

MINIREVIEW

Vitamin D and Multiple Sclerosis (44153A)

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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. [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 D₃ (1,25-[OH]₂D₃) synthesis, and the activity of this hormone as a selective immune system regulator.

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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 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 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 ≥ 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 D₃. In Switzerland, districts with high MS rates were at low altitudes (≤ 1000 m), whereas districts with low MS rates were at high altitudes (≥ 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 D₃ bio-

synthesis 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 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 prepubescent 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

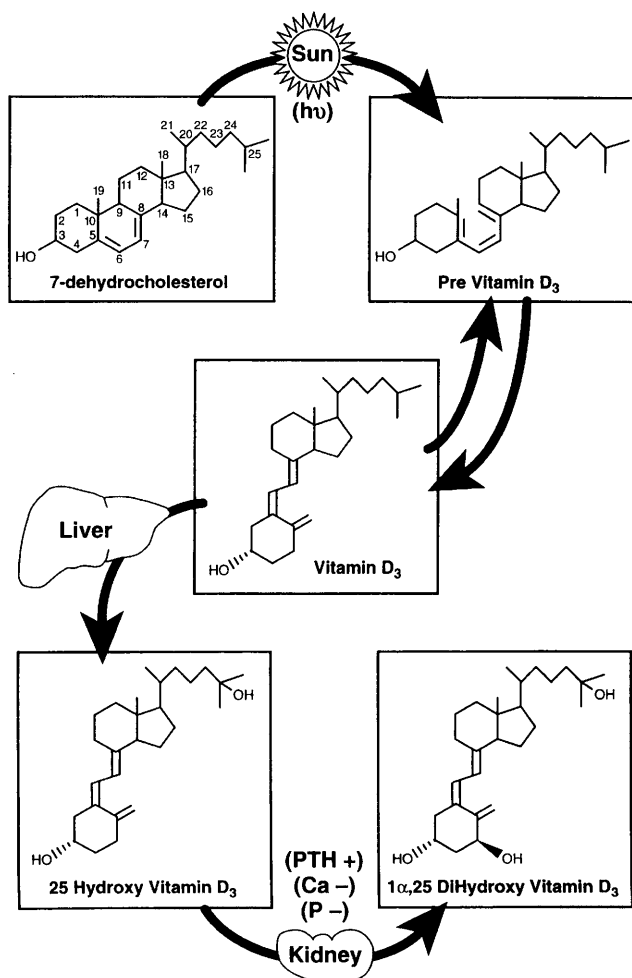


Figure 1. The production of vitamin D in skin by ultraviolet light from sun and its subsequent conversion to its active hormonal form, 1α -dihydroxyvitamin D₃. The chromophore of 7-dehydrocholesterol absorbs 282 to 310-nm ultraviolet light, resulting in the production of previtamin D. This slowly equilibrates to vitamin D₃, which is then transported to the liver and converted to 25-hydroxyvitamin D₃. It is finally activated to the hormonal form, $1\alpha,25$ -dihydroxyvitamin D₃ 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 μ g 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 D₃ is then transported in the serum to the liver.

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-D-1 α -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 calcitric 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)₂D₃ provided the benefit.

We studied the effects of 1,25-(OH)₂D₃ on relapsing and remitting EAE induced with a low dose of MBP in strain B10.PL mice (64). Supplying 1,25-(OH)₂D₃ 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)₂D₃. 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)₂D₃ 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)₂D₃ 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 D₃ 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 D₃ biosynthesis, while the geographic anomaly in Norway could be due to vitamin D₃ in the diet. One small study showed that vitamin D₃ deficiency was prevalent in MS patients (65), and two small studies showed that experimental diets rich in vitamin D₃ ameliorated MS symptoms (33, 34).

The precise function that vitamin D₃ might carry out to reduce the risk of MS is uncertain. Unlike Goldberg, who speculated that vitamin D₃ and calcium may be required for myelin biosynthesis, we theorize that 1,25-(OH)₂D₃ 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)₂D₃. 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 D₃ in MS, and that our experimental evidence in EAE is sufficiently compelling to warrant a clinical trial.

1. Steinman L. Multiple sclerosis: A coordinated immunological attack against myelin in the central nervous system. *Cell* **85**:299–302, 1996.
2. Ebers GC, Sadovnick AD. The role of genetic factors in multiple sclerosis susceptibility. *J Neuroimmunol* **54**:1–17, 1994.
3. Ebers GC, Bulman DE, Sadovnick AD, Paty DW, Warren S, Hader W, Murray TJ, Seland TP, Duquette P, Gray T, Nelson R, Nicolle M, Brunet D. A population-based twin study in multiple sclerosis. *N Engl J Med* **315**:1638–1642, 1986.
4. Haines JL, Ter-Minassian M, Bazyk A, Gusella JF, Kim DJ, Terwedow H, Pericak-Vance MA, Rimmler JB, Haynes CS, Roses AD, Lee A, Shaner B, Menold M, Seboun E, Fitoussi RP, Gartioux C, Reyes C, Ribierre F, Gyapay G, Weissenbach J, Hauser SL, Goodkin DE, Lincoln R, Usuku K, Garcia-Moreno A, Gatto N, Young S, Oksenberg JR. A complete genomic screen for multiple sclerosis underscores a role for the major histocompatibility complex. The Multiple Sclerosis Genetics Groups. *Nat Genet* **13**:469–471, 1996.
5. Sawcer S, Jones HB, Feakes R, Gray J, Smaldon N, Chataway J, Robertson N, Clayton D, Goodfellow PN, Compston A. A genome screen in multiple sclerosis reveals susceptibility loci on chromosomes 6p21 and 17q22. *Nat Genet* **13**:464–468, 1996.
6. Ebers GC, Kukay K, Bulman DE, Sadovnick AD, Rice G, Anderson C, Armstrong H, Cousin K, Bell RB, Hader W, Paty DW, Hashimoto S, Ager J, Duquette P, Warren S, Gray T, O'Connor P, Nath A, Auty A, Metz L, Francis G, Paulseth JE, Murray TJ, Pryse-Phillips W, Nelson R, Freedman M, Brunet D, Bouchard J-P, Hinds D, Ritsch N. A full genome search in multiple sclerosis. *Nat Genet* **13**:472–476, 1996.
7. Miller SD, Karpus WJ. The immunopathogenesis and regulation of T-cell-mediated demyelinating diseases. *Immunol Today* **15**:356–361, 1994.

8. Kurtzke JF. Epidemiologic evidence for multiple sclerosis as an infection. *Clin Microbiol Rev* **6**:382–427, 1993.
9. Brocke S, Veromaa T, Weissman I, Gijbels K, Steinman L. Infection and multiple sclerosis: A possible role for superantigens? *Trends Microbiol* **2**:250–254, 1994.
10. Wucherpfennig KW, Strominger JL. Selective binding of self peptides to disease-associated major histocompatibility complex (MHC) molecules: A mechanism for MHC-linked susceptibility to human autoimmune diseases. *J Exp Med* **181**:1597–1601, 1995.
11. Sibley WA, Bamford CR, Clark K. Clinical viral infections and multiple sclerosis. *Lancet* **1**:1313–1315, 1985.
12. Anderson O, Lygner P-E, Bergström T, Andersson M, Vahlne A. Viral infections trigger multiple sclerosis relapses: A prospective seroepidemiological study. *J Neurol* **240**:417–422, 1993.
13. Panitch HS. Influence of infection on exacerbations of multiple sclerosis. *Ann Neurol* **36**:S25–S28, 1994.
14. Johnson RT, Griffin DE, Hirsch RL, Wolinsky JS, Roedenbeck S, de Soriano IL, Vaisberg A. Measles encephalomyelitis—Clinical and immunologic studies. *N Engl J Med* **310**:137–141, 1984.
15. Goldberg P. Multiple sclerosis: Vitamin D and calcium as environmental determinants of prevalence (a viewpoint). Part 1: Sunlight, dietary factors and epidemiology. *Int J Environ Studies* **6**:19–27, 1974.
16. Goldberg P. Multiple sclerosis: Vitamin D and calcium as environmental determinants of prevalence (a viewpoint). Part 2: Biochemical and genetic factors. *Int J Environ Studies* **6**:19–27, 1974.
17. Acheson ED. The epidemiology of multiple sclerosis. In: McAlpine D, Lumsden CE, Acheson ED, Eds. *Multiple Sclerosis: A Reappraisal*. 2nd Edition. Baltimore: Williams and Wilkins, pp3–80, 1972.
18. Alter M, Loewenson R, Harshe M. The geographic distribution of multiple sclerosis: An examination of mathematical models. *J Chron Dis* **26**:755–767, 1973.
19. Kurtzke JF. Geography in multiple sclerosis. *J Neurol* **215**:1–26, 1977.
20. Davenport CB. Multiple sclerosis from the standpoint of geographic distribution and race. *Arch Neurol Psychiat* **8**:51–58, 1922.
21. Limburg CC. Geographic distribution of multiple sclerosis and its estimated prevalence in the US. *Res Publ Assoc Nerv Ment Dis* **28**:15–24, 1950.
22. Kurland LT. The frequency and geographic distribution of multiple sclerosis as indicated by mortality statistics and morbidity surveys in the United States and Canada. *Am J Hyg* **55**:457–476, 1952.
23. Ulett G. Geographic distribution of multiple sclerosis. *Dis Nerv Syst* **9**:342, 1948.
24. Acheson ED, Bachrach CA, Wright FM. Some comments on the relationship of the distribution of multiple sclerosis to latitude, solar radiation and other variables. *Acta Psychiat (Scand)* **35**(Suppl 147): 132–147, 1960.
25. Norman JE Jr., Kurtzke JF, Beebe GW. Epidemiology of multiple sclerosis in U.S. veterans: 2. Latitude, climate, and the risk of multiple sclerosis. *J Chron Dis* **36**:551–559, 1983.
26. Acheson ED. Epidemiology of multiple sclerosis. *Br Med Bull* **33**:9–14, 1977.
27. Dean G. Annual incidence, prevalence and mortality of multiple sclerosis in white South African-born and in white immigrants to South Africa. *Br Med J* **2**:724–730, 1967.
28. Kurtzke JF, Dean G, Botha DPJ. A method for estimating the age at immigration of white immigrants to South Africa with an example of MS importance. *S Afr Med J* **44**:663–669, 1970.
29. Kurtzke JF. On the fine structure of the distribution of multiple sclerosis. *Acta Neurol Scand* **43**:257–282, 1967.
30. Geiger R. *The Climate Near the Ground*. Cambridge, MA: Harvard University Press, pp 442–446, 1965.
31. Presthus J. Report on the multiple sclerosis investigations in West Norway. *Acta Psychiatr Neurol Scand* **35**(Suppl 147):88–92, 1960.
32. Westlund K. Distribution and mortality time trend of multiple sclerosis and some other diseases of Norway. *Acta Neurol Scand* **46**:455–483, 1970.
33. Goldberg P, Fleming MC, Picard EM. Multiple sclerosis: Decreased relapse rate through dietary supplementation with calcium, magnesium, and vitamin D. *Med Hypotheses* **21**:193–200, 1986.
34. Bates C, Cartlidge NEF, French JM, Jackson HJ, Nightenhale S, Shaw DA, Smith S, Woo E, Hawkins SA, Miller JH, Belin J, Conroy DM, Gill SK, Sidey M, Smith AD, Thompson RHS, Zilkha K, Gale M, Sinclair HM. A double-blind controlled trial of long chain n-3 polyunsaturated fatty acids in the treatment of multiple sclerosis. *J Neurol Neurosurg Psychiatry* **52**:18–22, 1989.
35. Newmark HL. Vitamin D adequacy: A possible relationship to breast cancer. In: Weisburger EK, Ed. *Diet and Breast Cancer*. New York: Plenum Press, pp 109–114, 1994.
36. DeLuca HF. Vitamin D: 1993. *Nutrition Today* **28**:6–11, 1993.
37. Velluz L, Amiard G. Chimie organique-equilibre de reaction entre precalciferol et calciferol. *Comput Rend* **228**:853–855, 1949.
38. Holick MF. The intimate relationship between the sun, skin and vitamin D: A new perspective. *Bone* **7**:66–69, 1990.
39. Holick MF. Photosynthesis of vitamin D in the skin: Effect of environmental and life-style variables. *Fed Proc* **46**:1876–1881, 1987.
40. Holick MF, MacLaughlin JA, Doppelt SH. Regulation of cutaneous previtamin D₃ photosynthesis in man: Skin pigment is not an essential regulator. *Science* **211**:590–593, 1981.
41. Clemens TL, Henderson SL, Adams JS, Holick MF. Increased skin pigment reduces the capacity of skin to synthesize vitamin D₃. *Lancet* **9**:75–76, 1982.
42. Holick MF. 1,25-Dihydroxyvitamin D₃ and the skin: A unique application for the treatment of psoriasis. *Proc Soc Exp Biol Med* **191**:246–257, 1989.
43. Holick MF. The cutaneous photosynthesis of previtamin D₃: A unique photoendocrine system. *J Invest Dermatol* **76**:51–58, 1981.
44. Holick MF. Photobiology, metabolism, and clinical aspects of vitamin D. In: Fotherby K, Pal SB, Eds. *Hormones in Normal and Abnormal Human Tissues*. Berlin: Walder de Gruyter, Vol. 2:pp 223–250, 1981.
45. DeLuca HF. Vitamin D: The vitamin and the hormone. *Fed Proc* **33**:2211–2219, 1974.
46. Clemens TL, Adams JA, Nolan JM, Holick MF. Measurement of circulating vitamin D in man. *Clin Chim Acta* **121**:301–308, 1982.
47. Boyle IT, Miravet L, Gray RW, Holick MF, DeLuca HF. The response of intestinal calcium transport to 25-hydroxy and 1,25-dihydroxy vitamin D in nephrectomized rats. *Endocrinology* **90**:605–608, 1972.
48. Holick MF, Garabedian M, DeLuca HF. 5,6-Trans-25-hydroxycholecalciferol: Vitamin D analog effective on intestine of anephric rats. *Science* **176**:1247–1248, 1972.
49. DeLuca HF. The vitamin D story: A collaborative effort of basic science and clinical medicine. *FASEB J* **2**:224–236, 1988.
50. Garabedian M, Holick MF, DeLuca HF, Boyle IT. Control of 25-hydroxycholecalciferol metabolism by the parathyroid glands. *Proc Natl Acad Sci U S A* **69**:1273–1276, 1972.
51. DeLuca HF, Schnoes HK. Vitamin D: Recent advances. *Annu Rev Biochem* **52**:411–439, 1983.
52. Ross TK, Darwish HM, DeLuca HF. Molecular biology of vitamin D action. In: Litwck G, Ed. *Vitamins and Hormones*. San Diego: Academic Press, Vol **49**:pp 281–326, 1994.
53. Pike JW. Vitamin D₃ receptors: Structure and function in transcription. *Annu Rev Nutr* **11**:189–216, 1991.
54. Bhalla AK, Amento EP, Clemens TL, Holick MF, Krane SM. Specific high-affinity receptors for 1,25-dihydroxyvitamin D₃ in human peripheral blood mononuclear cells: Presence in monocytes and induction in T lymphocytes following activation. *J Clin Endocrinol Metab* **57**: 1308–1311, 1983.
55. Provvedini DM, Manolagas SC. 1 α ,25-Dihydroxyvitamin D₃ receptor distribution and effects in subpopulations of normal human T lymphocytes. *J Clin Endocrinol Metab* **68**:774–777, 1989.
56. Tsoukas CD, Provvedini DM, Manolagas SC. 1,25-Dihydroxyvitamin D₃: a novel immunoregulatory hormone. *Science* **224**:1438–1440, 1984.
57. Yang S, Smith C, Prah JM, DeLuca HF. Vitamin D deficiency sup-

- presses cell-mediated immunity in vivo. *Arch Biochem Biophys* **303**:98–106, 1993.
58. Yang S, Smith C, DeLuca HF. $1\alpha,25$ -Dihydroxyvitamin D_3 and 19 -nor- $1\alpha,25$ -dihydroxyvitamin D_2 suppress immunoglobulin production and thymic lymphocyte proliferation in vivo. *Biochim Biophys Acta* **1158**:279–286, 1993.
59. Manolagas SC, Provvedini DM, Tsoukas C. Interactions of $1,25$ -dihydroxyvitamin D_3 and the immune system. *Mol Cell Endocrinol* **43**:113–122, 1985.
60. Rigby WFC. The immunobiology of vitamin D. *Immunol Today* **9**:54–57, 1988.
61. Lemire JM. Immunomodulatory role of $1,25$ -dihydroxyvitamin D_3 . *J Cell Biochem* **49**:26–31, 1992.
62. Lemire JM, Archer DC. $1,25$ -Dihydroxyvitamin D_3 suppresses the in vivo induction of murine experimental autoimmune encephalomyelitis. *J Clin Invest* **87**:1103–1107, 1991.
63. Branisteanu DD, Waer M, Sobis H, Marcelis S, Vandeputte M, Bouillon R. Prevention of murine experimental allergic encephalomyelitis: Cooperative effects of cyclosporine and $1\alpha,25(OH)_2D_3$. *J Neuroimmunol* **61**:151–160, 1995.
64. Cantorna MT, Hayes CE, DeLuca HF. $1,25$ -Dihydroxyvitamin D_3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc Natl Acad Sci U S A* **93**:7861–7864, 1996.
65. Nieves J, Cosman F, Herbert J, Shen V, Lindsay R. High prevalence of vitamin D deficiency and reduced bone mass in multiple sclerosis. *Neurology* **44**:1687–1692, 1994.