Vitamin D and Multiple Sclerosis
Colleen E. Hayes, Margherita T. Cantorna and Hector F. DeLuca

doi: 10.3181/00379727-216-44153A

Updated information and services can be found at:
http://ebm.rsmjournals.com/content/216/1/21

28 online articles that cite this article can be accessed at:
http://ebm.rsmjournals.com/content/216/1/21#otherarticles

© 2008 Society for Experimental Biology and Medicine
MINIREVIEW

Vitamin D and Multiple Sclerosis (44153A)

COLLEEN E. HAYES, MARGHERITA T. CANTORNA, AND HECTOR F. DELUCA
Department of Biochemistry, University of Wisconsin–Madison, Madison, Wisconsin 53706

Abstract. Recently, it has been clearly demonstrated that exogenous 1,25-dihydroxyvitamin D₃, the hormonal form of vitamin D₃, can completely prevent experimental autoimmune encephalomyelitis (EAE), a widely accepted mouse model of human multiple sclerosis (MS). This finding has focused attention on the possible relationship of this disease to vitamin D. Although genetic traits certainly contribute to MS susceptibility, an environmental factor is also clearly involved. It is our hypothesis that one crucial environmental factor is the degree of sunlight exposure catalyzing the production of vitamin D₃ in skin, and, further, that the hormonal form of vitamin D₃ is a selective immune system regulator inhibiting this autoimmune disease. Thus, under low-sunlight conditions, insufficient vitamin D₃ is produced, limiting production of 1,25-dihydroxyvitamin D₃, providing a risk for MS. Although the evidence that vitamin D₃ is a protective environmental factor against MS is circumstantial, it is compelling. This theory can explain the striking geographic distribution of MS, which is nearly zero in equatorial regions and increases dramatically with latitude in both hemispheres. It can also explain two peculiar geographic anomalies, one in Switzerland with high MS rates at low altitudes and low MS rates at high altitudes, and one in Norway with a high MS prevalence inland and a lower MS prevalence along the coast. Ultraviolet (UV) light intensity is higher at high altitudes, resulting in a greater vitamin D₃ synthetic rate, thereby accounting for low MS rates at higher altitudes. On the Norwegian coast, fish is consumed at high rates and fish oils are rich in vitamin D₃. Further, experimental work on EAE provides strong support for the importance of vitamin D₃ in reducing the risk and susceptibility for MS. If this hypothesis is correct, then 1,25-dihydroxyvitamin D₃ or its analogs may have great therapeutic potential in patients with MS. More importantly, current research together with data from migration studies opens the possibility that MS may be preventable in genetically susceptible individuals with early intervention strategies that provide adequate levels of hormonally active 1,25-dihydroxyvitamin D₃ or its analogs.

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) that is debilitating and can be fatal (1). The disease strikes typically between ages 20 and 40, and afflicts about 350,000 people in the United States alone. The key pathological mechanism in MS appears to involve either a breakdown in immunological self-tolerance and/or a neurotropic or peripheral infection, and a subsequent inflammatory demyelinating attack on the CNS. The initiation of this pathological process in MS represents a complex interaction of genetic and environmental forces that are not completely understood. Here, we review evidence supporting our hypothesis that sunlight performs a protective function in MS due to the action of ultraviolet (UV) light as a catalyst in an early biosynthetic step leading to hormonally active 1,25-dihydroxyvitamin D₃ (1,25-[OH]₂D₃) synthesis, and the activity of this hormone as a selective immune system regulator.
that for some reason triggers a loss of self-tolerance (1, 9).

22 MS AND VITAMIN D

Environmental Risk Factors

The clustering of MS within families suggests that genetic traits contribute to the disease mechanism (2). Biological first-degree relatives show a 20- to 40-fold increased risk of developing MS compared with unrelated individuals, and monozygotic twins show a further 10-fold increased risk compared with dizygotic twins (3). Three genome scans for MS susceptibility genes have been published. Significant linkage of MS susceptibility genes to the major histocompatibility complex HLA-DR locus at 6p21 was reported by two research teams (4, 5), but not a third group (6). The HLA-DR2 allele emerged as an important MS susceptibility factor. There were also significant linkages of MS susceptibility genes to the Chromosome 5p (6) and 17q22 (5) regions. Together these genome scans suggest that no single MS susceptibility locus is necessary and sufficient to cause disease. Rather, the genetic etiology likely results from the action of several genes.

Importantly, the 70% discordance rate for MS between monozygotic twins (3) emphasizes that nongenetic factors may well be of equal or greater significance than genetic risks in determining MS disease. These twin studies show incomplete penetrance of the susceptibility genes. In other words, inheritance of a susceptible genotype is not sufficient to cause the disease. If nongenetic risk factors are identifiable and avoidable, then MS disease may be preventable in genetically susceptible individuals.

The Immune System and CNS Pathology

In MS patients, the neurological symptoms (visual, sensory, and motor dysfunctions) are thought to arise through a neurodegenerative process (1, 7). The CNS shows lesions with a characteristic inflammatory cell infiltrate and demyelination. The cause of the neurodegenerative process is uncertain. One possibility is a persistent CNS infection (the neurotropic pathogen model) (8). A variety of neurotropic microorganisms have been implicated in MS, although conclusive evidence for causality has not been forthcoming (9).

In this model, the neurodegenerative process is thought to result from the CNS infection, and the lymphocyte and monocyte infiltration is viewed as a consequence of infection. A variation of the neurotropic pathogen model suggests that the neurodegenerative process might be attributable first to a CNS infection and secondarily to an autoimmune attack against CNS components. The primary CNS infection might precipitate a loss of immunological self-tolerance to CNS antigens, thereby initiating an autoimmune-mediated attack. In this model, some infiltrating T lymphocytes would be specific for epitopes of the pathogen and others for epitopes of CNS proteins.

A third model postulates that the neurodegenerative process may be a consequence of an autoimmune-mediated attack on the CNS pursuant to an infection in the periphery that for some reason triggers a loss of self-tolerance (1, 9). In this view, the infection could generate an inflammatory immune reaction in which epitopes of the infectious pathogen bearing strong structural homology to epitopes of self CNS proteins like myelin basic protein (MBP) might trigger self-reactive T cells. In the models that invoke autoimmunity, the HLA-DR-linked MS susceptibility gene is thought to encode a class II molecule capable of selectively binding peptides from both the infectious pathogen and from a CNS protein and triggering self-reactive T cells (10). No role has been proposed for the MS susceptibility genes that are not HLA-DR linked. Once triggered, T cells reactive with CNS antigens might migrate into the CNS and mediate neurodegeneration. The peripheral infection–autoimmunity model is most consistent with the clinical observation that several types of peripheral infections often precede the onset of MS symptoms and/or exacerbations (11–13).

Environment, Solar Radiation, and Diet

The incomplete penetrance of the MS susceptibility genes points directly to a crucial role for some environmental factor in disease development. Some investigators have argued that an infectious microorganism is the disease-determining environmental agent, based on clustering of cases in time and space (8). There is no doubt that viral infections can occasionally precipitate autoimmunization and CNS demyelination in humans (14). However, the strongest evidence against an infectious microorganism as the disease-determining environmental agent comes from the monozygotic twin studies (3). The infectious agent hypothesis predicts a high MS concordance rate among these twins, and the 30% concordance rate is actually quite low.

We favor an alternative theory, first suggested by Goldberg (15, 16; discussed below), that the disease-determining environmental agent may be an insufficiency of the UV light that is essential for vitamin D3 biosynthesis. Many epidemiological studies provide evidence that exposure to solar radiation may have a protective effect in MS (2, 17–19). Davenport (20) first noticed the striking geographic distribution of MS; he reported that soldiers born in the northern United States were more commonly afflicted with MS than those born in the southern United States. Three decades later, mortality, prevalence, and incidence statistics for MS in the United States and Canada confirmed these observations (21–23). Worldwide epidemiological studies have since shown that the MS prevalence rate is nearly zero in equatorial regions and increases to more than 50 per 105 population at latitudes greater than 45° in both the northern and southern hemispheres.

A plethora of environmental factors have been considered in an attempt to understand the puzzling MS geographic distribution. Acheson et al. (24) examined the birth counties of U.S. veterans diagnosed with MS between 1954 and 1958. These investigators found a significant positive correlation between MS rate and latitude, and impressive negative correlations between MS rate and average annual hours of sunshine or average December daily solar radiation. Norman et al. (25) reported similar findings in a study...
of U.S. World War II veterans. These and other reports ruled out air pollution, minerals in ground water, annual periods of high and low temperatures, annual rainfall, and average humidity as correlates with MS disease. Through multiple regression analyses, the Acheson group (24) showed that the contribution of latitude independently of average annual sunshine or December daily solar radiation was insignificant, whereas both sunshine and December daily solar radiation were significantly and inversely correlated with MS independently of latitude. These researchers concluded that sunshine “could conceivably act directly—a certain skin dose of sunshine per unit time protecting the individual in some way.”

One confounding problem in epidemiological studies is the difficulty of separating genetic and environmental factors. For example, the genotypes of individuals living in northern and southern Europe are quite different, so the comparison of MS prevalence rates between these two populations involves both genetic and environmental variables. One way to examine the effects of environment on a relatively homogeneous genotype is to study migrating populations. The migration studies have reinforced the concept of a disease-determining environmental agent and, more importantly, have indicated that there may be a win-

A second anomaly is the relatively high MS risk in Scandinavia except along the Atlantic coast of Norway (31, 32). This anomaly is also unlikely to have a genetic basis, but it may have a dietary basis. Most foods have little vitamin D content, but fish oil is a rich source of vitamin D3. Norwegians living in coastal fishing districts consumed an amount of fish and margarine that provided about 1300 IU of vitamin D3 daily, about 3-fold higher than individuals living in the inland agricultural districts (15). Separately, two controlled trials demonstrated that fish oil ingested with supplementary calcium and magnesium (33) or fish oil ingested alone (34) significantly lessened MS disease symptoms. Thus, the geographic anomaly in Norway may reflect differences in vitamin D3 intake attributable to dietary preferences.

UV Light, 1,25-(OH)2D3 Biosynthesis, and Multiple Sclerosis

Goldberg first theorized that insufficient UV light to support adequate vitamin D3 biosynthesis could be the environmental risk factor contributing to MS development in genetically susceptible individuals (15, 16). He further hypothesized that during pubescent CNS development, genetically predisposed individuals may require more vitamin D3 and calcium than other individuals for the biosynthesis of normal myelin. As a corollary, Goldberg speculated that inadequate vitamin D3 and calcium might alter lipid metabolism and yield unstable myelin of abnormal lipid composition and structure.

Based on the amount of sunshine in low–MS prevalence areas and the rate at which human skin produces vitamin D (7 IU/hr cm2), Goldberg estimated that 3800 IU/day of vitamin D might prevent MS (16). This is almost 10-fold higher than the dietary antirachitic dose that was used to establish the recommended daily allowance (RDA) for dietary vitamin D3. The RDA is 400 IU for youth (age 1–24 years) and 200 IU for adults (age >24 years). However, average dietary vitamin D3 intakes in the US are only 30% or so of this RDA (35).

It is likely that the major source of vitamin D for mammals is, in fact, not dietary but results from its manufacture by a chemical photolysis reaction in skin (36) as illustrated in Figure 1. A cholesterol metabolite, 7-dehydrocholesterol, is abundant in the epidermis probably because it is the substrate from which vitamin D is produced. UV light 282–310 nm in wavelength will penetrate into the epidermis where it is absorbed by 7-dehydrocholesterol (37, 38). This energy ruptures the 9,10-bonds; then a 1,7-sigmatropic shift produces previtamin D3. Previtamin D3 slowly isomerizes to vitamin D3, which is thermodynamically more stable. This equilibration at body temperature occurs over a 24- to 38-hr period (36). These reactions are strictly chemical, not involving proteins or enzymes. Vitamin D synthesis in skin is a relatively efficient process in which 10 min of summer sun
The production of vitamin D in skin by ultraviolet light from sun and its subsequent conversion to its active hormonal form, 1,25-dihydroxyvitamin D₃, is tightly regulated by the need for calcium and the need for phosphorus. The need for calcium is interpreted by the parathyroid glands that secrete parathyroid hormone in response to hypocalcemia. This peptide hormone has a major function in activating the 25-OH-D₁α-hydroxylase in the kidney. 1,25-(OH)₂D₃ is the functional form of vitamin D in all systems studied so far. All other metabolites, such as 24,25-(OH)₂D₃ and others, represent inactivation products.

Vitamin D₃ itself is a biologically inert molecule. It must be activated by 25-hydroxylation in the liver to produce the major circulating form of vitamin D, 25-hydroxyvitamin D₃ (25-OH-D₃) (45). This compound is generally assessed by clinical investigators to determine vitamin D status of a patient afflicted with metabolic bone disease or calcium disorders (46). However, 25-OH-D₃ is also biologically inactive at physiological concentrations (47, 48). It is finally activated in the proximal convoluted tubule cells of the kidney to produce the vitamin D hormone, 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) (45, 49). The production of 1,25-(OH)₂D₃ is tightly regulated by the need for calcium and the need for phosphorus (45, 49). The need for calcium is interpreted by the parathyroid glands that secrete parathyroid hormone in response to hypocalcemia. This peptide hormone has a major function in activating the 25-OH-D₁α-hydroxylase in the kidney (50). 1,25-(OH)₂D₃ is the functional form of vitamin D in all systems studied so far. All other metabolites, such as 24,25-(OH)₂D₃ and others, represent inactivation products (51). Ultimately, 1,25-(OH)₂D₃ is metabolized to calcitroic acid as its major excretory form (51). Full details of the metabolic fate of all vitamin D molecules are not entirely known (51).

1,25-(OH)₂D₃ functions through a nuclear receptor that is a member of the superfamily of transcriptionally active steroid hormone receptors. The receptor structure is known, and a great deal of detail is known concerning the molecular mechanism whereby 1,25-(OH)₂D₃ influences transcription of target genes. Interested readers are referred to recent reviews in this area (52, 53).

The discovery that peripheral blood monocytes and activated T lymphocytes have 1,25-(OH)₂D₃ receptors (VDR) first indicated that vitamin D might regulate some immune functions (54–56). More recently, the delayed hypersensitivity response (T cell mediated) was found impaired in vitamin D deficiency (57) and suppressed by 1,25-(OH)₂D₃ (58).

Despite many investigations of 1,25-(OH)₂D₃ action on immune cells in vitro, the immunomodulatory activity of this hormone remains largely undefined because the in vitro results are often conflicting. A comprehensive discussion of them is beyond the scope of this review; other reviews have summarized the in vitro studies (59–61).

A few reports have described 1,25-(OH)₂D₃ action on immune functions in vivo. Three of these studies are of special interest because they investigated experimental autoimmune encephalomyelitis (EAE), a widely accepted rodent model of MS. When susceptible rodent strains are immunized with CNS proteins like myelin basic protein (MBP) in an adjuvant, they develop an MS-like paralytic disease. The paralysis is acute and lethal in strain SJL mice, but relapsing and remitting in strain B10.PL mice.

Lemire and Archer (62) first explored possible effects of 1,25-(OH)₂D₃ on EAE in SJL mice. Branisteanu et al. (63) contributed a second, similar study. The mice were immunized with a very high dose of MBP (62) or mouse spinal cord (63), and the mice died of uncertain causes within 2 weeks. The immunized mice that received low 1,25-(OH)₂D₃ doses ip every other day starting 3 days prior to immunization all died within 2 weeks, whether they were fed a normal amount of calcium (62) or a low-calcium diet (63). When a higher 1,25-(OH)₂D₃ dose was injected as
above and the mice were also fed a low-calcium diet, the mice had prolonged survival compared with controls. It is not clear in either report whether the low-calcium diet and/or supplemental 1,25-(OH)_{2}D_{3} provided the benefit.

We studied the effects of 1,25-(OH)_{2}D_{3} on relapsing and remitting EAE induced with a low dose of MBP in strain B10.PL mice (64). Supplying 1,25-(OH)_{2}D_{3} in a diet high in calcium (2%) 1 day before EAE induction completely prevented the appearance of any disability whatsoever; in the control mice, the incidence was 100%, and the paralytic symptoms were uniformly severe. Secondly, EAE was induced in a large number of mice, and, when the mice first showed disease symptoms, half of them were treated with 1,25-(OH)_{2}D_{3}. The treated mice showed insignificant EAE disease progression, whereas the untreated mice progressed to severe paralysis. This experiment was continued and treatment was withdrawn from half of the treated mice. After a 10-day lag, the mice from whom treatment was withdrawn showed EAE disease progression. In a final experiment, mice were made vitamin D deficient, and EAE disease was induced as before. The vitamin D–deficient mice had a more rapid onset of disease compared with the D-sufficient littermate controls.

Our findings that 1,25-(OH)_{2}D_{3} fed in advance completely prevented EAE induction, that it reversibly blocked established EAE progression, and that vitamin D deficiency accelerated EAE onset (64) provide strong evidence that this hormone is a crucial regulator in the EAE disease model. Since the EAE model is known to require autoimmune T lymphocytes to establish the EAE disease symptoms (reviewed in Ref. 1), it follows that 1,25-(OH)_{2}D_{3} is a crucial and selective regulator of immune system function. If EAE accurately models MS, then our results support the hypothesis that vitamin D status may be a disease-determining environmental factor regulating MS development in genetically susceptible individuals. Also consistent with this theory is a recent report that vitamin D deficiency is prevalent in MS patients (65); whether vitamin D deficiency preceded or followed development of MS in these individuals is unknown.

### A Unifying Hypothesis

Effective intervention to control MS will require elucidating the biochemical nature of the environmental factor(s) that determines whether a pathological demyelinating attack on the CNS will occur in genetically susceptible individuals. The studies of MS geographic distribution, MS incidence in migrating populations, and MS in twins are mainly consistent with the idea that there is a disease-determining environmental risk factor, and, furthermore, that MS may be preventable in genetically susceptible individuals if that risk is eliminated. In the human life span, there may be a window when the environmental risk factor is particularly significant. Like Goldberg (15, 16), we believe that a disease-determining environmental risk factor for MS may be insufficient vitamin D_{3} from UV light–catalyzed biosynthesis or from the diet. The evidence that supports this hypothesis is indirect but compelling. The MS geographic distribution and the geographic anomaly in Switzerland could be due to availability of sunlight for vitamin D_{3} biosynthesis, while the geographic anomaly in Norway could be due to vitamin D_{3} in the diet. One small study showed that vitamin D_{3} deficiency was prevalent in MS patients (65), and two small studies showed that experimental diets rich in vitamin D_{3} ameliorated MS symptoms (33, 34).

The precise function that vitamin D_{3} might carry out to reduce the risk of MS is uncertain. Unlike Goldberg, who speculated that vitamin D_{3} and calcium may be required for myelin biosynthesis, we theorize that 1,25-(OH)_{2}D_{3} may be required as a selective regulator of immune system function. A wealth of information has been gathered on the neurodegenerative process in EAE and in MS, and the results are consistent with an autoimmune inflammatory attack on the CNS, perhaps triggered by structural homologies between infectious pathogens and self CNS proteins. Consistent with this paradigm, we can envision at least two possible functions for 1,25-(OH)_{2}D_{3}. First, the hormone may inhibit the development or function of encephalitogenic cells, and, second, the hormone may be required for the development or function of cells with a protective role. These possibilities are not mutually exclusive. Regardless of its mechanism of action, we believe that the circumstantial evidence supporting a protective role for vitamin D_{3} in MS, and that our experimental evidence in EAE is sufficiently compelling to warrant a clinical trial.

---

57. Yang S, Smith C, Prahl JM, DeLuca HF. Vitamin D deficiency sup-


