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Vitamin D and Multiple Sclerosis

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ABSTRACT

Vitamin D is a principal regulator of calcium homeostasis. However, recent evidence has indicated that vitamin D can have numerous other physiological functions including inhibition of proliferation of a number of malignant cells including breast and prostate cancer cells and protection against certain immune mediated disorders including multiple sclerosis (MS). The geographic incidence of MS indicates an increase in MS with a decrease in sunlight exposure. Since vitamin D is produced in the skin by solar or UV irradiation and high serum levels of 25-hydroxyvitamin D (25(OH)D) have been reported to correlate with a reduced risk of MS, a protective role of vitamin D is suggested. Mechanisms whereby the active form of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) may act to mediate this protective effect are reviewed. Due to its immunosuppressive actions, it has been suggested that $1,25(OH)_2D_3$ may prevent the induction of MS. J. Cell. Biochem. 105: 338–343, 2008.

KEY WORDS: VITAMIN D; SUNLIGHT; MULTIPLE SCLEROSIS; IMMUNE FUNCTION; 1,25-DIHDYROXYVITAMIN D_3; REVIEW

Multiple sclerosis (MS) is an inflammatory, demyelinating, and neurodegenerative disease of the central nervous system [Fontoura et al., 2006]. While the exact etiology of MS remains unknown, it is thought that many different genetic as well as environmental factors play a role [Hauser and Oksenberg, 2006]. Numerous epidemiological studies have indicated a negative correlation between increased sun exposure, which would result in a greater vitamin D synthetic rate, and diets rich in vitamin D (fortified dairy products and fish oils) and MS prevalence [Kurtzke, 1967, 2000; Goldberg, 1974; Freedman et al., 2000; van der Mei et al., 2001]. For example, MS is, for the most part, unknown in equatorial regions. The prevalence of MS increases with latitude. In Norway, although there is high MS prevalence inland, on the Norwegian coast, where there is high fish consumption, there is lower prevalence of MS. The mechanisms by which vitamin D may decrease the risk for MS, which have been suggested to involve normalization of the T-cell response, are only now beginning to be defined.

VITAMIN D

VITAMIN D METABOLISM AND MECHANISM OF ACTION

Vitamin D_3 (cholecalciferol) is taken in the diet or is synthesized in the skin from 7-dehydrocholesterol by ultraviolet irradiation. For biological activity vitamin D must be converted to its active form. Vitamin D is transported in the blood by the vitamin D binding protein (DBP; a specific binding protein for vitamin D and its metabolites in the serum) to the liver where it is hydroxylated by the 25-hydroxylase enzyme resulting in the formation of 25-hydroxyvitamin D_3 ($25(OH)D_3$), the major circulating form of vitamin D. $25(OH)D_3$ is transported by the DBP to the kidney. In the proximal convoluted and straight tubules of the kidney $25(OH)D_3$ is converted to the hormonally active form of vitamin D 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$) by the 25-hydroxyvitamin D_3 1-alpha-hydroxylase enzyme (1- alpha(OH)ase) [Christakos et al., 2003; Sutton and MacDonald, 2003; DeLuca, 2004]. Besides the proximal tubule of the kidney, placenta and macrophages are

Abbreviations used: MS, multiple sclerosis; EAE, experimental allergic encephalitis; SLE, systemic lupus erythematosus; IBD, inflammatory bowel disease; CNS, central nervous system; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; NFAT, nuclear factor of activated T-cells; NF-kappaB, nuclear factor kappa B; IL-2, interleukin 2; IL-4, interleukin 4; IL-10, interleukin 10; IL-12, interleukin 12; GM-CSF, granulocyte macrophage colony stimulating factor; DRIP, vitamin D receptor interacting protein complex; HAT, histone acetylase; VDR, vitamin D receptor; RXR, retinoid X receptor; VDRE, vitamin D response element; 1-alpha-(OH)ase: 25-hydroxyvitamin D3 1-alpha-hydroxylase; 24(OH)ase, 25-hydroxyvitamin D3 24-hydroxylase; $25(OH)D_3$, 25-hydroxyvitamin D3; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D3; KO, knock out; UV, ultraviolet.

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also sites of synthesis of 1- alpha(OH)ase. The presence of 1-alpha(OH)ase and the role for the local production of $1,25(OH)_2D_3$ in the function of additional cell types are still a matter of debate. Although the phenotype of the 1-alpha(OH)ase null mutant mice is identical to human vitamin D dependent rickets type I, it is of interest that immune dysfunction has also been noted in these mice, suggesting local production of $1,25(OH)_2D_3$ in cells in the immune system [Panda et al., 2001]. In an auto-regulatory mechanism $1,25(OH)_2D_3$ induces its own degrading enzyme, 25-hydroxyvitamin D_3 24-hydroxylase (24(OH)ase), thereby preventing vitamin D toxicity. $1,25(OH)_2D_3$ is a principal factor that regulates calcium and phosphate homeostasis. The classical target tissues are bone, kidney and intestine. However, $1,25(OH)_2D_3$ has been reported to have numerous other physiological functions including inhibition of proliferation of a number of malignant cells, including breast, and prostate cancer cells, effects on epidermal differentiation and effects on differentiation and function of cells in the immune system. $1,25(OH)_2D_3$, similar to other steroid hormones, regulates gene expression in target cells by binding stereospecifically to a high affinity, low capacity nuclear receptor (vitamin D receptor or VDR) resulting in the concentration of the $1,25(OH)_2D_3$ receptor complex in the nucleus and the activation or repression of target genes. Liganded VDR acts as a heterodimer with the retinoid X receptor (RXR), binds to vitamin D response elements (VDREs) within the promoter of target genes and, together with coactivators, affects target gene transcription [Rachez et al., 1999; Rachez and Freedman, 2000; Christakos et al., 2003; Sutton and MacDonald, 2003; DeLuca, 2004]. Coactivators include the p160 coactivators, SRC-1, SRC-2 and SRC-3 that have histone acetylase (HAT) activity. The HAT activity of the p160 coactivators is thought to destabilize the interaction between DNA and the histone core, liberating DNA for transcription. Recent studies have indicated that cooperativity between histone methyltransferases and p160 coactivators may also play a fundamental role in VDR mediated transcriptional activation [Christakos et al., 2007].

VDR mediated transcription is also mediated by the coactivator complex DRIP (vitamin D receptor interacting protein) [Rachez et al., 1999; Rachez and Freedman, 2000; Christakos et al., 2003]. This complex functions by recruitment of RNA polymerase II. It has been suggested that cell and promoter specific functions of VDR may be mediated through differential recruitment of coactivators.

SUNLIGHT AND VITAMIN D SUFFICIENCY

Total body sun exposure can provide an equivalent of 10,000 IU vitamin D per day [Holick, 1995, 2002]. However, the vitamin D_3 produced from 7-dehydrocholesterol depends on the intensity of UV irradiation which varies with latitude and season. For example in San Juan (18deg N) the skin produces vitamin D_3 all year. In Boston (42.2deg N) no vitamin D is produced from sun exposed skin from November to February and in Edmonton (52deg N) none is produced from October until April [Webb et al., 1988]. Clothing as well as sunscreen have been reported to prevent the conversion of 7-dehydrocholesterol to vitamin D_3 in the covered areas [Matsuoka et al., 1987, 1992]. Serum $25(OH)D_3$ levels, the most reliable indicator of vitamin D sufficiency, have been shown to vary with sunlight levels (there is a two month lag period between the highest and lowest level of sunlight and the respective peak and troughs in $25(OH)D_3$ levels) [Hine and Roberts, 1994; Holick, 2002]. Although there is still some controversy as to the normal and optimal blood levels of $25(OH)D_3$, most experts define vitamin D deficiency as levels of $25(OH)D_3$ below 30 ng/ml [Holick, 2007]. With this definition, a significant proportion of the world's population is vitamin D deficient. Due to the concerns regarding the association of sunlight and skin cancer and when enough sunlight is not available, vitamin D supplements are recommended. The general consensus is that the current recommended dosage 400 IU is inadequate [Vieth, 1999]. It is estimated that 1,000 IU vitamin D3/day is needed to maintain $25(OH)D_3$ level at the desired level [Heaney et al., 2003]. It has been reported that vitamin D toxicity with hypercalcemia has not been observed at doses lower than 10,000 IU per day [Hathcock et al., 2007].

VITAMIN D AND IMMUNE FUNCTION

MECHANISM OF VITAMIN D ACTION IN THE IMMUNE SYSTEM

Early studies indicating the presence of VDR in activated T-cells led to the suggestion that $1,25(OH)_2D_3$ may play a role in the regulation of the immune response. $1,25(OH)_2D_3$ can inhibit T-lymphocyte proliferation and activation [Bhalla et al., 1983]. This immunosuppressive effect is correlated with a decrease in IL-2 mRNA (one of the first genes to be expressed post-activation) as well as with a decrease in IFN-gamma and GM-CSF mRNA levels [Bhalla et al., 1986; Reichel et al., 1987; Tobler et al., 1987; Rigby, 1988]. For IL-2, IFN-gamma and GM-CSF, it has been reported that the mechanism involves VDR mediated inhibition of gene transcription [Alroy et al., 1995; Cippitelli and Santoni, 1998; Towers and Freedman, 1998]. The mechanism of the transcriptional repression of the human IL-2 gene by $1,25(OH)_2D_3$ involves block of the formation of the NFATp/AP-1 complex by the VDR/RXR heterodimer and stable association of VDR/RXR with the NFAT1 element in the IL-2 promoter [Alroy et al., 1995]. For inhibition of GM-CSF, VDR binds to the GM550 element that contains binding sites for NFAT1, Jun, and Fos. Unlike the mechanism of IL-2 repression, VDR binds to this element as a monomeric species, independent of RXR. VDR competes with NFAT for binding to the composite site, positioning itself adjacent to Jun-Fos, stabilizing the Jun-Fos complex [Towers and Freedman, 1998]. The selective interaction is between VDR and c-Jun (Fig. 1). Overexpression of c-Jun but not c-Fos is able to rescue the VDR repression. For GM-CSF repression, the model is that VDR would lock AP-1 in an off state. For the repression of IFN-gamma transcription, direct binding of VDR/RXR to a silencer region BED (−212/−183) in the human IFN-gamma promoter was suggested [Cippitelli and Santoni, 1998]. $1,25(OH)_2D_3$ has also been reported to up-regulate IL-4 under non-polarizing conditions [Cantorna et al., 1998b]. In addition, $1,25(OH)_2D_3$ has been shown to inhibit the differentiation and survival of dendritic cells, resulting in impaired alloreactive T-cell activation [Penna and Adorini, 2000; Griffin et al., 2001]. The inhibition of maturation and differentiation of dendritic cells results in a decrease in IL-12 and an increase in IL-10 secretion [Penna and Adorini, 2000]. The repressive effect of $1,25(OH)_2D_3$ on IL-12 production was reported to be at the level of

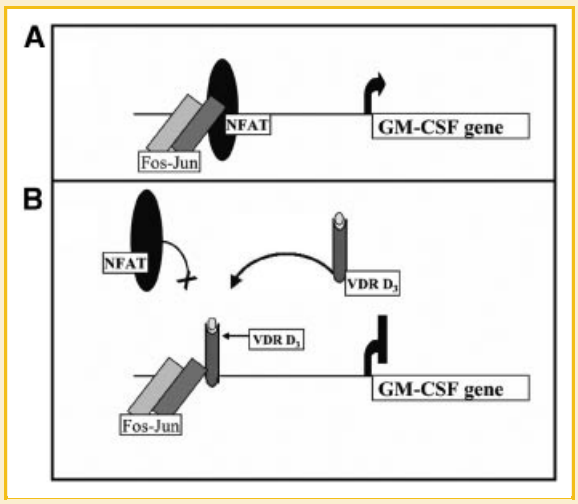

Fig. 1. Mechanism of repression of GM-CSF activated transcription by $1,25(OH)_2D_3$. Unlike most mechanisms involved in $1,25(OH)_2D_3$ mediated effects on transcription that require the VDR/RXR heterodimer, VDR bound to $1,25(OH)_2D_3$ ($VDRD_3$ in B) acts as a monomer on the GM-CSF promoter and competes with NFAT for binding to a composite NFAT AP1 site (composite site is shown in A). VDR bound to $1,25(OH)_2D_3$ also stabilizes the binding of Fos-Jun heterodimer by direct interaction with cJun (B). These two events result in $1,25(OH)_2D_3$ mediated transcriptional repression of GM-CSF [Adapted from Towers and Freedman, 1998].

transcription and required binding of the VDR/RXR heterodimer to the binding site for NF-kappaB in the IL-12 p40 promoter (p40-kB) [D'Ambrosio et al., 1998]. It is possible that the effects of $1,25(OH)_2D_3$ on IL-10, IL-4 and perhaps other cytokines may be also be indirect, resulting from an effect of $1,25(OH)_2D_3$ on other cells and genes resulting in a net change in cytokine expression.

Recent studies have indicated that $1,25(OH)_2D_3$ regulates not only adaptive but also innate immunity. $1,25(OH)_2D_3$ has been shown to induce the antimicrobial peptide cathelicidin with subsequent killing of bacteria including mycobacterium tuberculosis [Liu et al., 2007]. Thus, with regard to innate immunity, $1,25(OH)_2D_3$ may promote the host's response to a pathogen. The effect on adaptive immunity, on the other hand, may limit the immune response and may function in a process representative of tolerance.

AUTOIMMUNE DISEASES AND VITAMIN D

Previous studies have indicated that $1,25(OH)_2D_3$ can at least partially protect against a number of experimental autoimmune diseases. The experimental autoimmune diseases suppressed by $1,25(OH)_2D_3$ include experimental allergic encephalitis (EAE, the murine model of MS), experimental lupus erythematosus and autoimmune thyroiditis [Abe et al., 1990; Fournier et al., 1990; Lemire and Archer, 1991]. $1,25(OH)_2D_3$ has also been reported to prevent the progression of arthritis in murine models of human arthritis (infection of mice with Borrelia burgdorferi or immunization of mice with type II collagen) [Cantorna et al., 1998a]. $1,25(OH)_2D_3$ was also effective in collagen induced arthritis in inhibiting progression of arthritis when given to mice with early symptoms [Cantorna et al., 1998a].

In addition, $1,25(OH)_2D_3$ prevents autoimmune diabetes in nonobese diabetic (NOD) mice [Mathieu et al., 1994]. In the NOD mouse model and in the EAE model, it has been reported that an analogue of $1,25(OH)_2D_3$, 1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-vitamin D_3 and $1,25(OH)_2D_3$ respectively are effective not only in preventing the induction of the disease but also in inhibiting its progression when administered after disease onset [Cantorna et al., 1996; Gregori et al., 2002].

VITAMIN D AND MULTIPLE SCLEROSIS

INCIDENCE OF MULTIPLE SCLEROSIS VARIES WITH SUNLIGHT

Numerous epidemiological studies have reported a negative correlation between exposure to sunlight and sufficient dietary vitamin D_3 intake and prevalence of MS [Kurtzke, 1967, 2000; Goldberg, 1974; Freedman et al., 2000; van der Mei et al., 2001]. MS prevalence was shown to vary not only with latitude but also with altitude [Kurtzke, 1967]. There is lower MS incidence at high altitudes that corresponds to more optimal solar irradiation and increased cutaneous vitamin D synthesis. Also, it has been reported that immigrants, particularly if they move before the age of 15, acquire the MS risk of their new homeland, supporting the concept that early intervention to maintain vitamin D sufficiency may have a protective role against MS [Hammond et al., 2000; Pugliatti et al., 2002; van der Mei et al., 2003].

$1,25(OH)_2D_3$ INHIBITS EAE

Although numerous studies have shown that $1,25(OH)_2D_3$ inhibits EAE, the precise mechanisms involved have not been clearly defined and have been a matter of debate. $1,25(OH)_2D_3$ was found to inhibit EAE in mice lacking CD8 + T-cells but not in Rag-1 null mice [Nashold et al., 2001; Meehan and DeLuca, 2002]. The findings in the Rag-1 null mice suggest that $1,25(OH)_2D_3$ acts through a Rag-1 dependent cell to limit the occurrence of Th1 activation in the CNS. It has been suggested that CD4 + T-cells are a target of $1,25(OH)_2D_3$ immunosuppression in EAE and that $1,25(OH)_2D_3$ may sensitize CD4 + T-cells to apoptotic stimuli [Pedersen et al., 2007]. Both an increase and no change in IL-4 in lymph nodes in EAE after $1,25(OH)_2D_3$ treatment have been reported [Cantorna et al., 1998b; Nashold et al., 2001]. In response to $1,25(OH)_2D_3$, a reduction and no change in interferon gamma have been described [Nashold et al., 2001; Muthian et al., 2006]. The lack of consistency may be due to both indirect and direct effects of $1,25(OH)_2D_3$. Further studies need to be done to differentiate between these effects. With regard to IL-4, using IL-4 KO mice it has been reported that EAE is more severe in the absence of IL-4 and IL-4 KO mice are resistant to $1,25(OH)_2D_3$ treatment [Cantorna et al., 2000]. $1,25(OH)_2D_3$ is also unable to inhibit EAE in IL-10 or IL-10 receptor KO mice, suggesting IL-10 dependent protective effects of $1,25(OH)_2D_3$ [Spach et al., 2006]. It has also been reported that in vivo treatment with $1,25(OH)_2D_3$ or $1,25(OH)_2D_3$ analogue inhibits EAE in association with inhibition of IL-12 [Mattner et al., 2000].

Gender differences have also been noted in the effect of $1,25(OH)_2D_3$ or vitamin D on EAE. It has been reported that higher concentrations of $1,25(OH)_2D_3$ are needed to prevent EAE in male mice compared to female mice [Cantorna et al., 1999]. In addition, it was found that 5 μg/day of vitamin D_3 significantly inhibited EAE in

female but not male mice [Spach and Hayes, 2005]. In ovariectomized mice the protective effect of vitamin D was not observed. In the vitamin D study both male and female mice had equivalent levels of serum $1,25(OH)_2D_3$ [Spach and Hayes, 2005]. Further studies examining mechanisms involved in the gender differences in the response to $1,25(OH)_2D_3$ or vitamin D_3 are needed.

It has been suggested that the effects of $1,25(OH)_2D_3$ or vitamin D could include effects in the CNS. However, it should be noted, when suggesting central effects of $1,25(OH)_2D_3$, that $1,25(OH)_2D_3$ receptors are present in low levels in the brain and have a very localized distribution in the CNS [Stumpf et al., 1982; Stumpf and O'Brien, 1987]. $1,25(OH)_2D_3$ sites of action have been identified by autoradiography within certain structures in the forebrain, hindbrain, and spinal cord [Stumpf et al., 1988]. The central nucleus of the amygdala and the bed nucleus of the stria terminalis have the densest accumulation of $1,25(OH)_2D_3$. In the spinal cord nuclear retention of $1,25(OH)_2D_3$ is strongest in motor neurons in lamina IX. Nuclear retention is also observed in neurons of lamina II, VIII and X and in cells in the caudal spinal trigeminal nucleus. Immunocytochemical studies are generally in agreement with the autoradiographic findings [Prufer et al., 1999]. Also, restricted transport of vitamin D metabolites to the brain has been reported, at least in wild type rodent brain. Unlike estrogens, in which the protein steroid complex freely enters the brain, it has been reported that the vitamin D serum binding protein limits access of $1,25(OH)_2D_3$ into the CNS [Gascon-Barre and Huet, 1983]. In addition, although it has been suggested that $1,25(OH)_2D_3$ could be synthesized from $25(OH)D_3$ in the CNS and thus could account for observed effects of vitamin D, it should be noted that the presence of 1-alpha-(OH)ase at sites other than kidney, placenta and macrophages is a matter of debate [Shultz et al., 1983].

The role of calcium in the $1,25(OH)_2D_3$ suppressive effects in EAE also needs to be considered. Low calcium diets have been reported to result in resistance to treatment with $1,25(OH)_2D_3$ and it was found that calcium is required for the suppressive effect in EAE [Cantorna et al., 2000]. It is of interest that in the lupus model MRL/1 mice, low calcium plus $1,25(OH)_2D_3$ accelerated SLE while mice on the normal calcium (0.87%) diet showed reduced SLE with $1,25(OH)_2D_3$ treatment [DeLuca and Cantorna, 2001]. In addition, inflammatory bowel disease (IBD) is prevented by $1,25(OH)_2D_3$ administration also under conditions of normal calcium [DeLuca and Cantorna, 2001]. Unlike EAE, SLE and IBD, $1,25(OH)_2D_3$ can prevent collagen induced arthritis under low dietary calcium conditions [Cantorna et al., 1996; DeLuca and Cantorna, 2001]. Thus low calcium results in resistance to $1,25(OH)_2D_3$ in some but not all autoimmune diseases. These studies should help in the design of analogues that will be effective in vivo. It has recently been reported that the combination of the $1,25(OH)_2D_3$ analogue TX527 with interferon beta resulted in protection against EAE that was more effective than each treatment alone, suggesting consideration of a combination treatment for clinical intervention in MS [van Etten et al., 2007].

DOES VITAMIN D OR $1,25(OH)_2D_3$ ANALOG HAVE CLINICAL POTENTIAL FOR MS?

Although the effects of $1,25(OH)_2D_3$ in the EAE model are suggestive, the question that remains is whether vitamin D_3 or $1,25(OH)_2D_3$ analogues have clinical potential. A number of studies have shown that high serum levels of $25(OH)D_3$ are associated with a decreased risk of MS. The largest of these trials by Munger et al. in [2006] searched the US Army and Navy databases and found 257 cases of military personnel with diagnosed MS and stored serum available for testing of $25(OH)D_3$ levels [Munger et al., 2006]. A case matched analysis using 514 matched controls showed that MS incidence was lower in samples from 148 subjects with the highest circulating levels of $25(OH)D_3$. The inverse relationship between $25(OH)D_3$ levels and MS was particularly strong for $25(OH)D_3$ levels measured before age 20, suggesting that vitamin D supplements in adolescents and young adults, particularly those who are at high risk for MS (with a family history, for example), may have protective benefit. A protective effect of cod liver oil and regular fish consumption has also been reported [Kampman et al., 2007]. A large clinical trial will be required to determine whether vitamin D or analogue, alone or in combination with known treatments, is not only protective but is also effective in patients with active MS. A better understanding of the mechanisms by which $1,25(OH)_2D_3$ acts to affect the immune system is needed in order to aid in the design of $1,25(OH)_2D_3$ analogues with clinical potential for MS.

CONCLUSION

For individuals predisposed to MS, evidence indicates that maintenance of adequate vitamin D has a protective effect. One mechanism of vitamin D action may be to maintain balance in the T-cell response and thus avoid autoimmunity. Further studies are needed with regard to mechanisms involved in $1,25(OH)_2D_3$ mediated immune regulation and to determine whether vitamin D or analogue, alone or in combination with other treatments, is not only protective but is also effective in patients with active MS.

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