CHAPTER NINE

VITAMIN D EFFECTS ON LUNG IMMUNITY AND RESPIRATORY DISEASES

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Abstract

Our understanding of vitamin D metabolism and biological effects has grown exponentially in recent years and it has become clear that vitamin D has extensive immunomodulatory effects. The active vitamin D generating enzyme, 1α-hydroxylase, is expressed by the airway epithelium, alveolar macrophages, dendritic cells, and lymphocytes indicating that active vitamin D can be produced locally within the lungs. Vitamin D generated in tissues is responsible for many of the immunomodulatory actions of vitamin D. The effects of vitamin D within the lungs include increased secretion of the antimicrobial peptide cathelicidin, decreased chemokine production, inhibition of dendritic cell activation, and alteration of T-cell activation. These cellular effects are important for host responses against infection and the development of allergic lung diseases like...
asthma. Epidemiological studies do suggest that vitamin D deficiency predisposes to viral respiratory tract infections and mycobacterial infections and that vitamin D may play a role in the development and treatment of asthma. Randomized, placebo-controlled trials are lacking but ongoing.

I. INTRODUCTION

Emerging information on vitamin D physiology has revealed that vitamin D is not merely a micronutrient that plays a role in calcium homeostasis but a pluripotent hormone with extensive immunomodulatory functions. Studies have shown that the enzyme 1α-hydroxylase, which catalyzes the last and rate limiting step in the synthesis of active 1,25-dihydroxyvitamin D₃ (1,25D), and the vitamin D receptor (VDR), which mediates the actions of vitamin D, are expressed widely in the body, including the lungs and cells of the immune system. These observations have led to a surge of epidemiological and basic research studies examining the effects of vitamin D on immune responses, lung infections, and the development of lung diseases. Vitamin D insufficiency has been linked to increased risk of infections, in particular, viral respiratory tract infections (Cannell et al., 2006, 2008; Ginde et al., 2009b; Laaksi et al., 2007; Wayse et al., 2004) and tuberculosis (Bornman et al., 2004; Liu et al., 2007b; Martineau et al., 2007a,b; Roth et al., 2004, 2008; Wilkinson et al., 2000). Vitamin D may also play a role in the development of obstructive lung diseases like asthma and COPD (Brehm et al., 2010; Janssens et al., 2009; Sutherland et al., 2010). This chapter focuses on lung-specific vitamin D metabolism, immune effects of vitamin D, and the potential role of vitamin D in the development and treatment of lung diseases.

II. LUNG IMMUNE FUNCTIONS

The respiratory tract has a surface area of ~70 m² and is in direct and continuous contact with the surrounding environment. Despite continuous exposure to potential pathogens only rarely do the lungs become colonized or infected. A local defense system with components of both innate and adaptive immunity has evolved to discriminate between nonpathogenic antigens and potential pathogens and to clear pathogens.

The innate immune system involves a rapid, nonspecific, recognition and response to almost any pathogen. Only those antigens that penetrate the innate immune responses evoke the more specific adaptive immune responses. The main players in innate immunity in the lungs include the airway epithelium itself, alveolar macrophages, and dendritic cells. They all
express pattern recognition receptors (PRRs) and ligand engagement results in activation of intracellular signaling pathways that mobilize antimicrobial defenses, inflammation, and adaptive immune responses (Basu and Fenton, 2004). The airway epithelium is the first line of defense and functions as a physical barrier to prevent the entry of inhaled pathogens. When the airway epithelium recognizes the presence of a pathogen it responds by releasing antimicrobials, chemokines, and cytokines. Alveolar macrophages (AMs) recognize, phagocytose, and remove inhaled material. They are activated either in response to pathogens or through an autocrine/paracrine response to cytokines. Activation leads to enhanced phagocytosis and killing of pathogens as well as coordination of both innate and adaptive immune responses. The third major innate immune effector cells in the lung are dendritic cells. Dendritic cells use PRR’s to monitor the local environment for pathogens. When a pathogen is encountered, it is ingested and its proteins are processed into peptides which are then presented at the surface of the dendritic cell. Activated dendritic cells produce chemokines and migrate to local lymph nodes where they present the antigenic peptides bound to major histocompatibility complex (MHC) molecules to naive T-cells (CD4+ T-helper cells and CD8+ T-cytotoxic cells) and induce their activation and differentiation. Dendritic cells thus serve as a link between innate and adaptive immune responses. Vitamin D can influence all three innate immune effectors in the lungs and thus may play an important role in how the lung recognizes and responds to pathogens.

Activation of the innate immune system drives activation of the long-term adaptive immune system (Iwasaki and Medzhitov, 2010). Adaptive immune responses involve the ability of T- and B-lymphocytes to produce cytokines and immunoglobulins, respectively. All phases of the adaptive immune response are specific to unique antigen, from recognition of the antigen by antibody (humoral) or T-lymphocyte (cell-mediated) through lymphocyte activation, to effector function (elimination of antigen) and the development of immunologic memory (Mak and Saunders, 2005). Upon activation, memory T-cells downregulate lymphoid-tissue-homing receptors and upregulate tissue-specific-adhesion molecules and can now migrate to nonlymphoid tissues like the lungs (Holt et al., 2008). Further, once activated, \( T_{H} \) (CD4+) cells differentiate into \( T_{H1} \), \( T_{H2} \), or \( T_{H17} \) effector cells. The effector cells are characterized by the production of distinct set of cytokines (Medzhitov, 2007). Activation of B-cells and their differentiation into antibody-secreting plasma cells can be triggered directly by antigen but usually requires helper T-cells. Last, regulatory T-cells (\( T_{R,reg} \)) are important for the control of peripheral T-cell responses. In relation to the lungs, they are believed to have key roles in the protection against the inflammatory sequela of airway infections and in the protection against the induction and expression of atopic disease (Holt et al., 2008). There is data to support both
indirect (dendritic cells) and direct (T- and B-lymphocytes) effects of vitamin D on adaptive immune responses.

The respiratory tract is continuously exposed to antigens, some of which are pathogenic and some of which are not. A specialized lung immune system has evolved that can recognize and respond to potential pathogens but does not get activated by nonpathogenic antigens which would result in chronic inflammation and tissue damage. The following chapters will focus on how vitamin D may affect cells involved in lung immune responses at all levels, that is, airway epithelium, alveolar macrophages, dendritic cells, and T- and B-cells and thus can have significant overall immunomodulatory effects in the lungs.

III. 1,25-Dihydroxyvitamin D is Generated Locally in the Lungs

Humans get vitamin D through synthesis in the skin following UVB exposure and to a lesser extent from limited dietary sources. Vitamin D from the skin or diet is metabolized primarily in the liver to 25-hydroxyvitamin D$_3$ (25D; Ponchon et al., 1969). 25D is the “storage form” of vitamin D and is used to determine the vitamin D status of individuals. The last and rate limiting step in the synthesis of “active” 1,25D is catalyzed by the mitochondrial enzyme 1α-hydroxylase and is conventionally known to take place in the kidneys. Renal 1α-hydroxylase activity is under stringent regulation by parathyroid hormone, calcium, calcitonin, phosphorus, and 1,25D itself (Zehnder et al., 1999). Vitamin D is inactivated by the ubiquitous enzyme, 24-hydroxylase, whose expression is inducible by 1,25D, thus creating a negative feedback loop (Holick, 2007). The biological effects of vitamin D are achieved through the regulation of gene expression mediated by VDR (Baker et al., 1988). Active vitamin D binds to VDR, and upon ligand binding, the receptor dimerizes with the retinoic X receptor (RXR; MacDonald et al., 1993). The VDR/RXR complex binds to vitamin D responsive elements (VDREs) within the promoter regions of vitamin D-regulated genes (Sutton and MacDonald, 2003).

It is increasingly recognized that localized synthesis of 1,25D rather than systemic production is responsible for many of the immune effects of vitamin D. Extra-renal expression of 1α-hydroxylase has been found in various cells of the immune system including AMs (Adams et al., 1983; Reichel et al., 1987a,b), dendritic cells (Fritsche et al., 2003; Hewison et al., 2003; Sigmundsdottir et al., 2007), and lymphocytes (Chen et al., 2007; Sigmundsdottir et al., 2007) as well as in airway epithelia (Hansdottir et al., 2008; Table 9.1). Locally formed 1,25D acts in an autocrine or paracrine
Table 9.1  Local production and effects of 1,25D in the respiratory tract

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Conversion of 25D → 1,25D</th>
<th>1,25D effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airway epithelium</td>
<td>Constitutive</td>
<td>Increases CD14 and cathelicidin; dampens IFN-β and chemokine response during viral infection</td>
<td>Handsdottir et al. (2008, 2010)</td>
</tr>
<tr>
<td>Alveolar macrophages</td>
<td>Upon activation</td>
<td>Increases the antimicrobial peptide cathelicidin</td>
<td>Liu et al. (2006, 2007a,b)</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>Increases with differentiation</td>
<td>Inhibits dendritic cell differentiation, maturation, and function; decreases IL-12 and increases IL-10; alters T-cell activation</td>
<td>Fritsche et al. (2003), Sigmundsdottir et al. (2007), Piemonti et al. (2000), Penna et al. (2005)</td>
</tr>
<tr>
<td>T-lymphocytes</td>
<td>At least when activated</td>
<td>Inhibits proliferation; modulates cytokine production—inhibits Th1 and Th17 cytokines but induces T_{Reg}s</td>
<td>Sigmundsdottir et al. (2007), Lemire et al. (1995), Daniel et al. (2008), Mora et al. (2008), Penna et al. (2005)</td>
</tr>
<tr>
<td>B-lymphocytes</td>
<td>Unclear</td>
<td>Inhibits proliferation of activated B-cells and generation of plasma cells</td>
<td>Chen et al. (2007)</td>
</tr>
</tbody>
</table>
fashion to modulate cell proliferation, cell differentiation, and immune function (Bell, 1998; Hewison et al., 2007; White, 2008).

A. Airway epithelium

Defense systems have evolved to clear and inactivate inhaled pathogens so that despite continual exposure to potential antigens, the lung is generally maintained in a quiescent, noninflamed state (Kohlmeier and Woodland, 2008). The airway epithelium is constantly exposed to potentially pathogenic microorganisms. Recognition of pathogens by airway epithelial cells results in activation of intracellular signaling pathways and the end result is transcription of genes for a variety of effector molecules, including antimicrobials, type I interferons, and proinflammatory cytokines and chemokines (Bartlett et al., 2008).

Recent work has found that airway epithelium exposed to the “storage” form of vitamin D is able to generate “active” vitamin D, potentially creating localized areas with higher 1,25D levels than are seen in serum (Hansdottir et al., 2008). Primary human airway epithelial cells express relatively high mRNA levels of 1α-hydroxylase and lower levels of the inactivating 24-hydroxylase at baseline. Unlike alveolar macrophages, which need to be stimulated to convert 25D to 1,25D, airway epithelial cells constitutively generate 1,25D. Not only do airway epithelial cells produce active vitamin D at baseline, they also respond to pathogens by increasing the machinery needed to convert 25D to 1,25D. Viral infection induces expression of 1α-hydroxylase and increases conversion of 25D to 1,25D, which may be of benefit to the host response against the virus (Hansdottir et al., 2008).

Local generation of active vitamin D in the lung potentially regulates pulmonary immune responses. Active vitamin D, generated by airway epithelium, directly increases expression of VDR-regulated genes that are involved in recognition and killing of pathogens, including the TLR co-receptor CD14 and the antimicrobial peptide cathelicidin (Hansdottir et al., 2008). When airway epithelium is infected with a virus, 1,25D modulates the expression of inflammatory chemokines and cytokines in response to the virus (Hansdottir et al., 2010). This will be discussed further in the Section IV.B.

B. Alveolar macrophages

Like airway epithelium, AMs can also generate active vitamin D. In contrast to the constitutive activity of 1α-hydroxylase in airway epithelium, AMs need to be stimulated before converting inactive to active vitamin D. The first description of extrarenal 1α-hydroxylase was in patients with the granulomatous disease, sarcoidosis. It had been noted that some patients
with sarcoidosis had hypercalcemia and high vitamin D levels (Barbour et al., 1981; Papapoulos et al., 1979). Subsequently, Adams et al. (1983) showed that AMs from patients with sarcoidosis converted 25D to 1,25D whereas AMs from patients with idiopathic pulmonary fibrosis did not. It has since been shown that AMs from normal subjects do not constitutively express 1\(\alpha\)-hydroxylase and convert 25D to 1,25D but can do so if activated with TLR 2/1 ligands, IFN\(\gamma\) or LPS (Liu et al., 2006; Reichel et al., 1987a,b). This is different from renal 1\(\alpha\)-hydroxylase, which is mainly regulated by mediators of calcium and bone homeostasis. Moreover, activated macrophages lack negative feedback by 25D and 1,25D (Dusso et al., 1997). A nonfunctional alternatively spliced form of 24-hydroxylase has been found in the cytoplasm of macrophages that may be responsible for impeding the access of 25D and 1,25D to the enzyme and preventing their catabolism (Ren et al., 2005). The lack of a negative feedback system contributes to the increased serum vitamin D levels in patients with granulomatous diseases.

Expression of 1\(\alpha\)-hydroxylase by stimulated alveolar macrophages, production of 1,25D, and lack of active 24-hydroxylase cannot only have beneficial effects on host defense but also have pathological implications. However, TLR 2/1 ligands (mycobacterial antigen) activate alveolar macrophages, induce 1\(\alpha\)-hydroxylase and increase 1,25D which leads to an increase in the vitamin D-regulated antimicrobial peptide cathelicidin. Cathelicidin facilitates killing of Mycobacterium tuberculosis (Liu et al., 2006).

C. Dendritic cells

Dendritic cells play a key role in the initiation and regulation of adaptive immune responses to inhaled antigens. Dendritic cells form a contiguous network throughout the airway epithelium. In the steady state, dendritic cells are specialized for uptake and processing of environmental antigens but lack the capacity for efficient antigen presentation (Holt et al., 2008). If dendritic cells sense an abnormal state, they mature. Maturation is characterized by migration to regional lymph nodes, downregulation of antigen uptake, and an enhanced capacity to activate naïve T-cells. This process of dendritic cell maturation involves changes in the expression of chemokine receptors and is associated with upregulation of costimulatory molecules and markers of dendritic cell activation (Upham, 2003).

Human blood monocytes can be differentiated to dendritic cells by \textit{in vitro} culture with GM-CSF, IL-4, or IL-13. Monocyte-derived dendritic
cells constitutively express 1α-hydroxylase. Following terminal differentiation induced by TNFα, IFNγ, polyI:C, or LPS, there is marked increase in expression and function of 1α-hydroxylase (Fritsche et al., 2003; Hewison et al., 2003). Further, dendritic cells metabolize vitamin D precursors to active 1,25D (Fritsche et al., 2003; Hewison et al., 2003; Sigmundsdottir et al., 2007). In contrast, VDR expression may be downregulated during the maturation process (Hewison et al., 2003). 1,25D generated by the dendritic cells themselves and exogenous 1,25D inhibit dendritic cell differentiation, maturation, and function by decreasing the expression of MHC class II and costimulatory molecules, decreasing production of IL-12, and increasing secretion of IL-10 (D’Ambrosio et al., 1998; Mora et al., 2008; Penna and Adorini, 2000; Penna et al., 2005). By modulating dendritic cell activation, 1,25D alters T-cell activation favoring the induction of regulatory T-cells and leads to T-cell hyporesponsiveness (Penna and Adorini, 2000; Penna et al., 2005). It has been postulated that inhibition of dendritic cell maturation and T-cell hyporesponsiveness may explain some of the immunosuppressive activities of 1,25D including control of autoimmune diseases and transplantation tolerance (Adorini and Penna, 2008; Gregori et al., 2001; Griffin et al., 2001).

D. Lymphocytes

Vitamin D not only affects lymphocytes indirectly via its effects on dendritic cells as described above but also has direct effects on T-cells and likely B-cells. Activated T-lymphocytes and B-lymphocytes have been found to express VDR and 1α-hydroxylase and to convert 25D to 1,25D (Bhalla et al., 1983; Chen et al., 2007; Provvedini et al., 1986; Sigmundsdottir et al., 2007).

Antigen-mediated activation of naïve T-helper (T_H) cells results in the generation of pluripotent T_H0 lymphocytes that synthesize a broad spectrum of cytokines (IL-2, IL-4, IL-10, and IFNγ) (Adams and Hewison, 2008). Proliferating T_H0 lymphocytes are then able to differentiate into T_H1 (IL-2, IFNγ, TNF), T_H2 (IL-3, IL-4, IL-5, and IL-10), or T_H17 (IL-17) lymphocytes with a more distinct cytokine profile. Vitamin D suppresses the production of T_H1 and T_H17 (Daniel et al., 2008; Lemire et al., 1995; Mora et al., 2008) cytokines but its effects on the production of T_H2 cytokines is less clear. Early studies suggested that 1,25D enhanced the development of T_H2 cells (Boonstra et al., 2001) but subsequent studies indicate that 1,25D does not favor the T_H2 phenotype (Pichler et al., 2002; Staeva–Vieira and Freedman, 2002). Recent studies have shown that the effects of vitamin D are complex and include the generation of IL-10 producing T-regulatory lymphocytes (previously known as suppressor T-cells; Penna et al., 2005). IL-10 is a major anti-inflammatory and immunosuppressive cytokine that inhibits both T_H1 and T_H2 immune
responses (Moore et al., 2001). In general, the immunomodulatory effects of vitamin D on T-cell-mediated immunity may be beneficial for conditions in which the immune system is directed at self, that is, autoimmune diseases and graft rejection in transplantation (Barrat et al., 2002; Bikle, 2009; Gregori et al., 2001). In direct relation to the lungs, there is evidence that T_{Reg} function is impaired in allergic and asthmatic disease (Lloyd and Hawrylowicz, 2009). Vitamin D has been shown to reverse steroid resistance, through induction of IL-10 secreting T-cells, in patients with asthma (Xystrakis et al., 2006). The role of vitamin D in asthma pathogenesis and treatment will be discussed in the Section V.

The actions of 1,25D on B-cells are not well studied. A recent study found that 1,25D suppresses immunoglobulin production and B-cell proliferation and differentiation (Chen et al., 2007). This study also found that patients with systemic lupus erythematosus have low vitamin levels and hypothesized that low vitamin D levels may contribute to the B-cell hyperactivity that is seen in this disease.

IV. Vitamin D and Lung Infections

A. Mycobacteria

It was first noted over one century ago that UV light seemed to help in the treatment of mycobacterial infections. The 1903 Nobel Prize in Medicine was awarded to Niels Finsen for demonstrating that UV light is beneficial to patients with lupus vulgaris (tuberculosis of the skin). In the late nineteenth century, Hermann Brehmer built the first sanatorium for the treatment of tuberculosis (Liu et al., 2007a). Patients were exposed to plentiful amounts of high altitude, fresh air, and good nutrition. It has since been speculated that patients with tuberculosis benefitted from sanatoriums because of UV light exposure and increased production of vitamin D precursors in the skin. The first in vitro studies looking at vitamin D and M. tuberculosis were published in the 1980s. These studies demonstrated that adding 1,25D to M. tuberculosis-infected human monocytes and macrophages reduced the intracellular bacterial load (Crowle et al., 1987; Rook et al., 1986). This observation has been followed by a series of observational studies suggesting that individuals with low 25D levels are more susceptible to M. tuberculosis infection and often have a more severe course of disease (Gibney et al., 2008; Nnoaham and Clarke, 2008; Ustianowski et al., 2005; Wilkinson et al., 2000). Case-control studies have also found an association between VDR polymorphisms and susceptibility to tuberculosis, in particular, in individuals with low 25D levels (Bornman et al., 2004; Lewis et al., 2005; Roth et al., 2004; Wilkinson et al., 2000).
Several different mechanisms have been proposed for how vitamin D may increase antimicrobial actions of monocytes and macrophages. A multiplicity of studies has been published recently indicating that a vitamin D–induced antimicrobial peptide, cathelicidin, plays a key role. The first study was a translational study published in 2006, showing that adequate 25D levels are required for TLR2/1 activation (by a mycobacterial ligand) and subsequent 1α-hydroxylase and VDR–dependent expression of cathelicidin. This study also revealed increased killing of mycobacteria by macrophages in the presence of 25D (Liu et al., 2006). In a subsequent study of peripheral blood monocytes infected with recombinant mycobacteria, vitamin D strongly induced cathelicidin mRNA and reduced the growth of mycobacteria in a dose–dependent fashion (Martineau et al., 2007a,b). Another study showed a direct correlation between serum 25D levels and monocyte expression of cathelicidin following treatment with TLR 2/1 and TLR 4 ligands. In the same study, in vivo supplementation of vitamin D enhanced ex vivo innate immune responses by rescuing TLR–mediated suppression of cathelicidin expression (Adams et al., 2009). Last, a study using human monocytic cells found that siRNA knockdown of 1,25D induced cathelicidin resulting in complete loss of antimicrobial activity (Liu et al., 2007b; Fig. 9.1). Alternative mechanisms that have been proposed for the effects of vitamin D include 1,25D induction of superoxide burst and enhancement of phagolysosome fusion both of which are mediated through the phosphatidylinositol 3-kinase pathway (Hmama et al., 2004; Sly et al., 2001).

Human trials looking at vitamin D for prevention or treatment of tuberculosis have been performed. In a double-blinded randomized controlled trial, 192 healthy adult TB contacts were randomized to receive a single oral dose of vitamin D (2.5 mg = 100,000 IU) or placebo. Six weeks later, a functional whole blood assay to assess growth of recombinant reporter mycobacteria in vitro (BCG–lux assay) was performed. IFN–γ responses to M. tuberculosis antigens were also determined. The investigators found that vitamin D significantly enhanced the ability of participants’ whole blood to restrict growth of the reporter mycobacteria but did not affect antigen–stimulated IFN–γ secretion (Martineau et al., 2007a,b). Two small randomized studies have looked at adding vitamin D to treatment regimens for tuberculosis and showed faster resolution of symptoms and earlier sputum conversion to culture negativity in patients given vitamin D (Morcos et al., 1998; Nursyam et al., 2006). A larger randomized, double-blind, placebo control trial included 365 patients with TB starting treatment and gave 100,000 IU of vitamin D at inclusion and again 5 and 8 months after the start of treatment. No differences were found in a clinical severity score (TB score), sputum conversion, or 12–month mortality between patients treated with vitamin D or placebo (Wejse et al., 2009). Of note is that 25D levels in the two groups were similar when measured at 2 and 8 months, suggesting that perhaps the dose of vitamin D used was insufficient.
To date there is ample evidence that vitamin D inhibits growth of mycobacteria \textit{in vivo}. Epidemiological studies suggest that low vitamin D levels increase the susceptibility to and severity of tuberculosis. Clinical trials looking at vitamin D for the treatment of tuberculosis have provided conflicting results and it remains unclear whether vitamin D supplementation is beneficial. Several clinical trials are ongoing that are investigating the impact of vitamin D supplementation on response to treatment of \textit{M. tuberculosis} (\url{www.clinicaltrials.gov}).

### B. Respiratory infections

Seasonal variation in the incidence of communicable diseases, in particular, respiratory tract infections, is among the oldest observations in population biology, dating back to ancient Greece (Lipsitch and Viboud, 2009). Several mechanisms have been hypothesized to explain this observation, one of which is seasonal variation in vitamin D levels. It has been noted that the
peak incidence of respiratory tract infections coincides with the time of the year when there is insufficient UVB light to produce vitamin D, and vitamin D levels in the population are at a low (Cannell et al., 2006, 2008). As our understanding of the role of vitamin D in innate immunity has increased, this hypothesis has gained increased popularity. Further, circumstantial evidence supporting the role of vitamin D comes from epidemiological studies that have shown that children with rickets are at increased risk of respiratory infections (Muhe et al., 1997; Rehman, 1994). More recently, several epidemiological studies have consistently found an association between low vitamin D levels and increased susceptibility to respiratory infections (Aloia and Li-Ng, 2007; Ginde et al., 2009b; Laaksi et al., 2007; Wayse et al., 2004). The largest of those studies was a secondary analysis of the Third National Health and Nutrition Examination Survey (NHANES-III; Ginde et al., 2009b). This study looked at the association between 25D levels of nearly 19,000 participants and self-reported upper respiratory tract infections. After adjusting for demographic and clinical characteristics, lower 25D levels were independently associated with recent respiratory tract infections. Preliminary evidence also suggests an association between VDR polymorphisms and acute lower respiratory tract infection in children. A study of 56 children hospitalized with lower respiratory tract infection (predominantly viral bronchiolitis) found that the odds of infection were higher in children with the FokI ff VDR genotype (Roth et al., 2008) when compared with the FokI FF genotype (Roth et al., 2008).

At the basic science level, we have recently shown that airway epithelium converts 25D to 1,25D which raises the possibility of higher levels of 1,25D locally in the lungs than are seen in serum (Hansdottir et al., 2008). We have also shown that viral infection increases the amount of 1,25D generated by the airway epithelium. We believe that the increase in local 1,25D in airways will contribute to decreased tissue damage, while maintaining viral clearance. The studies supporting this conclusion are described below.

When examining the role of vitamin D in airway antiviral responses, the transcription factor, nuclear factor-κB (NF-κB) is a potential regulatory point. NF-κB is a well established key player in multiple physiological processes including innate- and adaptive-immune responses and inflammation (Holt et al., 2008). IκBα inhibits the NF-κB pathway by binding to NF-κB subunits in the cytoplasm and inhibiting translocation to the nucleus (Li and Verma, 2002). Relevant to vitamin D control of airway epithelial cell immune responses, we have shown that vitamin D induces IκBα in airway epithelium, leading to less induction of NF-κB-driven genes during viral infection. The end result is decreased secretion of inflammatory chemokines but no change in viral clearance (Hansdottir et al., 2010). While vitamin D dampens expression of inflammatory chemokines, we have also shown that it increases expression of CD14 and cathelicidin which serve a role in recognizing and eliminating pathogens, including viruses.
Combined, these results suggest that vitamin D may potentiate innate immunity while controlling the potentially harmful inflammatory response (Hansdottir et al., 2008, 2010; Fig. 9.1).

Two randomized placebo-controlled trials looking at vitamin D supplementation on respiratory tract infections were recently published. In the former study, 162 adults were given 2000 IU units of vitamin D daily or placebo for 12 weeks. A questionnaire was administered biweekly to record the incidence and severity of upper respiratory tract infection symptoms. This study found no difference in the incidence or severity between the groups (Li-Ng et al., 2009). The second randomized trial looked at the incidence of influenza A in school children treated with 1200 IU vitamin D daily or placebo. In this study, influenza A occurred in 18/167 (10.8%) of children in the vitamin D group compared with 31/167 (18.6) in the placebo group (relative risk 0.58; 95% CI 0.34–0.99; \(P = 0.04\)).

More rigorously designed randomized, placebo-controlled, clinical trials are warranted to further explore and establish the role of vitamin D in preventing and/or treating respiratory tract infections. A trial of vitamin D supplementation for the prevention of influenza and other respiratory infections is ongoing (www.clinicaltrials.gov).

V. Vitamin D and Obstructive Lung Diseases

A. Asthma

Asthma is a chronic inflammatory disorder that causes an increase in airways hyperresponsiveness leading to recurrent episodes of wheezing and shortness of breath. The prevailing consensus is that the immunological bases of allergic disease like asthma results from inappropriate \(T_{H2}\) responses to common, harmless, airborne antigens. These reactions are normally suppressed by \(T_{R eg s}\) which maintain airway tolerance (Lloyd and Hawrylowicz, 2009). There is increasing evidence that one mechanism for the development of asthma is imbalance between regulatory and effector T-cells and that the ability to enhance regulatory function may represent an effective treatment for asthma (Lloyd and Hawrylowicz, 2009; Robinson, 2009).

The prevalence of asthma has been steadily increasing over the past several decades and over the same period of time vitamin D insufficiency has also been on the rise. The prevalence of both conditions has been linked to African American race, obesity, and immigration to westernized countries (Litonjua and Weiss, 2007). These observations have prompted the hypothesis that vitamin D deficiency is an important contributor to the asthma epidemic. Epidemiological studies have found that vitamin D insufficiency is common in asthmatics and is associated with increased asthma severity and hospitalizations (Brehm et al., 2009, 2010; Freishtat et al., 2010;
Sutherland et al., 2010). If such an association exists, it may be mediated through increased risk of respiratory viral infection in vitamin D-deficient individuals or by the effects of vitamin D on adaptive immunity, in particular, T-regulatory cells (Litonjua, 2009).

Vitamin D modulates adaptive immunity both indirectly via inhibition of dendritic cell maturation and directly via its effects on T\textsubscript{Regs}. Regulatory T-cells can either develop as a normal part of the immune system (naturally occurring T\textsubscript{Regs}) or in response to particular antigenic exposure (induced/adaptive T\textsubscript{Regs}; Xystrakis et al., 2007). Naturally occurring T\textsubscript{Regs} are characterized by the expression of the forkhead winged transcription factor FoxP3, whereas induced T\textsubscript{Regs} may be FoxP3\textsuperscript{+} or FoxP3\textsuperscript{−} (Dimeloe et al., 2010). Pretreatment of dendritic cells with vitamin D and subsequent coculture with CD4\textsuperscript{+} cells leads to induction of CD4\textsuperscript{+}FoxP3\textsuperscript{+} T\textsubscript{Regs} with suppressive activity (Penna et al., 2005). 1,25D also acts directly on CD4\textsuperscript{+} T-cells and promotes an IL-10 secreting T\textsubscript{Reg} population (Barrat et al., 2002; Fig. 9.1). IL-10 inhibits many functions relevant to asthma and has been proposed to play a role in maintaining immune homoeostasis in the airways (Hawrylowicz and O’Garra, 2005). An inverse correlation exists between the presence of IL-10 and the incidence and severity of asthma.

Glucocorticosteroids are the principal controller therapy for patients with persistent asthma but there is a significant variability in the response to this treatment and a proportion of patients do not achieve optimal asthma control despite high doses (Sutherland et al., 2010). Glucocorticosteroids increase T\textsubscript{Regs} and IL-10 synthesis (Karagiannidis et al., 2004) and the induction may be enhanced by 1,25D (Barrat et al., 2002; Xystrakis et al., 2006, 2007). CD4\textsuperscript{+} T-cells from steroid-resistant asthmatics fail to demonstrate increased IL-10 synthesis following stimulation in the presence of a glucocorticosteroid (Hawrylowicz et al., 2002). This defect in steroid induced IL-10 can be overcome by the addition of 1,25D to the T-cell culture (Xystrakis et al., 2006). Epidemiological evidence supports a role for vitamin D on the effects of glucocorticosteroids. Low vitamin D levels have been associated with increased use of corticosteroids and reduced in vitro glucocorticoid response (Brehm et al., 2009; Searing et al., 2010; Sutherland et al., 2010).

To summarize, vitamin D deficiency is common in asthmatic patients and vitamin D supplementation may result in improvement in asthma severity and treatment response to corticosteroids, likely via induction of T\textsubscript{Regs} and secretion of IL-10. It should be noted that not all the data support a positive role for vitamin D on the development of asthma. The hypothesis that vitamin D may cause asthma because of inhibition of T\textsubscript{H}1 responses also exists (Gale et al., 2008; Hypponen et al., 2004). Several clinical trials are ongoing that are looking at vitamin D and asthma, ranging from maternal supplementation during pregnancy and prevention of childhood asthma to the use of vitamin D as a treatment in individuals with asthma (www.clinicaltrials.gov).
B. Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation that is not fully reversible. The airflow limitation is progressive and associated with an abnormal inflammatory response of the lungs to noxious stimulus or gases, like cigarette smoke. In addition to slow progressive loss of lung function, patients with COPD can have acute exacerbations that lead to a faster decline in FEV₁. Exacerbations are most often triggered by viral or bacterial infection. (Papi et al., 2006). Vitamin D deficiency is highly prevalent in COPD and correlates with the severity of COPD (Janssens et al., 2010). In line with new insights into the immunomodulatory effects of vitamin D, including anti-inflammatory and possibly antimicrobial effects, it has been postulated that vitamin D may affect the pathogenesis of COPD (Janssens et al., 2009). Epidemiological studies in healthy subjects and patients with COPD have suggested a dose-dependent association between serum 25D levels and lung function (FVC and FEV₁; Black and Scragg, 2005; Janssens et al., 2010). It is unclear at this time how vitamin D may affect lung function but variants in the vitamin D-binding gene have been linked to vitamin D deficiency and COPD risk (Janssens et al., 2010). These population-based studies do not prove that there is an association between vitamin D deficiency and lung function but they do provide preliminary data and justification for randomized controlled trials of vitamin D supplementation in COPD. A randomized, multicentre, double-blind, placebo-controlled trial of vitamin D supplementation in COPD is currently underway (www.clinicaltrials.gov).

VI. Conclusions and Future Directions

Vitamin D deficiency is on the rise in western countries including the US (Ginde et al., 2009a). Our understanding of vitamin D metabolism and function has grown exponentially over the past decade. It has become clear that vitamin D is not only important for bone and muscle health but has a wide spectrum of biological actions including significant immunomodulatory effects (Holick, 2007). The enzyme 1α-hydroxylase is expressed by a variety of cells and the 1,25D that is produced locally in tissues may have direct effects on nearby cells and be responsible for the broad actions of vitamin D.

Epidemiological studies suggest an association between low vitamin D levels and mycobacterial infections, respiratory viral infections, and asthma (Fig. 9.1). The enzyme 1α-hydroxylase is expressed by airway epithelium, macrophages, dendritic cells, and lymphocytes in the respiratory tract indicating that active vitamin D may be produced locally within the lungs.
Mechanistic studies have found the 1,25D influences cellular mechanisms that are important for recognition and killing of pathogens, inflammation, and control of adaptive immune functions within the lungs (Fig. 9.1).

Epidemiological and mechanistic studies indicate that vitamin D may play an important role in the development of respiratory diseases but many questions remain. Important clinical trials are ongoing looking at the effects of vitamin D supplementation on mycobacterial infections, respiratory tract infections, asthma, and COPD.

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