PSEBM

Proceedings of the Society for Experimental Biology and Medicine

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Colleen E. Hayes, Margherita T. Cantorna and Hector F. DeLuca

Proc Soc Exp Biol Med 1997, 216:21-27.
doi: 10.3181/00379727-216-44153A

MINIREVIEW

Vitamin D and Multiple Sclerosis (44153A)

COLLEEN E. HAYES,[1] 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 D_3, the hormonal form of vitamin D_3, 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 D_3 in skin, and, further, that the hormonal form of vitamin D_3 is a selective immune system regulator inhibiting this autoimmune disease. Thus, under low-sunlight conditions, insufficient vitamin D_3 is produced, limiting production of 1,25-dihydroxyvitamin D_3, providing a risk for MS. Although the evidence that vitamin D_3 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 D_3 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 D_3. Further, experimental work on EAE provides strong support for the importance of vitamin D_3 in reducing the risk and susceptibility for MS. If this hypothesis is correct, then 1,25-dihydroxyvitamin D_3 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 D_3 or its analogs.** [P.S.E.B.M. 1997 Vol 216]

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 D_3 (1,25-$[OH]_2D_3$) synthesis, and the activity of this hormone as a selective immune system regulator.

[1] To whom correspondence should be addressed at Department of Biochemistry, University of Wisconsin—Madison, 420 Henry Mall, Madison, WI 53706. No reprints are available from the authors.
This work was supported by the National Institutes of Health Grants DK 46820 (C.E.H.) and DK 14881 (H.F.D.), the National Multiple Sclerosis Society Grant PP0509 (C.E.H.), the National Foundation for Cancer Research (H.F.D.), and the Wisconsin Alumni Research Foundation (H.F.D.).

0037-9727/97/2161-0021$10.50/0

Genetics

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 D_3 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 10^5 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 window of the human life span within which this factor exerts its most significant influence (2, 19, 26).

Dean (27) and Kurtzke *et al.* (28) studied immigrants from the United Kingdom, a high-risk region, to South Africa, a low-risk region. Adult immigrants (age $\geq$15 years) showed a high relative risk equal to that in the United Kingdom and 6-fold greater than immigrant children, whose low risk was equal to that in South Africa. Studies of migrations from low-risk regions to high-risk regions have shown similar trends; adult immigrants mainly retain the relative risk of their country of origin, whereas prepubescent immigrants mainly acquire the relative risk of their new homeland (reviewed in Ref. 2). While migration studies must be interpreted cautiously because they involve small populations whose genetic makeup may not be representative of the country of origin, these studies have led to the belief that the pathological processes underlying MS begin during childhood, whereas the disease symptoms appear much later. Most significantly, these migration studies reinforce the possibility that MS is preventable in genetically susceptible individuals if the environmental risk factor is reduced.

Certain anomalies in the geographic distribution of MS prevalence hint that the environmental risk factor could be vitamin D_3. In Switzerland, districts with high MS rates were at low altitudes ($\leq$1000 m), whereas districts with low MS rates were at high altitudes ($\geq$2000 m) (29). Since the Swiss population is genetically relatively homogeneous and stable, this anomaly is unlikely to have a genetic basis (29). Geiger (30) documented that the high altitudes receive more intense solar radiation than the low altitudes, especially short-wavelength UV light. Thus, the geographic anomaly in Switzerland may reflect differences in vitamin D_3 biosynthesis attributable to intensity of short-wavelength solar radiation.

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 D_3. Norwegians living in coastal fishing districts consumed an amount of fish and margarine that provided about 1300 IU of vitamin D_3 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 D_3 intake attributable to dietary preferences.

UV Light, 1,25-$(OH)_2D_3$ Biosynthesis, and Multiple Sclerosis

Goldberg first theorized that insufficient UV light to support adequate vitamin D_3 biosynthesis could be the environmental risk factor contributing to MS development in genetically susceptible individuals (15, 16). He further hypothesized that during prepubescent CNS development, genetically predisposed individuals may require more vitamin D_3 and calcium than other individuals for the biosynthesis of normal myelin. As a corollary, Goldberg speculated that inadequate vitamin D_3 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 cm^2), 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 D_3. The RDA is 400 IU for youth (age 1–24 years) and 200 IU for adults (age >24 years). However, average dietary vitamin D_3 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 D_3. Previtamin D_3 slowly isomerizes to vitamin D_3, 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

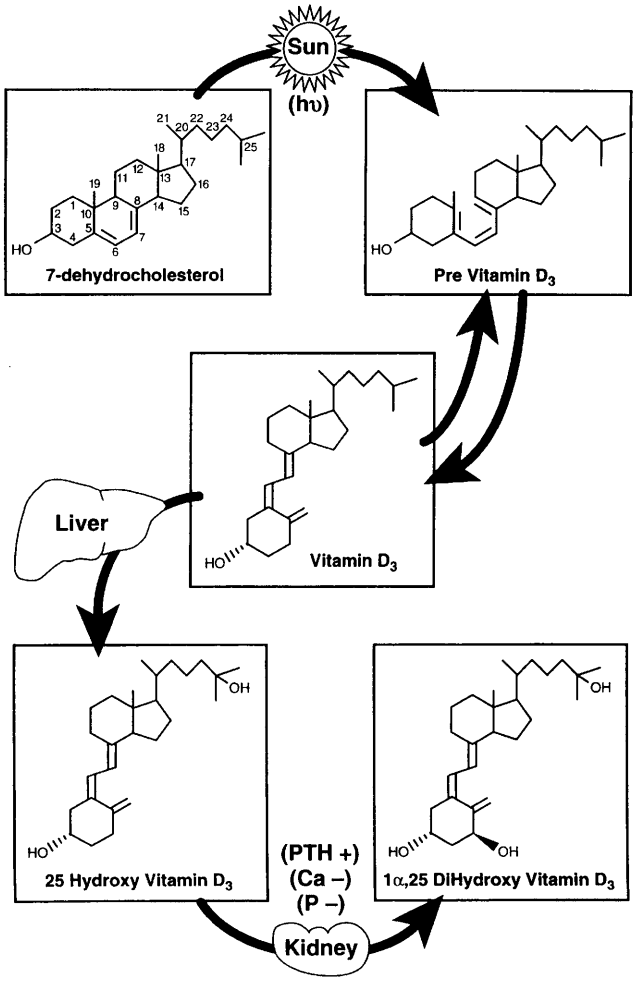

Figure 1. The production of vitamin D in skin by ultraviolet light from sun and its subsequent conversion to its active hormonal form, 1α-dihydroxyvitamin D_3. The chromophore of 7-dehydrocholesterol absorbs 282 to 310-nm ultraviolet light, resulting in the production of previtamin D. This slowly equilibrates to vitamin D_3, which is then transported to the liver and converted to 25-hydroxyvitamin D_3. It is finally activated to the hormonal form, 1α,25-dihydroxyvitamin D_3 in the proximal convoluted tubule cells of the kidney. The latter reaction is strongly regulated positively by parathyroid hormone (PTH +) and negatively by calcium (CA –) and phosphorus (P –) in the plasma. Calcium regulation may be entirely through regulation of the parathyroid hormone, or PTH.

at the latitude of Boston will produce the normal daily vitamin D requirement for humans (i.e., 10 μg or 400 IU) (39). Skin of the elderly is less able to produce vitamin D (39), and dark skin is also less efficient at vitamin D production (40, 41). Much is known concerning the production of vitamin D in skin and any interested readers are directed to available reviews (42–44). Bound to the vitamin D transport protein, vitamin D_3 is then transported in the serum to the liver.

Vitamin D_3 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 D_3 (25-OH-D_3) (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-D_3 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 D_3 (1,25-$[OH]_2D_3$) (45, 49). The production of 1,25-$(OH)_2D_3$ 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-D-1α-hydroxylase in the kidney (50). 1,25-$(OH)_2D_3$ is the functional form of vitamin D in all systems studied so far. All other metabolites, such as 24,25-$(OH)_2D_3$ and others, represent inactivation products (51). Ultimately, 1,25-$(OH)_2D_3$ 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-$(OH)_2D_3$ 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-$(OH)_2D_3$ 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-$(OH)_2D_3$ 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-$(OH)_2D_3$ (58).

Despite many investigations of 1,25-$(OH)_2D_3$ 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-$(OH)_2D_3$ 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-$(OH)_2D_3$ 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-$(OH)_2D_3$ 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-$(OH)_2D_3$ 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-$(OH)_2D_3$ provided the benefit.

We studied the effects of 1,25-$(OH)_2D_3$ on relapsing and remitting EAE induced with a low dose of MBP in strain B10.PL mice (64). Supplying 1,25-$(OH)_2D_3$ 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-$(OH)_2D_3$. 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-$(OH)_2D_3$ 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-$(OH)_2D_3$ 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-$(OH)_2D_3$ 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-$(OH)_2D_3$. First, the hormone may inhibit the development or function of encephalitogenic cels, 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.

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