaks may result in a complete suppression of the immune system. Alternatively, high peaks of 25-hydroxyvitamin D may upregulate catabolic enzymes (CYP24A1) leading to local deficiency of the active metabolite after the transient peak serum levels. In order to avoid supraphysiological levels as well as substantial fluctuations in serum 25-hydroxyvitamin D levels, daily supplementation is likely to be preferable to a bolus dosing regimen. Different authors suggested furthermore that intermittent bolus dosing may be less effective than daily dosing for inducing non-classical actions of vitamin D (Hollis and Wagner, 2013;Vieth, 2009).

Adding to the complexity of the vitamin D dosing regimen, the levels of 1,25-dihydroxyvitamin D required to effectively elicit immunomodulatory functions *in vivo* in the lungs remain largely unknown. *In vitro* studies demonstrating anti-inflammatory and

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antimicrobial functions of vitamin D often use remarkably higher concentrations of 1,25-dihydroxyvitamin D than those found in serum. As conversion of 25-hydroxyvitamin D into 1,25-dihydroxyvitamin D can occur locally in the lungs (Baeke, et al., 2010; Hansdottir, et al., 2008; Monkawa, et al., 2000; Overbergh, et al., 2000) and the negative feedback mechanisms to control this conversion appear to be absent in immune cells (Overbergh, et al., 2000; Ren, et al., 2005), local concentration of 1,25-dihydroxyvitamin D within the lungs may reach relatively higher levels in the lungs than levels found in serum. However, 25-hydroxyvitamin D and especially 1,25-dihydroxyvitamin D concentrations were found to be significantly lower in the bronchoalveolar lavage (BAL) fluid compared to serum (Anandaiah, et al., 2013; Liu, et al., 2012). It must be noted that concentrations of vitamin D within the BAL fluid may not be representative for the 1,25-dihydroxyvitamin D levels within the entire pulmonary system. Alternatively, assessment of local activation within the lungs compared to renal activation may be derived from the 1,25-dihydroxyvitamin D to 25-hydroxyvitamin D ratio in BAL compared to this ratio in serum.

As high serum levels of vitamin D are associated with adverse side effects, much effort has been made towards the development of vitamin D analogs that still exert the beneficial effects of vitamin D, but without the hypercalcemic side effects. Unfortunately, many vitamin D analogs that successfully dissociated beneficial immunomodulatory functions from undesired side effects on calcium metabolism have not progressed beyond the preclinical stage. Only a few vitamin D analogs are currently on the market for treatment of immune diseases (Vojinovic, 2014). TX527 and maxacalcitol, two less hypercalcemic vitamin D analogs, have already been shown to inhibit NF- κ B activity *in vitro* in several cell types, including monocytes (Gonzalez-Pardo, et al., 2013; Komine, et al., 1999; Stio, et al., 2007), showing the anti-inflammatory potential of vitamin D analogs.

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In order to reduce systemic side effects and maximize the desired effectiveness of treatment locally in the lungs, alternative routes of administration of vitamin D rather than peroral administration must also be considered. In this regard, in an animal model of acute lung injury, intratracheal administration of 1,25-dihydroxyvitamin D was found to be more effective in dampening neutrophil recruitment into the lungs after LPS inhalation compared to peroral administration (Takano, et al., 2011). Development of a drug for inhaled administration is often a common strategy to achieve high efficiency locally in the lungs and additionally reduce systemic side effects. Takano and colleagues have demonstrated the anti-inflammatory potential of inhalational delivery of vitamin D analogs, showing that the inhaled vitamin D analog TEI-A00114 inhibits neutrophil recruitment in an animal model of acute lung injury (Takano, et al., 2012). Therefore, delivery of vitamin D or alternatively vitamin D analogs via inhalation could be a promising and more directed approach for COPD patients. Liposome encapsulation could be an attractive delivery mode for targeting vitamin D to the lung via inhalation. Liposomal encapsulation could prolong therapeutic levels locally in the lungs, while reducing systemic toxicity (Allen and Cullis, 2013; Joshi and Misra, 2001). Furthermore, liposomal delivery could facilitate intracellular delivery of vitamin D, particularly to phagocytes.

As vitamin D has been shown to enhance the anti-inflammatory activities of corticosteroids (Xystrakis, et al., 2006), vitamin D may also be administered as an adjuvant therapy, rather than as a sole replacement for current therapies. This could be an effective strategy, since the majority of COPD patients are resistant to even high doses of inhaled or oral corticosteroids. In asthma, low serum levels of 25-hydroxyvitamin D are associated with increased corticosteroid use (Searing, et al., 2010) and reduced GC responses *in vitro* (Sutherland, et al.,

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2010). Vitamin D could not only increase the responsiveness to GCs, but may also reduce the required dose of GCs, therefore limiting the systemic side-effects of GCs, including reduced bone density, increased fracture risk, and adrenal suppression (Lipworth, 1999). Moreover, reducing the required dose of inhaled GCs may also dampen the dose-dependent risk for bacterial pneumonia (Calverley, et al., 2007;Dransfield, et al., 2013;Ernst, et al., 2007;Wedzicha, et al., 2008).

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Conclusion

The airways and lung parenchyma of COPD patients are characterized by exaggerated inflammation, increased oxidative stress, impaired host defense and lung remodeling. As vitamin D could positively affect each of these processes, vitamin D therapy may have great therapeutic potential in COPD patients, who often present with vitamin D deficiency. Appropriate antimicrobial treatment is essential in the treatment of acute exacerbations of COPD. However, repetitive and long-term treatment with antibiotics should still be avoided as they contribute to the multi-resistance of colonizing strains. Therefore, vitamin D could be a potential alternative due to its dual antimicrobial and anti-inflammatory properties in the (pulmonary) immune system. Moreover, as vitamin D has been shown to improve responsiveness to GCs, co-administration of inhaled GCs and vitamin D may enhance the anti-inflammatory effects of existing GC therapies in COPD patients. A subsequent lower but still efficacious dose of inhaled GCs may be beneficial by reducing the risk for pneumonia associated with high doses (Calverley, et al., 2007;Dransfield, et al., 2013;Ernst, et al., 2007;Wedzicha, et al., 2008).

Although an abundance of epidemiological and mechanistic studies support the potential benefit of vitamin D therapy in COPD patients, more well-designed trials are necessary to unravel the potential efficacy of vitamin D therapy in COPD patients. Here, the administration dose, form of vitamin D and route of administration should be taken into consideration. Additionally, it must be noted that vitamin D therapy may only be effective in COPD patients with defined vitamin D deficiency, as demonstrated in two intervention trials with high-dose vitamin D supplementation in COPD patients (Lehouck, et al., 2012;Martineau, et al., 2014). Taken together, despite the clear potential of vitamin D in reversing a disturbed innate immune defense in COPD, many unknowns remain. Further research is needed to implicate

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the therapeutic potential of local upregulation of vitamin D signaling in the lungs of patients with COPD.

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Authorship contributions

Wrote or contributed to the writing of the manuscript: Heulens, Korf, and Janssens

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Figure legends

Fig. 1. Potential therapeutic innate immune targets for vitamin D in the pathogenesis of COPD

Cigarette smoke activates epithelial cells and alveolar macrophages, leading to the activation of major inflammatory pathways, including NF- κ B and p38 MAPK pathways. Activation of these intracellular pathways results in the production of several cytokines and chemokines, which attract inflammatory cells (neutrophils, T cells and monocytes) to the lungs. Cigarette smoke itself and activated inflammatory cells contribute to oxidative stress in the airways of COPD patients. Several cell types, including neutrophils and alveolar macrophages, release proteases, which break down connective tissue and thereby contribute to emphysema. Moreover, epithelial cells release TGF- β , which stimulates the proliferation of fibroblasts and consequently fibrosis in the small airways, resulting in small airway disease (chronic bronchiolitis). In COPD, the phagocytic function of alveolar macrophages has shown to be defective, potentially resulting in increased bacterial colonization of COPD airways. Vitamin D may have therapeutic potential in COPD by inhibition of TGF- β signaling, inhibition of NF- κ B and p38 MAPK pathways and production of inflammatory cytokines and chemokines, inhibition of oxidative stress, stimulation of antimicrobial peptide production and potentially by stimulating the phagocytic capacity of alveolar macrophages. MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor- kappa B; TGF- β , transforming growth factor-beta

