In: Vitamin D: New Research
Editors: Veronica D. Stolzt, pp. 1-23
ISBN 1-60021-000-7

Chapter I

High Levels of Active 1,25-Dihydroxyvitamin D Despite Low Levels of the 25-Hydroxyvitamin D Precursor – Implications of Dysregulated Vitamin D for Diagnosis and Treatment of Chronic Disease

J. C. Waterhouse*[1], *Trevor G. Marshall,
Belinda Fenter, Meg Mangin and Greg Blaney
Autoimmunity Research Foundation, Thousand Oaks, CA, USA 91360;
The Stillpoint Project, Vancouver, BC, Canada

Abstract

The active secosteroid hormone 1,25-dihydroxyvitamin-D (1,25D) often reaches excessive levels in normocalcemic patients suffering from chronic Th1 inflammatory illnesses, including sarcoidosis and rheumatoid arthritis. This is due to unregulated production of 1,25D in the mitochondria of activated macrophages. Phagocytic cells parasitized by cell wall deficient (CWD) L-forms of bacteria drive this dysfunction of vitamin D metabolism. The paracrine levels of 1,25D rise and the level of substrate 25-D falls. If studies measure only the 25D precursor, a low 25D may be misinterpreted as indicating the patient requires vitamin D supplementation. Our data show that active 1,25D hormone may be elevated, even with a low level of 25D substrate because of the inflamed macrophages' hyperactive conversion to the active hormone. In sarcoidosis, for example, this dysregulated vitamin D conversion can mean that even a moderate intake of vitamin D through ingestion or solar exposure can cause the 1,25D hormone to become high enough to stimulate osteoclastic action, and bone resorption. Data presented here suggest that this extra-renal synthesis of 1,25D is more widespread than previously

[1] Corresponding author. E-mail: jcw@autoimmunityresearch.org

thought and because it leads to vitamin D hypersensitivity, has important implications for research, diagnosis and treatment of chronic disease. The relationship of our data to past research on the role of vitamin D in several diseases is discussed. The assay of both the active 1,25D and inactive 25D metabolites will lead to additional clinical data, potentially improving both diagnosis and care of a variety of autoimmune and other Th1 illnesses. The high recovery rate using a new antibacterial protocol (initially developed for treating sarcoidosis) and the normalization of 1,25D levels, subsequent to such treatment, emphasizes the importance of measuring both the active 1,25D hormone and the 25D substrate. It should be noted that serum must be properly handled and transported frozen in order to obtain accurate 1,25D test results. Further, an increase in 25D levels induced by vitamin D supplementation may lead to long-term disease progression by facilitating proliferation of the intracellular CWD bacterial pathogens.

Introduction

New insights into the role of the active vitamin D hormone 1,25D in immune system modulation may soon lead to a new approach to vitamin D laboratory measurement and supplementation in clinical practice. It is now well-known from molecular medicine that, besides its role in calcium and phosphorus metabolism, the vitamin D hormone (1,25D) is closely tied to Th1 immune activity [1, 2, 3]. Research on the role of vitamin D in the Th1 autoimmune disease sarcoidosis has provided new insights into vitamin D's possible role in Th1 diseases [3, 4, 5]. These insights lead to the conclusion that measurement of the levels of both the relatively inactive form of vitamin D (25D) and the active 1,25D form of vitamin D may prove to be key in research, as well as diagnosis and treatment of many inflammatory diseases [3]. This may be especially important because it may lead to improved diagnosis of a treatable bacterial cause, as has been found in the case of sarcoidosis [3, 4, 5, 6, 7].

There is evidence that occult bacteria capable of living parasitically inside cells and lacking the usual type of rigid cell wall are the likely cause of Th1 inflammation in idiopathic diseases. These include *Chlamydia pneumoniae* and *Mycoplasma spp.*, as well as cell wall deficient forms of classical bacteria (CWD, also called L forms, cysts, pleomorphic, mollicutes). Over 20 years ago, CWD bacterial forms were found in tissue samples from sarcoidosis patients [8]. More recently, these bacteria have been photographed and studied independently by a number of researchers [9, 10, 11, 12, 13, 14, 15, 16]. Recently, bacteria have even been photographed in the process of replication inside immune cells in sarcoidosis [17]. Many bacteria escape destruction by the immune system or certain antibiotics by transforming into cell wall deficient forms and hiding within cells [12]. The Lyme disease pathogen, *Borrelia burgdorferi*, is an example of a CWD-capable bacterium, and research shows that it can transform back and forth between its spirochete and cyst forms [18, 19, 20].

The success of antibiotic treatment in sarcoidosis [3, 7], rheumatoid arthritis [21, 22, 23], scleroderma [24], multiple sclerosis [25] and Crohn's Disease [26, 27] provide evidence for the role of bacteria in Th1 disease. We discuss a treatment approach that combines immune modulation, and the use of synergistic pulsed, low dose antibiotics to improve antibiotic treatment outcome by overcoming the resistance mechanisms of these elusive bacteria. We present case histories and discuss the literature indicating that evaluating vitamin D

metabolite levels is important for immune modulation as well as for the detection of Th1 inflammation.

Aberrant Vitamin D Metabolism Linked to the Th1 Inflammatory Diseases

In healthy people with a normally functioning immune system, the kidneys tightly regulate conversion of inactive vitamin D (25D) into the active steroid hormone form (1,25D), keeping 1,25D within a fairly narrow range. Under the assumption that this tight control by the kidneys is nearly universal, there has been a tendency to only measure the precursor 25D, as this has been considered to be adequate for the detection of vitamin D deficiency states and it is easy to measure [28]. It has long been recognized, however, that in certain granulomatous diseases, such as sarcoidosis, tuberculosis, some cancers and other diseases, hypersensitivity to vitamin D can cause increased levels of 1,25D due to unregulated conversion of 25D to 1,25D by activated macrophages. Dysregulated vitamin D metabolism has been considered to be rare, and it has been assumed that measuring serum calcium was sufficient for detecting dangerous elevations that might occur when supplementing with vitamin D [29].

Bonnema et al. **[**30**]** first recommended measuring 1,25D as an accurate marker of disease activity in sarcoidosis, a chronic inflammatory disease with predominant Th1 activity. When evaluating osteoporosis treatments, the U.S. FDA's recommendations for studies include testing of the active 1,25 D metabolite [31]. It seems wise to apply this more thorough level of testing to other Th1 diseases that may involve vitamin D abnormalities, considering the recently widening understanding of hormone 1,25D's important immune system functions. Currently, many studies fail to do this, basing conclusions about D-metabolite status only on 25D levels. Research in sarcoidosis and other diseases supports the FDA in its recommendation to measure 1,25D as well.

Hypersensitivity to vitamin D occurs because, as part of the Th1 inflammatory response, activated macrophages possess 1-hydroxylase activity and are able to metabolize 1,25D from 25D [3, 32, 33]. Regulation of 1,25D levels is canceled by the effects of the Th1 cytokine, Interferon Gamma, which inhibits 1,25D induction of 24-hydroxylase, a key enzyme in 1,25D inactivation [34]. Interferon Gamma may additionally suppress the ability of increased levels of 1,25D to slow down its own synthesis through feedback inhibition of 1-hydroxylase [33]. It has been noted that the effect on regulation may extend to the kidney and intestinal 24-hydroxylases that breakdown 1,25D in order to regulate its levels. This lack of down-regulation is supported by the high serum concentrations of 1,25D in sarcoidosis, in which there is no significant increase in the metabolic clearance rate of 1,25D [33, 35].

At very high levels, the elevated 1,25D in sarcoidosis is known to sometimes cause frank hypercalcemia, bone loss and other symptoms of excessive vitamin D. Research has shown that at more moderate levels, elevated 1,25D can cause bone loss without hypercalcemia [36, 37]. The increased 1,25D can also cause increased fatigue, paresthesias [4, 6], muscle dysfunction and even facial paralysis [38]. There are published reports of exacerbations and even heart attacks in sarcoidosis patients during sunny holidays [39]. Marshall *et* al. [4, 6]

reported several cases where sarcoidosis patients had symptomatic relief from fatigue and paresthesias by avoiding sunlight, shielding their eyes from bright lights and eliminating dietary vitamin D. Marshall et al. used the "D-ratio" the ratio of 1,25D:25D as a marker of the level of inflammation in sarcoidosis and patients were able to reduce this D-ratio through avoidance of vitamin D.

An angiotensin receptor blocker, olmesartan, was also found to be very helpful through its ability to block angiotensin II from binding to its receptors on the macrophages, thus slowing the increase in activated macrophage production of 1,25D [3, 4]. Marshall et al. [4] found an elevated D-ratio ranging from 2.0 to more than 4.5 in sarcoidosis. In a large Danish study of smokers and nonsmokers, the data showed that a normal D-ratio for healthy nonsmokers could be calculated to be 1.3 [40], although this ratio may vary some depending on the latitude, for example 1.7 or 1.8 [41, 42].

Clearly, when vitamin D hypersensitivity due to extra-renal synthesis and lack of regulation is a potential issue, measurement of 25D alone is not sufficient to detect excessive 1,25D. Most of the sarcoidosis patients with elevated 1,25D are normocalcemic and monitoring serum calcium will not generally detect whether hypersensitivity to vitamin D is occurring due to the role of activated macrophages [43]. Data presented here suggest that nonrenal synthesis of vitamin D associated with low serum 25D is more widespread than previously thought and has important implications for research, diagnosis and treatment in chronic disease.

Measuring and Interpreting Vitamin D Levels

As has been noted, many of the studies concluding there is a vitamin D deficiency in various autoimmune and other conditions have only measured inactive vitamin D (25D). This metabolite tends to reflect the intake of vitamin D and largely misses the role of processes that cause dysregulation of the 1,25 vitamin D hormone levels, such as the conversion of 25 D to 1,25D by activated macrophages. Since the active form of vitamin D (1,25D) tends to degrade easily, the serum sample must be frozen for transport, and not all labs require this. We found Quest Diagnostics, the largest owner of laboratories in the United States, to have more rigorous standards than most other labs, and to give more accurate results for serum 1,25D.

We also found that the normal reference ranges used by most labs are quite broad. This is because the population used to determine the reference range is heavily biased with sick patients, no doubt including some with undiagnosed vitamin D dysregulation [6]. As testing for 1,25D becomes more routine, and not confined just to a sick population, the lab reference ranges will no doubt become more meaningful. As a point of comparison, Quest Labs currently cites a reference range of 15-60 pg/ml for 1,25D, while Labcorp uses a reference range 15-75 pg/ml. We regard the Merck Manual's reference range as more appropriate [44]. It gives an upper limit of 45 pg/ml for 1,25D (117 pmol/ml).

In addition, evaluation of serum levels of 25D may be misleading, since the inflammation from Th1 activity leads to paracrine extra-renal conversion of inactive 25D to active 1,25D, often depleting serum levels of measured 25D in order to increase active vitamin D (1,25D).

This may further contribute to a false impression of vitamin D insufficiency in D hypersensitivity due to this increased extra-renal 1,25D production. We typically see 25D presentations in Th1 disease at the lower limit of range, around 20ng/ml.

Illnesses where signs or symptoms are affected by vitamin D supplementation must be studied with an eye to identifying vitamin D dysregulation and extra-renal synthesis of 1,25D. Although our purpose is not to determine optimal levels of vitamin D for the general population, there are several points which merit special attention. First, it seems that the reliance on serum 25D levels in people getting nearly full-body solar exposure in a sunny climate as indicators of safe and/or desirable vitamin D levels [28, 97] seems questionable (sunbathers and lifeguards are an example sometimes used). Adams et al. [98] found hypercalciuria and bone loss at levels of 25D above approximately 50 ng/ml (> 125 nmol/ml), levels that many now regard as safe [28, 97] and there might be other long-term unknown consequences from prolonged elevations in D levels. For instance, Rajasree et al. [99] found an elevated risk of ischemic heart disease when 25D was above 89 ng/ml.

Caucasians evolved in northern latitudes, where there is little sun much of the year and where weather would have required some sort of covering or clothing much of the time. A large proportion of the land was forested until the last few thousand years, which would have tended to reduce solar exposure. In general, much of the world was more heavily forested, providing a less sunny environment for most human populations than at this time. This should be taken into account when assessing the environmental conditions under which humans evolved, as well as when considering what is a "natural" level of solar exposure and a desirable level of vitamin D metabolites. Future studies should consider whether low serum levels of 25D in African Americans and other people of color may be due, at least in some cases, to efficient increased extra-renal conversion of 25D to the active metabolite 1,25D and possible elevated 1,25D.

Analysis of Published Data on Vitamin D Metabolites in Some Diseases

Inflammatory Bowel Disease

A recent study showed elevated 1,25 D levels (>60 pg/ml) in 40% of Crohn's disease and 7% of ulcerative colitis patients tested [37]. If the study had used the upper limit for elevated 1,25 D of 45 pg/ml from the Merck Manual [44], the percentages with elevated 1,25D levels would have been much higher. We made a crude estimate from Figure 2 in Abreu et al. [37] and found that approximately 45% of ulcerative colitis patients and 68% of Crohn's Disease patients had 1,25D levels above 45 pg/ml. Abreu et al. [37] found that elevated 1,25 D was related to a negative effect on bone mineral density that was independent of glucocorticoid use. The authors concluded that the elevated levels of active 1,25 vitamin D might be due to inflamed tissue involved in the disease process. This was supported by measurement of elevated levels of 1 alpha hydroxylase (CYP27B1), the enzyme that converts 25D to 1,25D, from colonic biopsies of Crohn's patients [37]. Bacteria have been detected and treated with

some success in Crohn's disease [26, 27] indicating occult bacteria may be a source of chronic inflammation.

Rheumatoid Arthritis

Mawer et al. [45] demonstrated abnormal vitamin D metabolism in rheumatoid arthritis (RA). They challenged a cohort of 19 RA patients with 250 ug of the precursor, 25D, and found that RA patients generated peak levels of 1,25D significantly higher than controls. Levels of 1,25D were particularly elevated in the synovial fluid and were correlated with 25D levels, providing strong evidence for extra-renal synthesis of 1,25D in patients with RA. Studies of macrophages from synovial fluid in vitro in RA also showed this extra-renal synthesis [45, 46]. This supports the view that in RA, as in sarcoidosis, macrophages are causing excess conversion of 25D to 1,25D in areas of inflammation.

The level of inflammation in RA is typically not as extensive as in active sarcoidosis, thus resulting in smaller increases in the serum D-ratio and less elevated serum 1,25D. Also, since the joints are not as well perfused as areas like the gastrointestinal tract or lungs, the amount of 1,25D reaching the blood stream from sites of inflammation is also lessened. And, as Mawer et al. [45] point out, since the 1,25 D from the synovium is limited, the kidneys may be able to compensate by suppressing its own synthesis, resulting in serum levels of 1,25D staying near normal for most patients.

The results of the above study [45] suggest supplementation with the substrate 25D could lead to a local increase in 1,25D in the joint, whether or not it was reflected in elevated serum 1,25D levels. As has been observed in sarcoidosis [3], this increased 1,25D in inflamed tissues might lead to greater inflammation through promotion of macrophage activity and eventual bacterial increase. Supplementation also might increase osteoclastic activity and bone loss near the joint due to a local increase in 1,25D [45, 47]. Inaba found elevated 1,25D to be related to elevation of the inflammatory cytokines IL-1 and IL-2 [46]. IL-1 has been implicated in increased bone loss and together with elevated 1,25D may contribute to periarticular bone loss [48].

In fact, it is interesting to note that of 17 patients with rheumatoid arthritis whom Sambrook et al. [48] found to have bone loss near the wrist joint during a two-year study (mean loss rate 6.1%), one patient actually had a slight increase in bone density near the wrist joint. This patient was also the only one to take hydoxychloroquine during the study period, a drug sometimes used to block the conversion of 25D to 1,25D in sarcoidosis. From examining Table 2 and Fig. 2B [48], one can determine that this patient also had the lowest 1,25D level in the study, and this may account for the lack of periarticular bone loss. Two other patients with almost no bone loss also had very low 1,25D.

Systemic Lupus Erythematosis

In systemic lupus erythematosis (SLE) patients Muller et al. [49] provided data that could be consistent with an abnormal D metabolism, showing a mean of 27 pg/ml for 1,25D and a

mean of 13 ng/ml for 25D, yielding a D-ratio of 2.1. However, they did not separate patients according to which drugs patients were on. This is important in SLE, because some patients are treated with hydroxychloroquine, a drug that inhibits conversion of 25D to 1,25D. Huisman et al. [50] found a mean of 37.6 pg/ml for 1,25D and a mean of 17.4 ng/ml for 25D, with a mean D-ratio of 2.2 in systemic lupus erythematosis (SLE) for patients not taking hydroxychloroquine. The combined group of lupus patients, most of whom were taking hydroxychloroquine, showed a mean D-ratio of 1.6. The 25D levels were identified as being low, but the 1,25D levels were above average, and even high (using the Merck Manual's upper limit of 45 pg/ml) in many of the patients not taking hydroxychloroquine. This study suggests extra-renal synthesis of 1,25D is occurring, just as in Crohn's, RA and sarcoidosis, and supports the view that lupus might also benefit from the antibiotic approach used in sarcoidosis. Sometimes SLE is referred to as a TH2 disease, however we believe that the above evidence of vitamin D dysregulation indicates that it is more accurately viewed as a Th1 disease, in which Interferon Gamma is predominant, as in sarcoidosis.

If one looked only at the 25D levels in the above study, one might conclude that vitamin D supplementation was warranted. However, the above-normal 1,25D and the sensitivity to light found in SLE, leads us to propose that light exposure and D supplements would lead to disease progression in the long term, as has been found in sarcoidosis [4]. It should be noted that Grant [51] found increased SLE mortality was associated with UVB radiation in a geographical correlation study. Evidence for the role of bacteria in SLE has been found and thus chronic infection may account for the ongoing inflammation [10, 52].

Patients with SLE frequently have flares of symptoms when exposed to UVB, and some report sensitivity to fluorescent light or the UVA light entering through car windows [53]. For this reason, SLE patients are often told to avoid solar exposure and encouraged to use sunscreen. It may be that this difficulty with UVB exposure results from increased 1,25D production as a result of increased synthesis of vitamin D in the skin.

Sjogren's Syndrome

In Sjogren's Syndrome, evidence for abnormal vitamin D metabolism has been found by Muller et al. [54]. This Danish study provides an example of 25D and 1,25D values in a chronic illness in which the patients were not receiving immunosuppressive drugs, and unlike SLE, would not have been told to avoid solar exposure. The mean 1,25D was 33 pg/ml and the mean 25D was 12.4 ng/ml, yielding a mean D-ratio of 2.7. The data included 9 patients with 1,25D levels above 45 pg/ml, and it included 9 patients with undetectable levels of 25D. Muller et al. [54] concluded that the explanation for the disturbed vitamin D metabolism was unclear. Experience with sarcoidosis, Crohn's disease and RA, suggest the need to seek for extra-renal synthesis through further experimentation. The low 25D values may reflect rapid conversion of 25D to 1,25D by activated macrophages, rather than low values due to deficient vitamin D from lack of solar exposure or dietary sources.

Fibromyalgia, Generalized Chronic Pain, Chronic Fatigue Syndrome

Studies have differed in the vitamin D levels found in chronic pain and fatigue states and more study is needed, but the incomplete data are suggestive and are in keeping with our case histories (see below). The only study we found that measured both vitamin D metabolites found a mean of 20.6 ng/ml for 25D and a mean of 34.6 pg/ml for 1,25D, with a D-ratio of 1.7 [50]. This is slightly higher than the mean D-ratio of 1.3 found for controls at a similar latitude [40]. Plotnikoff et al. [55] reported a very high prevalence of low 25D levels among chronic musculoskeletal pain patients in a multi racial inner city Minnesota environment. Block [56] found a lower prevalence of low 25D among non-urban Caucasian fibromyalgia patients, but neither study was controlled. Of six patients who had low levels and were supplemented with 50,000 IU of vitamin D weekly for 8 weeks, only one believed the vitamin D was helpful [56]. Al-Allaf et al. [57], in a study in Scotland, found significantly more patients with FM had levels of 25D below 8 ng/ml than controls. The slightly elevated D-ratio [50] and the tendency to low levels of 25D is consistent with our hypothesis of extra-renal conversion by activated macrophages. Whether or not the low 25D is partly due to low D intake and/or lack of sun, it would be made even lower by extra-renal conversion to 1,25D.

In chronic fatigue syndrome, only one study reported on 25D and it did not publish the levels, only reporting that the CFS patients did not differ from controls [58]. However, they did report a correlation between hip bone mineral density (BMD) near the trochanter and 25D levels. Although a deficient level of 25D is one possible explanation for the correlation, an alternative explanation is that 25D is lowered by conversion to 1,25D by infected macrophages and thus its low level might reflect severity of the inflammation, which might affect BMD. A high rate of *Mycoplasma spp*. [59, 60] and *Borrelia burgdorferi* [61] have been found in FM and CFS patients, supporting a bacterial cause of inflammation.

Multiple Sclerosis

There are several lines of evidence that have been used to suggest that vitamin D may be beneficial to prevent or treat multiple sclerosis [28, 62]. Some of the insights from sarcoidosis, rheumatoid arthritis and Crohn's disease suggest a need to reevaluate this evidence.

Geographic and epidemiological data are subject to many confounding factors, and multiple alternative explanations are possible for any given pattern. For example, Poser [63] noted an increased genetic tendency toward multiple sclerosis (MS) in those of Scandinavian descent and their diaspora. An alternative explanation for the geographic pattern of MS that is in agreement with bacterial causation would be the geographic pattern of occurrence of the pathogen. Fritzsche [64] related the geographical and seasonal pattern of MS to that of the tick that carries *Borrelia burgdorferi*, the causative agent of Lyme disease. He particularly examined the role of migratory birds carrying ticks to various regions, including the Faroe Islands, which had a well-studied outbreak of MS. Other organisms, such as *Chlamydiae pneumoniae* have seasonal patterns and the more time spent indoors, which is naturally favored at a higher latitudes, as well as winter, the more likely one would be to acquire

certain organisms like this one. *Chlamydiae pneumoniae* has been linked to progressive MS [65].

The fact that one analysis implicates *Chlamydiae pneumoniae* and another implicates *Borrelia burgdorferi* can be reconciled by the theory that various combinations of species of bacteria are likely to be responsible for producing various diseases or symptom patterns. Multiple infections are likely because the immune dysregulation initiated by the first pathogen would facilitate infection with additional species. Diversity in particular bacterial species and strains among patients would also be in keeping with the diversity of symptoms and temporal patterns of illness, even within a given diagnostic category, like MS. This diversity would also partly explain the difficulty in linking one particular species to one particular disease.

Another type of study that contains many uncontrolled variables are those that relate vitamin D consumption or serum 25D levels and rates of disease. Many unknown factors might bias the results. For instance, if the cause of the disease was a chronic bacterial infection that required a long length of time for its development, it might produce a very gradual and subtle increase in fatigue or cognitive symptoms. These subtle effects might reduce the tendency of the subjects to consume certain "healthier" vitamin D-rich foods or to take supplements or give them to their infant (who they might have infected). Those in early stages of illness prior to diagnosis might sense they felt less well when they had a lot of solar exposure and this might affect their lifestyle and measured 25D levels. These ideas are only speculative at this time, but it suggests how effects of this type could influence results of this type of study.

Whether or not vitamin D is truly helpful in prevention or treatment of MS or other diseases cannot be proven by these studies due to their methodological limitations. Randomized controlled trials would be needed. However, even if future controlled trials were to show a preventative effect of vitamin D, this might be due to enhancement of immune response to the initial phase of bacterial infection through correction of very low levels of D, for which there is some limited evidence [66, 67]. If this effect occurs, it might even be limited to a subpopulation with one of the genetic polymorphisms of the vitamin D receptor that has been identified [68]. And any protective effect, if one were found, would not necessarily imply a benefit of D supplementation in patients once the chronic illness is established, since later in the illness, extra-renal synthesis of 1,25D reaches significant levels.

Studies in the animal model of MS, murine experimental allergic encephalomyelitis (EAE), have shown a benefit from vitamin D in preventing and treating EAE in mice [69]. However, applying animal models to a disease of unknown cause in humans has many possible pitfalls. Some of the pitfalls that may apply in this case, are the differences in physiology in humans and mice, different disease causation being involved, the short life span of the mice and short time span of the experiment. Additionally, the ability of the murine model to assimilate bacterial pathogens is vastly different from that of *homo sapiens*.

Even if the EAE model did apply to human MS to some degree, giving large amounts of 1,25D might be able to halt the disease process in EAE due to immuno-suppression, but the short time span of the experiment would fail to detect a long-term worsening. This objection is also relevant to studies of seasonal reductions in lesions in MS [70] and some short-term trials of vitamin D supplementation [see 71] in which greater 1,25D is thought to be

favorably affecting disease activity. It may affect visible signs of inflammation or short-term symptomology, yet fail to have a positive effect in the long run, particularly if the proposed bacterial infection is actually being promoted by the immunosuppressive effects of the elevated 1,25D.

The only way to determine if supplementation of vitamin D is therapeutic in MS is to do randomized double blind controlled trials of supplementation. A long time frame would be necessary (3 to 5 years) in order to determine whether the therapy was not just providing a short-term palliative effect, with long-term negative effects, as would be expected if an immunosuppressive drug were given to someone with a slow growing infection. The results should be compared to similar trials of an antibiotic protocol, such as the one described below, that has been shown to be capable of overcoming the strategies that certain intracellular bacteria have developed that allow them to avoid elimination by the immune system and most antibiotics. Research that has detected, cultured and photographed *Borrelia burgdorferi* from MS patients support the need for such studies of antibiotic protocols [12, 64, 72].

Bacteria-Induced Inflammation, Vitamin D Dysregulation and a New Antibiotic Protocol

In previous sections, we described evidence for the role of intracellular bacteria in Th1 disease. Here we briefly discuss the relationship between bacterial parasitization of macrophages and vitamin D dysregulation and its implications for treatment for Th1 inflammatory diseases. Lipopolysaccharides, which are a major component of the cell walls of gram-negative bacteria, have been observed to be able to induce conversion of 25D to 1,25D by macrophages [66], supporting the role of bacteria in the excessive extra-renal conversion.

Nilsson's [17] demonstration of the ability of bacteria to reproduce within macrophages, the very cells that are meant to destroy them, led Marshall et al. [3, 4, 5] to understand the etiology of sarcoidosis and design a treatment that would combat multiple species of treatment-resistant intracellular bacteria. This approach includes immune modulation by restricting vitamin D while using higher-than-usual doses of an angiotensin receptor blocker, olmesartan. Together, these modulating factors tend to reduce inflammation and enhance antibiotic effectiveness. The inclusion of immune modulation and the specific combinations of pulsed, low doses of several classes of antibiotics has been effective in overcoming the bacterial resistance that has resulted in only limited success of other antibiotic regimens. The role of angiotensin receptor blockade has been discussed elsewhere [3, 4, 73], but more should be said about the role of vitamin D in the context of the immune response to Th1 disease.

The effect of 1,25D on the immune system is complex and can even seem paradoxical in that it reduces "lymphocyte activity while stimulating monocyte/macrophage function" [1], as well as providing an important negative feedback mechanism to control innate and inflammatory responses of activated macrophages [102]. This paradox may lead to a complex effect of 1,25D on inflammation in Th1 disease, sometimes increasing and sometimes

suppressing inflammatory symptoms. In sarcoidosis, lymphocytes actually become depleted in the central region of granuloma, especially in later stages of the disease and we find that the cytokines and elevated 1,25D resulting from the activated macrophages in the granuloma make the role of the CD4+ lymphocytes minimal in comparison to the macrophage activity [3,4].

In contrast, in other cases, the lymphocyte's role may be more dominant and high levels of 1,25D may suppress inflammation by suppressing Th1 lymphocytes, causing short-term improvement in symptoms (e.g., multiple sclerosis, see above). Which scenario applies may depend on the degree to which Th1 CD4 helper cell lymphocytes are involved in the ongoing reaction, which might differ among Th1 illnesses or the stage of illness. In later stages of certain Th1 illnesses, the lymphocytes may decrease in number and importance due to the dominating effects of the bacteria-infected macrophages.

So, under some circumstances, increasing 1,25D may suppress inflammation and in other situations it may increase inflammation. However, in our experience, in either case, elevated levels of 1,25D are immunosuppressive in the sense that they reduce the ability of the immune system to function effectively to eradicate the CWD forms of bacteria and resolve the infection.

Experience with this antibiotic approach in sarcoidosis has shown [3, 4] that the higher the 25D level, the more 25D fuels conversion to active 1,25D by mitochondria in the macrophages in inflamed tissue cells. This conversion is exacerbated by paracrine Interferon Gamma secreted by the activated macrophages, which is reportedly increased by a factor of 30 or more [74]. Thus, the antibiotic protocol described requires a reduction in vitamin D intake and solar exposure in order to minimize 1,25D and the 25D that fuels its production.

Besides the gradual improvement achieved through this antibiotic protocol, the short-term reaction to the antibiotics also provides evidence for the role of bacteria. Patients with virtually all of the conditions discussed in the case histories presented experienced what we refer to as a Jarisch Herxheimer reaction (JHR) following effective antibiotic dosing [75]. This JHR is due to the proinflammatory cytokine release as a result of the immune reaction to bacterial die-off and the death of the cells they have parasitized. If the antibiotics were effective only due to an anti-inflammatory effect, as some postulate, repeated exacerbations following pulsed antibiotic dosing would clearly not be the expected response. Allergy to the antibiotics cannot explain the reactions, since the same dose without immune modulation usually does not produce the same reaction and the JHR decreases over time, as the bacterial load decreases with treatment. For sarcoidosis patients who have been treated long enough, the JHR eventually ceases altogether. It is noteworthy that we found elevated levels of serum 25D inhibited antibiotic-induced bacterial killing, as evidenced by lessened Jarisch Herxheimer reactions in patients until they succeeded in lowering their vitamin D levels.

In seven of nine of the granuloma-forming illnesses involving an overproduction of extra-renal vitamin D that have known causes, the cause is infection [1]: Leprosy, histoplasmosis, cryptococcosis, tuberculosis, candidiasis, cocccidiomycosis, and cat scratch fever. Now, it appears that one of the previously unexplained granulomatous diseases – sarcoidosis - has an infectious intracellular bacterial cause [3]. Future research could reveal whether other idiopathic granulomatous diseases and other diseases with less obvious overproduction of extra-renal 1,25D also have an infectious cause.

Case Histories Illustrating Aberrant Vitamin D Metabolism and Apparent Bacterial Causation

We present here a few cases, of the hundreds we have observed, illustrating how a range of painful and fatiguing chronic conditions may exhibit aberrant D metabolism involving excessive extra-renal production of 1,25D by activated macrophages in the same manner as sarcoidosis. In most of these cases, the initial response to the antibiotic protocol closely resembles the response in sarcoidosis, thus supporting a bacterial cause (see above). All but the last three cases are from a medical practice specializing in the treatment of pain in Vancouver, British Columbia in Canada [100].

Many of these cases involve family members, illustrating how common case clustering is and reflecting possible person-to-person transmission. Case clustering within families has been documented for chronic fatigue syndrome among families of *Mycoplasma*-positive Gulf War Illness patients [60, 76]. However, these clusters are by no means always the case. Relatives may also have little, if any, symptoms and yet be at higher risk of developing some illness involving inflammation later in life.

Patient 1 is a 59-year-old male, suffering from chronic debilitating headaches, chronic fatigue, decreased cognitive function, heart block and allergies. His 1,25D was 115 pg/ml and his 25D was 29.2 ng/ml, yielding a D-ratio of 3.9. After a week on olmesartan, used as part of the immune modulation of the antibiotic protocol, his headaches disappeared. After 2 months on the low dose minocycline, his elevated PSA dropped from 27 to 9.2 ng/ml.

Patient 2 (wife of patient 1) is 53 years old, was positive for tick bite, suffered from neck pain, cognitive impairment restless leg syndrome, eye twitches, anxiety and depression and had a 1,25D of 69 pg/ml and a 25D ng/ml of 48, with a D-ratio of 1.43. When 25D is elevated due to supplementation, the D-ratio is less useful, and the elevated 1,25D level is relied upon more. She began the antibiotic protocol with immune modulation and experienced Jarisch Herxheimer reactions and an overall pattern of improvement.

Patient 3 (first of 3 sisters) is 50 years old, had a car accident when young and had her spleen removed, was positive for tick bite and *Bartonella* and suffered from neck pain, fatigue and muscle twitching. Her 1,25D was 61.5 pg/ml and her 25D was 30 ng/ml, with a D-ratio 2.1. She felt much better due to reduced inflammation when she began olmesartan, the angiotensin receptor blocker that modulates the immune system, but became very symptomatic due to the JHR from the alternate day dosing of minocycline used in the protocol. She reported an extreme exacerbation when she took a holiday during which she allowed her hands and feet to be exposed to the sun.

Patient 4 (son of patient 3) is 20 years old and is extremely fatigued, having to sleep much of the day. He tested positive for *Bartonella*. His 1,25D was 57.7 pg/ml and his 25D was 13.2 ng/ml , with a D-ratio of 4.4.

Patient 5 (sister of patient 3) suffered from CFS and had a 1,25D of 80.8 pg/ml and a 25D of 36 ng/ml, with a D-ratio of 2.2. She had previously developed aplastic anemia, but was in remission.

Patient 6 (sister of patients 3 and 5) is 45 years old and suffered from chronic headaches, fatigue and dermatitis. She had a 1,25D of 48.9 pg/ml and a 25D of 22 ng/ml, with a D-ratio of 2.2.

Patient 7 is a 49-year-old female with chronic fatigue syndrome, osteoporosis, ovarian cysts, chronic fungal infections, dermatitis and severe varicose veins. This patient had a 1,25 D of 50 pg/ml and a 25 D of 16.8 ng/ml, with a D-ratio of 3.1.

Patient 8 (husband of patient 7) is 57 years old, had been incapacitated for more than 10 years and suffered from the following conditions: CFS/FM, cognitive loss, skin granulomas, neuropathy, fungal infections, reduced kidney function, lesions on pons and cerebral peduncle shown by MRI, L5 disc degeneration, porphyria and prostatitis, and was positive for *Rickettsia* and *Bartonella*. His 1,25 D was 42 pg/ml and his 25 D was 21.6 ng/ml, with a D-ratio of 1.9. When placed on olmesartan and pulsed minocycline he experienced JHR in response to the antibiotics, as expected.

Patient 9 is a 39-year-old female who was diagnosed with multiple sclerosis 9 years ago. Her 1,25D was 53 pg/ml and her 25D was 35 ng/ml, with a D-ratio of 1.5. She is experiencing JHR symptoms following the low doses of minocycline. After 2 months on the protocol she has already experienced some improvements in strength, although it is too soon to come to know if the improvements are significant.

Patient 10 is a 43-year-old male who was diagnosed with amyotrophic lateral sclerosis 2 years ago. His 1,25D was 58.9 pg/ml and his 25D was 36ng/ml, with a D-ratio of 1.6. He has not yet begun antibiotic treatment. The high 1,25D suggests it is not under tight control by the kidneys and is consistent with excessive extra-renal synthesis.

Patient 11 is a 54-year-old female who was diagnosed with rheumatoid arthritis 9 years ago. Her 1,25D level was 65 pg/ml and her 25D was 32 ng/ml, with a D-ratio of 2.0. The reduction of inflammation achieved by the angiotensin receptor blocker, olmesartan, as part of the antibiotic/immune modulation protocol, reduced her 1,25D levels by more than half to 31 pg/ml in 2 weeks. After the immune modulation accomplished through the olmesartan and reducing vitamin D and solar exposure, she began experiencing JHR in response to even very low doses of minocycline and has improved significantly after 1 year on the protocol. Previously, on high doses of almost all the antibiotics she had tried, including minocycline, she had no JHR or significant improvement after 6 years. Her relatively high 25D is explained by vitamin D supplementation recommended by her doctor, which gradually climbed from 800 to 2400 IU daily. During this 2-year period on vitamin D supplementation, her condition worsened.

Patient 12 is a 46-year-old female, disabled for 20 years by CFS, fibromyalgia syndrome, and irritable bowel syndrome, who also received a diagnosis of chronic Lyme disease more recently. Her 1,25D was 64 pg/ml and her 25D was 11 ng/ml, with a D-ratio of 5.8. Since she was supplementing with 500 IU/day of vitamin D and her 1,25D was elevated, it seems much more likely that her low 25D was due to extra-renal conversion to 1,25D than a vitamin D deficiency. This is supported by the fact that she felt better when she lowered her vitamin D intake and her tendency to feel worse, including having an elevated heart rate, when in the sun for even a few minutes. After being on the protocol for 5 months, her D-ratio went down to 3.7 (22 pg/ml 1,25D:6 ng/ml 25D). She experienced JHR exacerbations in response to antibiotics and improved significantly over 8 months of treatment.

Future Research Directions

There is little data on vitamin D metabolite levels and their corresponding extra-renal sources in the majority of chronic diseases so clearly more studies are needed. Although, in most diseases, 1,25D may not be as elevated as it is in sarcoidosis, a situation analogous to RA may occur, in which 1,25D is generated locally, often without elevating the serum levels significantly. In this case, experiments could be performed similar to those done by Mawer et al. [45], as discussed above, in which a fairly large challenge dose of 25D is given to a group of patients, as well as age- and sex-matched controls. The serum levels of 1,25D could be followed in the days after the challenge, and inflamed tissues or fluids could be assayed as well (e.g., cerebrospinal fluid might be tested in the case of MS). This type of study could reveal whether significant extra-renal synthesis of 1,25D was occurring, as in RA, Crohn's disease and sarcoidosis. *In vitro* studies of hydroxylase enzymes and D metabolites produced by macrophages from various inflamed tissues would also be useful [37, 45, 46].

Chronic diseases provide good candidates for studies using these methods. In particular, whenever vitamin D abnormalities have been found or whenever a connection between seasonality, latitude, solar exposure or D supplementation has been identified as having either a positive or negative effect on the disease, we believe that looking for extra-renal synthesis and bacterial involvement is essential.

It should be remembered that positive short-term effects resulting from vitamin D supplementation would not be beneficial in the long run if these high 1,25D levels are blocking the immune system's attempts to combat an occult infection underlying the disease. To put it simply, stopping the immune system's bacterial killing may make the patient feel better, but does not mean they will be healthier in the long run.

We believe that any similarity to sarcoidosis in vitamin D dysregulation indicated by these types of studies would then indicate the need for a trial of the same antibiotic and immune-modulating protocol found to achieve a 90% success rate in sarcoidosis [3].

Examples of diseases that might merit these types of investigation, besides the ones discussed previously, include heart disease, psychiatric disorders, Parkinson's disease, Alzheimer's disease, autism and cancer. Seasonal variation in heart events may relate to infection with organisms like *Chlamydia pneumoniae*, which also show a seasonal pattern [77, 78]. Mattman [79] has reported cases in which strokes or heart attacks seem to have been communicable via *Chlamydia pneumoniae*, which was also detected in the pleural fluid. Nanobacteria, which are probably CWD bacterial forms, have also been found in calcified human arteries and cardiac valves [80] and may respond to antibiotics [81].

In psychiatric illnesses affected by solar or light exposure, such as depression [82] or bipolar disorder [83], studies looking for extra-renal synthesis of vitamin D should be conducted. The prevalence of heart disease, depression and other cognitive and affective symptoms in sarcoidosis, and their improvement during antibiotic treatment provide evidence for the role of bacteria-caused inflammation in these symptoms and support the need for such studies. It has long been known that Lyme disease can produce heart damage, and Borreliosis is also known to be able to cause psychiatric symptoms virtually indistinguishable from schizophrenia, depression and bipolar disorder [84]. Fritzsche linked seasonal and geographic patterns in schizophrenia to the tick that carries *Borrelia burgdorferi* [85].

Parkinson's disease has shown evidence of vitamin D dysregulation [3] and some case clusters have been found [86, 87], which we think might support an infectious cause. There have even been studies showing antibiotics to be at least partially effective in treating Alzheimer's disease [88] and even temporarily helpful in a subset of autism patients [89]. More research on vitamin D dysregulation and potential bacterial pathogens seems warranted.

A number of observations suggest that these types of research approaches might be profitably applied to cancer. Stomach cancer is known to be related to infection with *Helicobacter pylori* and in other cases, cancer has been increasingly linked to chronic inflammation [90, 101, 103]. Bacteria have also been linked to prostate cancer [91, 92, 93, 94]. Tuohimaa [95] found a U shaped curve of 25D levels in relation to prostate cancer, with high levels of 25D being detrimental, as well as low levels, which seems to us to be suggestive of D dysregulation. Mawer [96] found some patients with early breast cancer showed high 1,25D levels, suggesting extra-renal synthesis.

Some of the reviews, [28,62] suggest a benefit of vitamin D in cancer. A more recent study by Townsend et al. [101] shows that the role of vitamin D in cancer is a lot more complex than anticipated in these reviews. We have noticed that the calcium metabolism is affected only rarely in Th1 patients with hypervitaminosis D. There are also other types of confounding that may occur in many of these correlational studies, as we discussed previously in the section on multiple sclerosis, and these issues should be examined further. Most importantly, we note that dysfunction of vitamin D metabolism seems to be an integral part of the body's reaction to intra-phagocytic CWD bacteria that our work demonstrates are likely behind the Th1 diseases and which have been postulated to be involved in the pathogenesis of cancer [90, 92].

Once significant extra-renal synthesis of 1,25D due to activated macrophages is established, one might proceed to determine whether the vitamin D dysregulation is due to a bacterial pathogen by conducting a therapeutic trial with the antibiotic and immune modulating protocol discussed previously [3]. In the short term, one can obtain evidence for bacterial causation by monitoring for Jarisch Herxheimer reactions in response to antibiotics, and by detecting increases in 1,25D that are associated with these reactions. One can also monitor longer-term changes in 25D and 1,25D during treatment.

We believe multiple bacterial species are involved in these Th1 diseases and the protocol we have described was designed to use several antibiotics to cover virtually all species and strains of resistant bacteria likely to be encountered. Nicholson [76] has observed multiple species of bacteria in chronic fatigue syndrome patients and found that the more species infecting a patient, the sicker they are. We believe the tendency to multiple infections is largely due to the excessive cytokine production and vitamin D dysregulation allowing subsequent infections to be acquired more easily. Th1 patients also tend to acquire difficult-to-eradicate chronic viral, fungal and parasitic infections over time. Experience so far suggests these organisms may be controlled adequately by improved immune function following eradication of intracellular bacteria and these nonbacterial coinfections may not need to be individually treated in many cases.

Conclusion

We have shown [3,4] that in Th1 disease, increasing levels of 25D fuel the conversion to the active 1,25D metabolite by the mitochondria in activated macrophages within inflamed tissue. To date, we have identified a pattern of vitamin D dysregulation which may include elevated 1,25D, depleted 25D, or an elevated D-ratio in a variety of Th1 diseases, including rheumatoid arthritis, Hashimoto's thyroiditis, lupus pernio, Meniere's disease, CFS/ME, tertiary Lyme disease, attention deficit hyperactivity disorder, sarcoidosis and fibromyalgia. In all of these diseases, our clinical experience has been that increasing the availability of 25D to the inflamed tissue decreases the ability of the immune system to kill the intra-phagocytic pathogens. Short term, this may result in a palliation of the patient's symptoms, but in the long term facilitates progression of the chronic disease.

There has recently been evidence presented in favor of increasing recommended levels of vitamin D supplementation in the general population [97]. However, until the degree of vitamin D dysregulation due to chronic disease is clarified, extreme caution is warranted. There are many Th1 chronic conditions that initially present with mild symptoms, but in which subclinical vitamin D dysregulation is manifest.

A lack of awareness of this issue may cause harm, in that vitamin D supplementation or increased solar exposure, will insidiously worsen the condition. Illnesses where signs or symptoms improve with vitamin D supplementation must be studied with an eye to identifying vitamin D dysregulation and extra-renal synthesis of 1,25D.

It may not be appropriate to apply the same upper limit of normal for 1,25D to those with chronic diseases as might apply to healthy people.

Our experience indicates that further study of vitamin D dysregulation will provide a window onto the functioning of the immune system, potentially leading to a pathogenic description, improved diagnosis and effective treatment for many chronic idiopathic diseases.

References

[1] Hewison, M; Gacad, MA; Lemire, J; Adams, JS. Vitamin D as a cytokine and hematopoetic factor: *Rev Endocr Metab Disord*. 2001 2(2), 217-27.

[2] Dusso, AS; Brown, AJ; Slatopolsky, E. Vitamin D. *Am J Physiol Renal Physiol*. 2005 289(1), F8-28.

[3] Marshall, TG; Marshall, FE. Sarcoidosis succumbs to antibiotics - implications for autoimmune disease. *Autoimmun Rev*. 2004 3(4), 295-300.

[4] Marshall, TG; Marshall, FE; Remission in Sarcoidosis: Clinmed. 2002. Available at: URL: *http://clinmed.netprints.org/cgi/content/full/2002080004*.

[5] Marshall, TG; Marshall, FE. Antibiotics in sarcoidosis - reflections on the first year. JOIMR 2003 1(3). Available at: URL: *http://www.joimr.org/phorum/read.php?f=2andi=38andt=38*.

[6] Marshall, TG; Marshall, FE. New treatments emerge as sarcoidosis yields up its secrets. Clinmed 2003. Available at: URL: *http://clinmed.netprints.org/cgi/content/full/2003010001*.

[7] Bachelez, H; Senet, P; Cadranel, J, Kaoukhov, A; Dubertret, L. The use of tetracyclines for the treatment of sarcoidosis. *Arch Dermatol*. 2001 137(1), 69-73.

[8] Cantwell, AR Jr. Histologic observations of variably acid-fast pleomorphic bacteria in systemic sarcoidosis - a report of 3 cases. *Growth*. 1982 46(2), 113-25.

[9] Cantwell, AR Jr. The eccrine sweat gland as a possible focus of infection with acid-fast cell wall deficient bacteria. JOIMR 2003 1(1). Available at: URL: *http://www.joimr.org/phorum/read.php?f=2andi=2andt=2*.

[10] Cantwell, AR Jr; Cove, JK. Variably acid-fast bacteria in a necropsied case of systemic lupus erythematosus with acute myocardial infarction. *Cutis*. 1984 33(6), 560-7.

[11] Moscovic, EA. Sarcoidosis and mycobacterial L-forms. A critical reappraisal of pleomorphic chromogenic bodies (Hamazaki corpuscles) in lymph nodes. *Pathol Annu.* 1978 13(2), 69-164.

[12] Mattman, LH. *Cell Wall Deficient Forms: Stealth Pathogens*. Third Edition. Boca Raton, FL: CRC Press; 2000.

[13] Almenoff, PL; Johnson, A; Lesser, M; Mattman, LH. Growth of acid fast L forms from the blood of patients with sarcoidosis. *Thorax* 1996 51(5), 530-3.

[14] Wirostko, E; Johnson, L; Wirostko, B. Sarcoidosis associated uveitis. Parasitization of vitreous leucocytes by mollicute-like organisms. *Acta Ophthalmol.* 1989 67(4), 415-24.

[15] Wirostko, E; Johnson, L; Wirostko, W. Juvenile rheumatoid arthritis inflammatory eye disease. Parasitization of ocular leukocytes by mollicute-like organisms. *J Rheumatol*. 1989 16(11), 1446-53.

[16] Johnson, LA; Wirostko, E; Wirostko, WJ. Crohn's disease uveitis. Parasitization of vitreous leukocytes by mollicute-like organisms. *Am J Clin Pathol*. 1989 91(3), 259-64.

[17] Nilsson, K; Pahlson, C; Lukinius, A; Eriksson, L; Nilsson, L; Lindquist, O. Presence of Rickettsia helvetica in granulomatous tissue from patients with sarcoidosis. *J Infect Dis*. 2002 185(8), 1128-38.

[18] Brorson, O; Brorson, SH. In vitro conversion of Borrelia burgdorferi to cystic forms in spinal fluid, and transformation to mobile spirochetes by incubation in BSK-H medium. *Infection*. 1998 26(3), 144-50.

[19] Brorson, O; Brorson, SH. Transformation of cystic forms of Borrelia burgdorferi to normal, mobile spirochetes. *Infection*. 1997 25(4), 240-6.

[20] Mursic, VP; Wanner, G; Reinhardt, S; Wilske, B; Busch, U; Marget, W. Formation and cultivation of Borrelia burgdorferi spheroplast-L-form variants. *Infection*. 1996 24(3), 218-26.

[21] Tilley, BC; Alarcan, GS; Heyse, SP; Trentham, DE; Neuner, R; Kaplan, DA; Clegg, DO; Leisen, JC; Buckley, L; Cooper, SM; Duncan, H; Pillemer, SR; Tuttleman, M; Fowler, SE. Minocycline in rheumatoid arthritis. A 48-week, double-blind, placebo-controlled trial. MIRA Trial Group. *Ann Intern Med*. 1995 122(2), 81-9.

[22] O'Dell, JR ; Haire, CE; Palmer, W; Drymalski, W; Wees, S; Blakely, K; Churchill, M; Eckhoff, PJ; Weaver, A; Doud, D; Erikson, N; Dietz, F; Olson, R; Maloley, P; Klassen, LW; Moore, GF. Treatment of early rheumatoid arthritis with minocycline or placebo: results of a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum*. 40(5): 842-8 1997.

[23] Clark, HW; Coker-Vann, MR; Bailey, JS; Brown, TMcP. Detection of mycoplasmal antigens in immune complexes from rheumatoid arthritis synovial fluids. *Ann of Allergy*. 1988 60(5), 394-8.

[24] Le, C; Morales, A; Trentham, DE. Minocycline in Early Diffuse Scleroderma, *Lancet* 1998 352(9142), 1755-6.

[25] Zemke, D; Majid, A. The potential of minocycline for neuroprotection in human neurologic disease. *Clin Neuropharmacol*. 2004 27(6), 293-8.

[26] Colombel, JF; Cortot, A; van Kruiningen, HJ. Antibiotics in Crohn's disease. Comment in: *Gut*. 2001 49(6), 873-4.

[27] Hulten, K; Almashhrawi, A; El-Zaatari, FA; Graham, DY. Antibacterial therapy for Crohn's disease: a review emphasizing therapy directed against mycobacteria. *Dig Dis Sci*. 2000 45(3), 445-56.

[28] Holick, MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr*. 2004 80, 1678S-88S.

[29] Vasquez, A; Manso, G; Cannell, J. The clinical importance of vitamin D (cholecalciferol): a paradigm shift with implications for all healthcare providers. *Alternative Therapies*. 2004 10(5), 28-36.

[30] Bonnema, SJ; Moller, J; Marving, J; Kvetny, J. Sarcoidosis causes abnormal seasonal variation in 1,25-dihydroxy-cholecalciferol. *J Intern Med*. 1996 239(5), 393-8.

[31] Division of Metabolic and Endocrine Drug Products, Food and Drug Administration: *Guidelines for Preclinical and Clinical Evaluation of Agents Used in the Prevention or Treatment of Postmenopausal Osteoporosis*. 5600 Fishers Lane, HFD-510, Rockville, MD 20857-1706.

[32] Dusso, A; Brown, A; Slatopolsky, E. Extrarenal production of calcitriol. *Semin Nephrol* 1994 14(2), 144-55.

[33] Dusso, AS; Kamimura, S; Gallieni, M; Zhong, M; Negrea L; Shapiro, S; Slatopolsky, E. Interferon-induced resistance to 1,25-(OH)2 D3 in human monocytes and macrophages: a mechanism for the hypercalcemia of various granulomatoses. *J Clin Endocr Metab*. 1997 82(7), 2222-32.

[34] Vidal, M; Ramana, CV; Dusso, AS. Stat1-vitamin D receptor interactions antagonize 1,25-dihydroxyvitamin D transcriptional activity and enhance stat1-mediated transcription. *Mol Cell Biol*. 2002 22(8), 2777-87.

[35] Insogna, KL; Dreyer, BE; Mitnick, M; Ellison, AF; Broadus, AE. Enhanced production rate of 1,25-dihydroxyvitamin D in sarcoidosis. *J Clin Endocrinol Metab*. 1988 66(1), 72-5.

[36] Bouillon, R; Okamura, WH; Norman, AW. Structure-function relationships in the vitamin D endocrine system. *Endocrine Reviews* 1995 16(2), 200-257.

[37] Abreu, MT; Kantorovich, V; Vasiliauskas, EA; Gruntmanis, U; Matuk, R; Daigle, K; Chen, S; Zehnder, D; Lin, Y-C; Yang, H; Hewison, M; Adams, JS. Measurement of vitamin D levels in inflammatory bowel disease patients reveals a subset of Crohn's disease patients with elevated 1,25-dihydroxyvitamin D and low bone mineral density. *Gut* 2004 53,1129-1136.

[38] Muler, H; Paquelin, F; Cotin, G; Luboinski, B; Henin, JM. Facial paralysis in children. *Ann Otolaryngol Chir Cervicofac*. 1975 92(4-5), 229-34.

[39] Cronin, CC; Dinneen, SF; O'Mahony, MS; Bredin, CP; O'Sullivan, DJ. Precipitation of hypercalcaemia in sarcoidosis by foreign sun holidays: report of four cases. *Postgrad Med J*. 1990 66(774), 307-9.

[40] Brot, C; Jorgensen, NR; Sorensen, OH. The influence of smoking on vitamin D status and calcium metabolism. *Eur J Clin Nutr* 1999 53(12), 920-6.

[41] Marks, R; Foley, PA; Jolley, D; Knight, KR; Harrison, J; Thompson, SC.The effect of regular sunscreen use on vitamin D levels in an Australian population. Results of a randomized controlled trial. *Arch Dermatol*. 1995 131(4), 415-21.

[42] Farrerons, J; Barnadas, M; Rodriguez, J; Renau, A; Yoldi, B; Lopez-Navidad, A; Moragas, J. Clinically prescribed sunscreen (sun protection factor 15) does not decrease serum vitamin D concentration sufficiently either to induce changes in parathyroid function or in metabolic markers. *Br J Dermatol.* 1998 139(3), 422-7.

[43] Stern, PH. De Olazabal, J; Bell, NH. Evidence for abnormal regulation of circulating 1 alpha,25-dihydroxyvitamin D in patients with sarcoidosis and normal calcium metabolism. *J Clin Invest*. 1980 66(4), 852-5.

[44] *The Merck Manual of Diagnosis and Therapy - Vitamin D Deficiency and Dependency*. 17th Edition, Section 1, Chapter 3.

[45] Mawer, EB; Hayes, ME; Still, PE; Davies, M; Lumb, GA; Palit, J; Holt, PJ. Evidence for nonrenal synthesis of 1,25-dihydroxyvitamin D in patients with inflammatory arthritis. *J Bone Miner Res.* 1991 6(7), 733-9.

[46] Inaba, M; Yukioka, K; Furumitsu, Y; Murano, M; Goto, H; Nishizawa, Y; Morii, H. Positive correlation between levels of IL-1 or IL-2 and 1,25(OH)2D/25-OH-D-ratio in synovial fluid of patients with rheumatoid arthritis. *Life Sci* 1997 61(10), 977-85.

[47] Hayes, ME; Bayley, D; Stil, P; Palit, J; Denton, J; Freemont, AJ; Cooper, RG; Mawer, EB. Differential metabolism of 25-hydroxyvitamin D3 by cultured synovial fluid macrophages and fibroblast-like cells from patients with arthritis. *Ann Rheum Dis*. 1992 51(2), 220-6.

[48] Sambrook, PN; Shawe, D; Hesp, R; Zanelli, JM; Mitchell, R; Katz, D; Gumpel, JM; Ansell, BM; Reeve, J. Rapid periarticular bone loss in rheumatoid arthritis: Possible promotion by normal circulating concentrations of parathyroid hormone or calcitriol (1,25-dihydroxyvitamin D3). *Arthritis Rheum*. 1990 33(5), 615-22.

[49] Muller, K; Kriegbaum, NJ; Baslund, B; Sorensen, OH; Thymann, M; Bentzen, K. Vitamin D3 metabolism in patients with rheumatic diseases: low serum levels of 25-hydroxyvitamin D3 in patients with systemic lupus erythematosus. *Clin Rheumatol*. 1995 14(4), 397-400.

[50] Huisman, AM; White, KP; Algra, A; Harth, M; Vieth, R; Jacobs, JW; Bijlsma, JW; Bell, DA. Vitamin D levels in women with systemic lupus erythematosus and fibromyalgia. *J Rheumatol*. 2001 28(11), 2535-9.

[51] Grant, WB. Solar UV-B radiation is linked to the geographic variation of mortality from systemic lupus erythematosus in the United States, *Lupus* 2004 13, 281-2.

[52] Ginsburg, KS; Kundsin, RB; Walter, CW; Schur, PH. Ureaplasma urealyticum and Mycoplasma hominis in women with systemic lupus erythematosus. *Arthritis Rheum*. 1992 35(4), 429-33.

[53] Millard, TP; Hawk, JL; McGregor, JM. Photosensitivity in lupus. *Lupus*. 2000 9, 3-10.

[54] Muller, K; Oxholm, P; Sorensen, OH; Thymann, M; Hoier-Madsen, M; Bendtzen, K. Abnormal vitamin D3 metabolism in patients with primary Sjogren's syndrome. *Ann Rheum Dis*. 1990 49(9), 682-4.

[55] Plotnikoff, GA; Quigley, JM. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. *Mayo Clin Proc*. 2003 78(12), 1463-70.

[56] Block, SR. Vitamin D deficiency is not associated with nonspecific musculoskeletal pain syndromes including fibromyalgia. *Mayo Clin Proc*. 2004 79(12),1585-6.

[57] Al-Allaf, AW; Mole, PA; Paterson, CR; Pullar, T. Bone health in patients with fibromyalgia. *Rheumatology*. 2003 42(10),1202-6.

[58] Hoskin, L; Clifton-Bligh, P; Hansen, R; Fulcher, G; Gates, F. Bone density and body composition in young women with chronic fatigue syndrome. *Ann NY Acad Sci.* 2000 904, 625-7.

[59] Vodjani, A; Franco, AR. Multiplex PCR for the detection of Mycoplasma fermentans, M. hominis, and M. penetrans in patients with chronic fatigue syndrome, fibromyalgia, rheumatoid arthritis and Gulf War syndrome. *J of Chronic Fatigue Syndrome* 1999 5(3/4),187-197.

[60] Nicholson, GL; Nasralla, NY; Nicholson, NL; Haier, J. High prevalence of Mycoplasmal infections in symptomatic (Chronic Fatigue Syndrome) family members of Mycoplasma-positive Gulf War Illness patients. *J Chronic Fatigue Syndrome* 2003 11(2), 21-36.

[61] Wright, A. Is CFS a bacterial illness? *Presentation at Autoimmunity Research Foundation Conference: Recovering from Chronic Disease*, March 12-13, 2005.

[62] Cantorna, MT; Zhu, Y, Froicu, M; Wittke, A. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. *Am J Clin Nutr*. 2004 80(6), 1717S-20S.

[63] Poser, CM. The epidemiology of multiple sclerosis: a general overview. *Ann. Neurol*. 1994 36, S181-S193.

[64] Fritzsche, M. Chronic Lyme borreliosis at the root of multiple sclerosis--is a cure with antibiotics attainable? *Med Hypoth.* 2005 64(3), 438-48.

[65] Munger, KL; Peeling, RW; Hernan, MA; Chasan-Taber, L; Olek, MJ; Hankinson, SE; Hunter, D; Ascherio, A. Infection with Chlamydia pneumoniae and risk of multiple sclerosis. *Epidemiology*. 2003 14(2),141-7.

[66] Reichel, H; Koeffler, HP; Barbers, R; Norman, AW. Regulation of 1,25-dihydroxyvitamin D3 production by cultured alveolar macrophages from normal human donors and from patients with pulmonary sarcoidosis. *J Clin Endocrinol Metab*. 1987 65(6), 1201-9.

[67] Ustianowski, A; Shaffer, R; Collin, S; Wilkinson, RJ; Davidson, RN. Prevalence and associations of vitamin D deficiency in foreign-born persons with tuberculosis in London. *Infection*. 2005 50(5), 432-7.

[68] Wilkinson, RJ; Llewelyn, M; Toossi, Z; Patel, P; Pasvol, G; Lalvani, A; Wright, D; Latif, M; Davidson, RN. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. *Lancet*. 2000 355(9204), 618-21.

[69] Cantorna, M; Hayes, C; DeLuca, H. 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc Natl Acad Sci*. 1996 93, 7861-7864.

[70] Koziol, JA; Feng, AC. Seasonal variations in exacerbations and MRI parameters in relapsing-remitting multiple sclerosis. *NeuroEpidemiology* 2004 23(5), 217-23.

[71] Cantorna, MT; Mahon, BD. Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. *Exp Biol Med*. 2004 229(11),1136-42.

[72] Brorson, O; Brorson, SH; Henriksen; TH; Skogen, PR; Schoyen, R. Association between multiple sclerosis and cystic structures in cerebrospinal fluid. *Infection*. 2001 29(6), 315-9.

[73] Marshall, TG; Fenter, B; Marshall, FE. Putative antibacterial mechanisms for angiotensin receptor blockers. JOIMR 2004 2(2). Available at: URL: *http://www.joimr.org/phorum/read.php?f=2andi=53andt=53*.

[74] Omdahl, JL; Morris, HA; May, BK. Hydroxylase enzymes of the vitamin D pathway: expression, function, and regulation. *Annu Rev Nutr*. 2002 22, 139-66.

[75] Mangin, M. Jarisch-Herxheimer reactions in sarcoidosis. JOIMR 2004 2(1):1 Comment on: Antibiotics in sarcoidosis - reflections on the first year. JOIMR 2003 1(3). Available at: URL: *http://www.joimr.org/phorum/read.php?f=2andi=51andt=51*.

[76] Nicholson, GL; Gan, R Haier J. Multiple co-infections (Mycoplasma, Chlamydia, human herpes virus-6) in blood of chronic fatigues syndrome patients: association with signs and symptoms. *Acta Pathol Microbiol Immunol Scand*. 2003 111,557-66.

[77] Aso, N; Tamura, A; Kadota, J; Nasu, M. Association of peripheral mononuclear cells containing Chlamydia pneumoniae DNA with acute coronary syndrome and stable coronary artery disease in Japanese subjects. *Clin Infect Dis*. 2004 39(3), 366-72.

[78] Spencer, FA; Goldberg, RJ; Becker, RC; Gore, JM. Seasonal distribution of acute myodardial infarction in the second National Registry of Myocardial Infarction. *J Am Coll Cardiol.* 1998 31(6), 1226-33.

[79] Mattman, LH. 'Stealth pathogens.' *Presentation at conference: Recovering from Chronic Disease*. Chicago, March 12, 2005.

[80] Miller, VM; Rodgers, G; Charlesworth, JA; Kirkland, B; Severson, SR, Rasmussen, TE; Yagubyan, M; Rodgers, JC; Cockerill, FR; Folk, RL; Rzewuska-Lech, E; Kumar, V; Farell-Baril, G; Lieske, JC. Evidence of nanobacterial-like structures in calcified human arteries and cardiac valves. *Am J Physiol Heart Circ Physiol*. 2004 287(3), H1115-24.

[81] Maniscalco, BS; Taylor, KA. Calcification in coronary artery disease can be reversed by EDTA-tetracycline long-term chemotherapy. Pathophysiology. 2004 11(2), 95-101.

[82] Gloth, FM 3rd; Alam, W; Hollis, B. Vitamin D vs. broad spectrum phototherapy in the treatment of seasonal affective disorder. *J Nutr Health Aging*. 1999 3(1), 5-7.

[83] Carney, PA; Fitzgerald, CT, Monaghan, CE. Influence of climate on the prevalence of mania. *Br J Psychiatry*. 1988 152, 820-3.

[84] Fallon, BA; Nields, JA. Lyme disease: a neuropsychiatric illness. *Am J Psychiatry*. 1994 151(11), 1571-83.

[85] Fritzsche, M. Geographical and seasonal correlation of multiple sclerosis to sporadic schizophrenia. *Int J Health Geogr*. 2002 1(1), 5.

[86] Goldsmith, JR; Herishanu, Y; Abarbanel, JM; Weinbaum, Z. Clustering of Parkinson's disease points to environmental etiology. *Arch Environ Health*. 1990 45(2), 88-94.

[87] Kumar, A; Calne, SM; Schulzer, M; Mak, E; Wszolek, Z; Van Netten, C; Tsui, JK; Stoessl, AJ; Calne, DB. Clustering of Parkinson disease: shared cause or coincidence? *Arch Neurol*. 2004 61(7), 1057-60.

[88] Loeb, MB; Molloy, DW; Smieja, M; Standish, T; Goldsmith, CH; Mahony, J; Smith, S; Borrie, M; Decoteau, E; Davidson, W; McDougall, A; Gnarpe, J; O'Donnell, M; Chernesky, M. A randomized, controlled trial of doxycycline and rifampin for patient with Alzheimer's disease. *J Am Geriatr Soc*. 2004 52(3), 381-7.

[89] Bolte, ER. Autism and Clostridium tetani. *Med Hypotheses*. 1998 51(2),133-44.

[90] Broxmeyer, L. Is cancer just an incurable infectious disease? *Med Hypoth.* 2004 63(6), 986-96.

[91] Cohen, RJ; Shannon, BA; McNeal, JE; Shannon, T; Garrett, KL. Propionibacterium acnes associated with inflammation in radical prostatectomy specimens: a possible link to cancer evolution? *J. Urol*. 2005 173, 1969-1974.

[92] Cantwell, AR Jr. Acid-fast bacteria in-vivo in prostate cancer and the connection between prostate cancer, other cancers, and the Kaposi's sarcoma virus. JOIMR 2004 2(3). Available at: URL*: http://www.joimr.org/phorum/read.php?f=2andi=57andt=57.*

[93] Cimolai, N. Do mycoplasmas cause human cancer? *Can J Microbiol*. 2001 47(8), 691-7.

[94] Huan, S; Li, JY; Wu, J; Meng, L; Shou, CC. Mycoplasma infections and different human carcinomas. *World J Gastroenterol*. 2001 7(2), 266-9.

[95] Tuohimaa, P; Tenkanen, L; Ahonen, M; Lumme, S; Jellum, E; Hallmans, G; Stattin, P; Harvei, S; Hakulinen, T; Luostarinen, T; Dillner, J; Lehtinen, M; Hakama, M. Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: a longitudinal, nested case-control study in the Nordic countries. *Int J Cancer*. 2004, 108(1), 104-8.

[96] Mawer, EB; Walls, J; Howell, A; Davies, M; Ratcliffe, WA; Bundred, NJ. Serum 1,25-dihydroxyvitamin D may be related inversely to disease activity in breast cancer patients with bone metastases. *J Clin Endocrinol Metab*. 1997 82(1), 118-22.

[97] Vieth, R. Why the optimal requirement for vitamin D3 is probably much higher than what is officially recommended for adults. *J Steroid Biochem Mol Biol*. 2004 89-90(1-5), 575-9.

[98] Adams, JS; Lee, G. Gains in bone mineral density with resolution of vitamin D intoxication. *Ann Intern Med*. 1997 127, 203-206.

[99] Rajasree, S; Rajpal, K; Kartha, CC; Sarma, PS; Kutty, VR; Iyer, CS; Girija, G. Serum 25-hydroxyvitamin D3 levels are elevated in South Indian patients with ischemic heart disease. *Eur J Epidemiol* 2001 17(6), 567-71.

[100] Blaney, G.; 'Familial Aggregation Panel Session.*' Conference: Recovering from Chronic Disease.* Chicago, March 12, 2005.

[101] Townsend K; Evans KN; Campbell MJ; Colston KW; Adams JS; Hewison M; Biological actions of extra-renal 25-hydroxyvitamin D-1alpha-hydroxylase and implications for chemoprevention and treatment. *J Steroid Biochem Mol Biol*. In press Aug 2005. Available from: URL*: http://tinyurl.com/bpxyl*

[102] Helming L; Bose J; Ehrchen J; Schiebe S; Frahm T; Geffers R; Probst-Kepper M; Balling R; *Lengeling A. 1{alpha},25-dihydroxyvitamin D3 is a potent suppressor of interferon-{gamma} mediated macrophage activation. Blood*. In press Aug 2005. Available from: URL*: http://tinyurl.com/7a69q*

[103] Cohen RJ; Shannon BA; McNeal JE; Shannon T; Garret KL. Propionibacterium acnes associated with inflammation in radical prostatectomy specimens: a possible link to cancer evolution? *J Urol* 2005 Jun;173(6):1969-74.