Published in final edited form as:

Mol Aspects Med. 2008 December ; 29(6): 369–375. doi:10.1016/j.mam.2008.04.004.

The paradoxical effects of vitamin D on Type 1 mediated immunity

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Abstract

Low vitamin D status is associated with an increased risk of Th1 mediated autoimmune diseases like inflammatory bowel disease. 1,25(OH)₂D₃ treatments have been shown to suppress Th1 mediated immunity and protect animals from experimental autoimmunity. Th1 mediated immunity is important for clearance of a number of different infectious diseases. For tuberculosis 1,25(OH)₂D₃ treatment is associated with decreased Th1 mediated immunity but increased bactericidal activity. Systemic candidiasis is unaffected by 1,25(OH)₂D₃ treatment. The seemingly paradoxical effects of 1,25(OH)₂D₃ and vitamin D on Th1 mediated autoimmunity versus infectious immunity point to a broad array of vitamin D targets in the immune system. The interplay of these vitamin D targets and their impact on the host-immune response then dictate the outcome.

Keywords

Vitamin D; inflammatory bowel disease; infectious immunity; immune regulation

1. Introduction

The classical role of vitamin D is in the regulation of calcium homeostasis. Recently it has become clear that vitamin D regulates immune function. In particular, poor vitamin D status has been linked to the development of a number of different type-one (Th1) mediated autoimmune diseases including type-1 diabetes, multiple sclerosis (MS), and inflammatory bowel diseases (IBD) (Cantorna et al. 2004). In addition the active form of vitamin D (1,25(OH)₂D₃) has been shown to completely block the development of experimental models of these autoimmune diseases (Cantorna et al. 1996; Cantorna et al. 2000; Zella et al. 2003). Th1 mediated immunity is critical for the ability of the host to mount a protective immune response to many different infections. Paradoxically, 1,25(OH)₂D₃ treatments did not compromise the ability of infected mice to fight a fungal infection that depends on Th1 mediated immunity (Cantorna et al. 1998; Kaposzta et al. 1998). Furthermore, vitamin D status and 1,25(OH)₂D₃ have been suggested to be protective against tuberculosis (TB) where host immunity depends on Th1 production of the cytokine IFN-γ. Clouding the picture further is the evidence that patients with Th1 driven diseases like IBD (Crohn's disease), sarcoidosis and tuberculosis show hypercalcemia and calcification of granulomatous lesions and suggest that the local immune response may produce 1,25(OH)₂D₃ (Yang et al. 2000; Tuohy et al. 2005; Volpicelli et al. 2005; Falk et al. 2007). How can 1,25(OH)₂D₃ selectively regulate the immune system

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to suppress autoimmune disease without compromising the host's ability to fight infection? Furthermore, is the local production of $1,25(\text{OH})_2\text{D}_3$ in granulomatous lesions protective or pathogenic? The effects of vitamin D, $1,25(\text{OH})_2\text{D}_3$ and vitamin D metabolism on the susceptibility and resistance to Th1 driven immune responses will be discussed in order to clarify the seemingly paradoxical effects of vitamin D.

2. Vitamin D and the hygiene hypothesis

The incidence of immune mediated diseases like IBD, MS, allergies and asthma have all increased in developed countries over the last 50 years. This rapid increase in disease incidence cannot be explained genetically. In addition, the concordance rate between identical twins is only between 20–50% for IBD and MS (Cantorna 2006). Therefore, environmental factors impact the development of autoimmunity. The prevailing theory about the environmental cause of the increase in immune mediated diseases has been called the hygiene hypothesis (Christen et al. 2005). The hygiene hypothesis states that because of vaccination, improved sanitation, and reduced rates of infection the immune system is missing important signals that prevent over-activation and disease (Christen et al. 2005). If the hygiene hypothesis is relevant to diseases from IBD to asthma there is likely going to be common mechanisms of infectious disease stimulated protection in these very different diseases.

The hygiene hypothesis for IBD centers around the critical findings that uncontrolled immune responses to the bacterial flora in the gut result in disease development. Changes in the normal bacterial flora and or the decreased incidence of intestinal parasites including worm infections are cited as the basis for the hygiene hypothesis in IBD (Khan et al. 2002; Strober et al. 2007; Koloski et al. 2008). Experimental evidence shows that gastrointestinal worm infections can suppress IBD in experimental animals and humans (Khan et al. 2002; Strober et al. 2007; Koloski et al. 2008). In addition, approaches that aim to alter the types of bacteria in the gastrointestinal track including the use of probiotics to replace the existing bacteria with “good” bacteria have been successful in some circumstances (Rolfe et al. 2006). However, there are also infections that are associated with disease induction and therefore it is likely that other environmental factors including pollution, diet etc. affect the development of these complex diseases.

We propose a modification to the hygiene hypothesis that states that vitamin D is one of the environmental factors that affects the development of immune mediated diseases. Current research in the vitamin D field shows that people in developed countries especially those that experience winter have large fluctuations in their vitamin D status. A major source of vitamin D results from it being manufactured via a photolysis reaction in the skin and vitamin D availability from sunlight exposure is significantly less in northern climates, and is especially low during the winter (Clemens et al. 1982; DeLuca 1993). The fluctuations in vitamin D are a direct result of decreased outdoor activity, changes in season, increased pollution and diets that lack adequate vitamin D levels. It seems possible that vitamin D exposure especially early in life may be an environmental factor that affects the development of the immune system. Vitamin D exposure and exposure to other environmental elements would determine which genetically susceptible individuals develop autoimmunity.

3. Autoimmunity, IBD and Th1 driven immune responses

The etiology of autoimmune diseases and asthma are very different and cells that are pathogenic for one disease are beneficial for the other. For most forms of autoimmunity the immune response is characterized by overproduction of type-1 helper cell (Th1) associated cytokines including interferon (IFN)- γ and tumor necrosis factor (TNF)- α (Fig. 1). Th1 mediated immune responses are induced when a combination of circumstances occur. Antigen specific T cells that are primed in the presence of interleukin (IL)-12 and little IL-4 result in the development

of Th1 cells (Fig. 1). Th1 mediated responses are inhibited by regulatory T (T reg) and NKT cells that produce IL-4, IL-10 and transforming growth factor (TGF)- β (Fig. 1). The cell types that are involved include macrophage and dendritic cells (DC) that act as antigen presenting cells (APC), Th1 cells, T reg and NKT cells. Type 2 helper cells (Th2) produce IL-4 and IL-10 that inhibit Th1 development and are the pathogenic cells in allergic asthma (Fig. 1). The pathogenesis of asthma and autoimmune diseases are complex and although autoimmune diseases can be classified as Th1 driven diseases other cells including Th17 cells are pathogenic (Fig. 1) (Iwakura et al. 2006). For asthma the NKT cells are required for disease pathology since mice that are NKT cell deficient fail to develop asthma (Akbari et al. 2003). NKT cells are early innate regulatory cells that can suppress autoimmunity (Taniguchi et al. 2003). Induction of T reg cells that produce IL-10 and TGF- β are protective for both Th1 and Th2 driven diseases including experimental asthma and IBD (Akbari et al. 2003; Maul et al. 2005).

IBD are immune mediated diseases of unknown etiology affecting the gastrointestinal tract. In North America and Europe about 1 in 1000 people are affected with IBD (Podolsky 1991; Podolsky 1991). There are at least two distinct forms of IBD, ulcerative colitis and Crohn's disease. IBD are chronic recurring illnesses most commonly involving inflammation of the terminal ileum and colon, although these diseases can also affect many sites throughout the alimentary tract. Crohn's disease is a Th1 mediated disease and ulcerative colitis has more of a mixed Th1/Th2 phenotype. Both genetic and environmental factors predispose individuals to develop IBD. Cells from Crohn's disease patients have been shown to secrete high amounts of IFN- γ and TNF- α among other cytokines (Fig. 2) (Neissner et al. 1995). There is no cure for Crohn's disease, although there are some very good treatments available that mainly suppress inflammation. TNF- α blocking drugs have been shown to be beneficial for Crohn's disease patients. Experimental approaches to treating Crohn's disease include induction of T reg cells (Fig. 2). However, there is no cure and repeated treatments and maintenance drugs are required to keep Crohn's patients healthy long-term. The major side effect associated with all therapies is an increased risk of infection and cancer as a result of the immunosuppression.

4. Vitamin D and the immune system

The identification of the vitamin D receptor (VDR) in peripheral blood mononuclear cells sparked the early interest in vitamin D as an immune system regulator (Bhalla et al. 1983; Provvedini et al. 1983). All cells of the immune system that have been evaluated express the VDR and at least in T cells activation induces the expression of additional VDR (Veldman et al. 2000; Mahon et al. 2003). 1,25(OH) $_2$ D $_3$ is a potent *in vivo* suppressor of experimental autoimmune diseases such as arthritis, type-1 diabetes, lupus, IBD and experimental MS models (reviewed in (Cantorna 2000)). Regulation of macrophage and DC function by 1,25(OH) $_2$ D $_3$ is important for the inhibition of experimental autoimmunity. 1,25(OH) $_2$ D $_3$ treatment of DC *in vitro* inhibited differentiation and maturation of DC and resulted in DC that when transferred induced *in vivo* suppression of T cells (Griffin et al. 2003). Similarly, 1,25(OH) $_2$ D $_3$ induced myeloid progenitors to differentiate into macrophage and inhibited the production of IL-12 and TNF- α *in vitro* (Fig. 2) (Koeffler et al. 1984; Clohisy et al. 1987). *In vivo* the efficacy of 1,25(OH) $_2$ D $_3$ to suppress experimental IBD has been shown to correlate with the inhibition of TNF- α and other downstream genes activated by TNF- α (Fig. 2) (Zhu et al. 2005). Overall, the effects of 1,25(OH) $_2$ D $_3$ on the APC are to decrease production of IL-12, and TNF- α and to suppress maturation and differentiation such that the immature APC fail to prime Th1-like immune responses (Fig. 2).

T cells are also vitamin D targets and vitamin D regulates T cell function both directly and indirectly by regulating APC function. 1,25(OH) $_2$ D $_3$ treatments have been shown to directly inhibit IFN- γ *in vitro* (Staeva-Vieira et al. 2002; Mahon et al. 2003). Furthermore 1,25

(OH)₂D₃ inhibits the development of Th1 cells *in vivo* and the inhibition of the Th1 mediated response correlates with the protection from disease pathology (Cantorna et al. 2000). More recently 1,25(OH)₂D₃ has been shown to induce T reg cells directly through upregulation of FoxP3 and indirectly through APC (Barrat et al. 2002; Adorini et al. 2003). Induction of T reg and suppression of Th1 cells by 1,25(OH)₂D₃ has been correlated with suppression of experimental autoimmunity. In addition, the VDR deficient host is more susceptible to experimental IBD in a number of different models (Froicu et al. 2003; Froicu et al. 2007). The immune system of VDR KO mice has normal CD4+, CD8+, and NK cell numbers. However, CD4+ T cells from VDR KO mice overproduce IFN- γ , have a strong mixed lymphocyte reaction that is Th1 mediated and are lacking NKT cells (Froicu et al. 2003; Froicu et al. 2006; Yu et al. 2008). iNKT cells are early innate regulatory cells that can alter the outcome of autoimmunity (Taniguchi et al. 2003). Furthermore 1,25(OH)₂D₃ treatments induced NKT cell function *in vitro* and *in vivo* (Yu et al. 2008). Two types of cells are induced by 1,25(OH)₂D₃ the T reg and the NKT cell; induction of these regulatory cells and direct inhibition of Th1 cells are the mechanisms by which 1,25(OH)₂D₃ suppresses experimental autoimmunity (Fig.2).

5. Vitamin D and Th1 mediated host immunity to infection

Th1 mediated immune responses and especially the ability to produce IFN- γ is important for host immunity to a number of infectious diseases. In particular, immunity to intracellular pathogens including many viruses, bacteria such as *Mycobacteria* and *Listeria* species and parasites like *Leishmania* require IFN- γ as part of the host response for successful clearance. Regulatory cell induction and IL-10 production are associated with prolonged infections and chronicity with these same organisms (Scott-Browne et al. 2007). *In vitro*, 1,25(OH)₂D₃ has been shown to suppress IFN- γ and IL-12 production from human leukocytes infected with *Mycobacteria tuberculosis* (Vidyanani et al. 2007). In addition, it should be noted that regulatory T cell induction suppresses macrophage anti-TB effects and 1,25(OH)₂D₃ has been shown to induce T reg cells (Adorini et al. 2003; Hougardy et al. 2007; Scott-Browne et al. 2007). 1,25(OH)₂D₃ addition to murine macrophages inhibited the IFN- γ induced killing of *Leishmania major* *in vitro* (Ehrchen et al. 2007). Furthermore, mouse monocytes treated with 1,25(OH)₂D₃ induced the growth of *Listeria* organisms in bone marrow derived macrophage cultures (Helming et al. 2005). Mice that lack the VDR showed enhanced IFN- γ responses and increased clearance rates of *L. major* *in vivo* (Ehrchen et al. 2007). The evidence is consistent that in the presence of 1,25(OH)₂D₃ there is an inhibition of Th1 mediated immune responses to infectious organisms that includes depressed IFN- γ and IL-12 production and induction of T reg cells.

1,25(OH)₂D₃ has some paradoxical effects on host resistance to infections. Even though IFN- γ production is critical for survival from *Candida albicans* infection; 1,25(OH)₂D₃ treatment of mice had no effect on survival from systemic candidiasis (Cantorna et al. 1998; Kaposzta et al. 1998). For TB there is anecdotal evidence that perhaps vitamin D deficiency and reduced sunlight exposure result in more severe TB (Gibney et al. 2008). Vitamin D deficiency in mice resulted in the increased replication of *M. bovis* (Waters et al. 2004). In addition, 1,25(OH)₂D₃ has been shown to inhibit replication of TB *in vitro* by inducing anti-bacterial peptides (Liu et al. 2006). More work is needed to determine *in vivo* what the net effects of vitamin D status are on the whole organisms resistance to Th1 mediated diseases like TB. Although there has been a flurry of activity aimed at studying the anti-bacterial effects of 1,25(OH)₂D₃ to date no information is available to address the potential 1,25(OH)₂D₃ mediated effects of Th1 inhibition or T reg induction in the context of TB host resistance.

6. Extra-renal production of the 1alpha hydroxylase

Cyp27B1 is the gene encoding the 1 alpha hydroxylase that produces active $1,25(\text{OH})_2\text{D}_3$ and is expressed classically in the kidneys. Extra-renal production of Cyp27B1 has been implicated to occur in a number of inflammatory conditions including sarcoidosis, and Crohn's disease and may explain hypercalcemia that occurs in these patients (Conron et al. 2000; Abreu et al. 2004). Other granulomatous diseases including TB are associated with disordered vitamin D metabolism and hypercalcemia (Yang et al. 2000). Interestingly enough the granulomatous diseases are Th1 mediated events. Although these conditions exist it is unclear whether extra-renal production of $1,25(\text{OH})_2\text{D}_3$ is beneficial or pathological in these diseases. Often resolution of the disease corresponds with normalization of the vitamin D and calcium status (Conron et al. 2000; Abreu et al. 2004). Immune cells including macrophage and DC express the 1alpha hydroxylase enzyme (Hewison et al. 2007). The possibility that the immune system can produce locally active $1,25(\text{OH})_2\text{D}_3$ is an attractive idea. *In vitro* induction of the Cyp27B1 enzyme in macrophage induces anti-bacterial peptides that kill TB (Liu et al. 2006). At present the *in vivo* relevance of immune mediated production of Cyp27B1 is not known.

One group (TG Marshall, Autoimmunity Research Foundation, Thousand Oaks, CA) has recommended and continues to recommend lowering vitamin D status to treat a number of chronic inflammatory conditions (Marshall 2008). The basis for this recommendation are the reports of production of $1,25(\text{OH})_2\text{D}_3$ by the immune system during disease pathology and the possibility that this induction of $1,25(\text{OH})_2\text{D}_3$ may be contributing to diseases from obesity to other chronic diseases including those mediated by Th1 cells like sarcoidosis and Crohn's disease (Marshall et al. 2004; Marshall 2008). The argument is that the hormone activity of $1,25(\text{OH})_2\text{D}_3$ induces transcription of a large number of genes that somehow cause these diseases and that $1,25(\text{OH})_2\text{D}_3$ should be measured because it is the biologically active form of vitamin D that binds the receptor (Marshall 2008). Current assays for $25(\text{OH})\text{D}_3$ that are used to assess vitamin D levels in the general population are inaccurate and lack standards (Glendenning et al. 2006). $1,25(\text{OH})_2\text{D}_3$ measurements are even more difficult to do, are not standardized, and are also inaccurate. Measurements of $1,25(\text{OH})_2\text{D}_3$ are meaningless in the face of these measurement problems. Furthermore, excess $1,25(\text{OH})_2\text{D}_3$ has been given to animals with Th1 mediated diseases and the symptoms of the disease as well as the Th1 response were significantly reduced in many experiments by many investigators (Lemire et al. 1991; Cantorna et al. 1998; Cantorna et al. 2000; Zella et al. 2003; Mathieu et al. 2004). More work is needed to determine whether immune mediated production of $1,25(\text{OH})_2\text{D}_3$ is biologically significant, pathogenic, or protective. At present there is not strong evidence to recommend decreasing vitamin D status in any population.

7. Conclusions

The effects of vitamin D on immunity are beginning to be appreciated. Regardless of whether Th1 mediated autoimmunity or infectious immunity is being monitored, $1,25(\text{OH})_2\text{D}_3$ inhibits IL-12 and IFN- γ production. Induction of regulatory T cells by $1,25(\text{OH})_2\text{D}_3$ is an additional mode for inhibition of immune responsiveness. In addition, regulatory NKT cells depend on vitamin D for development and are upregulated by $1,25(\text{OH})_2\text{D}_3$. For *L. monocytogenes* and *L. major* $1,25(\text{OH})_2\text{D}_3$ suppression of the Th1 immune response is associated with decreased clearance of the microorganism. Conversely, $1,25(\text{OH})_2\text{D}_3$ increases the clearance of TB in infected human macrophage. Furthermore, clearance of a systemic infection with *C. albicans* was unaffected by $1,25(\text{OH})_2\text{D}_3$ treatment. Vitamin D should not be labeled as broadly anti-infectious or immunosuppressive and caution should be used in interpreting studies in isolation. The *in vivo* effects of $1,25(\text{OH})_2\text{D}_3$ on immune function likely depend on the vitamin D status of the host, the local expression of the VDR and Cyp27B1 and additional factors that impact on host resistance to infection or autoimmunity. The seemingly paradoxical effects of

1,25(OH)₂D₃ on Th1 mediated immunity point to a role of vitamin D and 1,25(OH)₂D₃ on multiple aspects of immunity that are still only partially understood.

Acknowledgements

This work was supported by National Institutes of Health Grant #R01 DK070781 to MTC.

Abbreviations

APC	antigen presenting cell
DC	dendritic cell
IBD	inflammatory bowel disease
IFN	Interferon
IL	interleukin
MS	multiple sclerosis
T_{reg}	regulatory T
TGF	transforming growth factor
TB	tuberculosis
TNF	tumor necrosis factor
Th1	type 1 helper
Th2	type 2 helper
VDR	vitamin D receptor
Th2	type 2 helper

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Fig. 1. CD4⁺ T helper cell subsets and functions. The black arrows ($\rightarrow$) indicate cytokines that induce differentiation and the black lines ($-$) indicate cytokines that inhibit Th cell development. The grey arrows and lines indicate induction ($\rightarrow$) or suppression ($-$) by 1,25(OH)₂D₃ treatment. Th1 immune responses are inhibited by Th2 and T reg cells and are important for protection from intracellular infections, cancer immunity and participate in autoimmune disease. Th2 cell immune responses are also important for fighting infections, can be suppressed by T reg cells and are pathogenic in asthma and allergies. Regulatory NKT cells can be either pathogenic or protective for autoimmune responses and are required for experimental asthma disease induction. All T cells that have been tested express the VDR. 1,25(OH)₂D₃ suppresses Th1 driven cytokine responses, induces T reg cells, induces IL-4 production and enhances NKT cell function.

Fig. 2.

Model of the effects of vitamin D status and 1,25(OH)₂D₃ treatment on Chron's disease. When vitamin D is low NKT cells and T reg cells fail to develop, Th1 cells are overactive and the incidence of Th1 mediated diseases increase. Vitamin D regulates the APC such that Th1 cell responses are inhibited, T reg cell function is induced and NKT cells develop. The net result is decreased Chron's disease and normalization of the Th cell response.