Reversing Bacteria-induced Vitamin D Receptor Dysfunction Is Key to Autoimmune Disease

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Vitamin D research is discussed in light of the hypothesis that the lower average levels of vitamin D frequently observed in autoimmune disease are not a sign of deficiency. Instead, it is proposed that the lower levels result from chronic infection with intracellular bacteria that dysregulate vitamin D metabolism by causing vitamin D receptor (VDR) dysfunction within phagocytes. The VDR dysfunction causes a decline in innate immune function that causes susceptibility to additional infections that contribute to disease progression. Evidence has been accumulating that indicates that a number of autoimmune diseases can be reversed by gradually restoring VDR function with the VDR agonist olmesartan and subinhibitory dosages of certain bacteriostatic antibiotics. Diseases showing favorable responses to treatment so far include systemic lupus erythematosis, rheumatoid arthritis, scleroderma, sarcoidosis, Sjogren's syndrome, autoimmune thyroid disease, psoriasis, ankylosing spondylitis, Reiter's syndrome, type I and II diabetes mellitus, and uveitis. Disease reversal using this approach requires limitation of vitamin D in order to avoid contributing to dysfunction of nuclear receptors and subsequent negative consequences for immune and endocrine function. Immunopathological reactions accompanying bacterial cell death require a gradual elimination of pathogens over several years. Practical and theoretical implications are discussed, along with the compatibility of this model with current research.

Key words: **bacteria; autoimmune diseases; vitamin D receptor; vitamin D; cholecalciferol; immunosuppression; 25-hydroxyvitamin D; 1,25-dihydroxyvitamin D; L-form bacteria; biofilm; natural immunity; metagenomic**

Introduction

The conventional view of autoimmune disease is that it results from the adaptive immune system "gone awry," leading to inflammation and destruction of human tissue. Consequently, immunosuppressive agents are frequently used to curb what is considered to be inappropriate immune activation. One of the agents proposed for this purpose is vitamin D.[1] The use of vitamin D in various forms has had particular appeal because of a lower level of the precursor form 25-hydroxyvitamin D (25-D) often being associated with autoimmune disease.[1,2] This inverse association has fostered the view that adding vitamin D is correcting a deficiency.

The new model discussed here is based on a different view of vitamin D and the pathogenesis of autoimmune disease.[2,3] Vitamin D is a secosteroid with a close resemblance in structure to immunosuppressive steroids. The levels of each of the vitamin D metabolites are affected by a complex network of feedback mechanisms involving multiple enzymes and receptors,[2] indicating vitamin D is regulated more

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like a steroid than a nutrient. A low level of serum 25-D is seen as the result of downregulation rather than a causal factor leading to illness. Although vitamin D metabolite levels are seen as playing an important role, it is vitamin D receptor (VDR) dysfunction that is proposed to be the key factor in the disease process.

An intraphagocytic bacterial microbiota is proposed to be the primary cause of VDR dysfunction.[2] Because VDR is key to the innate immune response, VDR dysfunction would lead to chronic infections with a wide range of pathogens, leading to inflammation and frequent elevation in autoimmune disease markers. Remission has been induced in a range of autoimmune and inflammatory diseases, using an antibacterial approach in which restoration of VDR function is used to aid in the elimination of the underlying infectious cause.[3–8]

Vitamin D Receptor and the Innate Immune System

Vitamin D is produced in the skin upon exposure to UV radiation, and varying amounts are also found in food (Table 1). The next step is conversion in the liver to 25-D and then a final hydroxylation step in the kidney to produce the active hormonal form 1,25-D (1,25-dihydroxyvitamin D).[2] However, it is now known that this last hydroxylation step can occur in many other tissues, notably in activated macrophages and dendritic cells.[2] The precursor form, 25-D, is the form that is most commonly measured.

The view of vitamin D metabolites as primarily of interest for bone metabolism has been superseded by the recognition that when the only active form of vitamin D, 1,25-D, activates the VDR, it affects the expression of more than 900 genes.[2,9] One of the most important effects of VDR activation is its ability to increase the innate immune response, including the production of antimicrobial peptides, which are important in controlling a wide variety of pathogens.[2,10] The importance of innate/natural immunity has been increasingly recognized in autoimmune disease as well.[2,11]

Nature of Bacterial Microbiota Implicated in Autoimmune Disease

The potential for chronic infectious agents to be a causal factor for autoimmune disease has long been recognized and has recently been receiving increased attention.[12,13] Shoenfeld *et al.*[14] noted that autoimmune disease markers can be highly elevated during chronic mycobacterial infections. Advances in detection techniques using improved genome-based[12] and culturing methodologies[15] will almost certainly greatly expand the number of pathogens implicated in chronic disease.

It is increasingly recognized that bacteria can persist as cell wall-deficient variants (L-forms)[15–17] and as "persister" forms within metagenomic bacterial communities.[18] Biofilm communities are protected by a polymeric matrix that allows bacteria to survive both immune system attack and antibiotic administration.[18] Bacteria have been observed in intracellular inclusions in a number of autoimmune diseases[17,19] and have been observed persisting within phagocytes.[15]

Recent examples of how advanced molecular techniques can uncover previously undetected bacteria include a study of bacterial diversity in wounds[20] and a study of biofilm communities in patients with Barrett's esophagus,[21] a precancerous condition. It would seem that more studies applying advanced microbiological techniques to autoimmune diseases are called for as well.

Marshall[2] has noted that any intracellular bacteria capable of producing a substance that blocks the VDR would have an effective strategy for disabling the immune system. Molecular modeling has indicated that one type of bacteria does produce a substance capable of disabling the VDR, providing proof of concept.[2] It is the sulfonolipid capnine, which is produced by some gliding bacteria. Similar

TABLE 1. A Simple Overview of Several Important Vitamin D Metabolites and their Significance in Marshall's Vitamin D Receptor (VDR)-dysfunction Model of Autoimmune/Inflammatory Disease

Vitamin D Metabolite	Sources	Measurement in Clinical Setting	Effect on VDR
Vitamin D	skin, food, supplements	not measured	concentration-dependent antagonist
25-D	converted from vitamin D	most commonly measured, diagnostically useful	concentration-dependent antagonist
1,25-D: Active Form	converted from 25-D	seldom measured, proper handling important, diagnostically useful	concentration-dependent agonist – ineffective when VDR is blocked

At higher concentrations, all of the vitamin D metabolites act upon other receptors, with potentially damaging effects on hormonal and immune function. For a detailed model of the feedback system affecting vitamin D metabolite levels, see Marshall.[2]

gliding bacteria have been identified in biofilm communities on prosthetic hip joints.[2,6]

Immune activation accompanies bacterial killing, and this leads to transient increases in symptoms that vary greatly in nature and severity. These reactions have been referred to as being similar to Jarisch–Herxheimer reactions,[3,8] immune reconstitution inflammatory syndrome, or para-inflammation[22] and have been related to apoptosis of infected cells and cytokine increases.[6] We will refer to this range of reactions as immunopathological reactions. Immunosuppressive agents will tend to reduce these reactions and minimize symptoms. However, over the long term, such an approach is seen as counterproductive as decreased immune function allows chronic pathogens to persist and spread with greater ease (Table 2).

VDR Dysfunction Leads to Lowered 25-D and Elevated 1,25-D

A model of vitamin D metabolic pathways was presented by Marshall,[2] showing how bacteria-induced VDR dysfunction could explain the low 25-D and high 1,25-D levels observed in a variety of autoimmune diseases. The model also provides the basis for a treatment that restores normal VDR and innate immune system function (Table 2).

Although usually tightly regulated by the kidneys, 1,25-D production can rise to high levels in inflammatory diseases, such as sarcoidosis, from unregulated extrarenal production by activated macrophages and dendritic cells.[2,7] High levels of 1,25-D can also bind to the nuclear receptor PXR and inhibit the ability of the enzyme CYP24A1 to break down 1,25-D and thus regulate its levels.[2] This generally results in elevated serum 1,25-D in autoimmune diseases.[2,7]

Elevated levels of 1,25-D bound to the PXR also inhibit the conversion of vitamin D to 25-D in the liver. This ability of high levels of 1,25-D to downregulate the production of 25-D has been observed *in vivo* and has been linked to diseases such as sarcoidosis.[23]

The pattern of vitamin D metabolite levels in VDR knockout mice[24] appears to be supportive of the model presented here. An absent VDR achieved through genetic manipulation, as in the VDR knockout mice, can be compared to a VDR disabled by chronic pathogens, with both causing low 25-D and high 1,25-D levels.

Vitamin D Metabolites, Vitamin D Receptor Dysfunction, and the Innate Immune System

Recent *in silico* data indicate that vitamin D supplementation, if it achieves high enough levels of the precursor forms (vitamin D and

TABLE 2. Marshall's VDR-dysfunction Model of Autoimmune/Inflammatory Disease[2,3,8,22]—Summary of Main Components of Model's Etiology, Treatment, and Predictions

Model Facet	Model Component
Etiology	1. Intraphagocytic bacteria produce ligands that block the VDR.
	2. Blocked VDR reduces innate immune function, allowing pathogens to increase.
	3. High 25-D and 1,25-D can contribute to inhibition of innate immune function.
	4. Development of autoimmune/inflammatory disease, with the particular diagnosis varying with the organisms acquired and host factors.
Treatment	1. VDR agonist olmesartan partially displaces bacterial products that block VDR.
	2. Vitamin D metabolites lowered by reducing ingested vitamin D and sun exposure.
	3. Selected, low-dosage, pulsed bacteriostatic antibiotics are used.
	4. To reduce the risk of severe immunopathology reactions, low dosages of minocycline alone are used at first, other antibiotics being added later over several years.
Prediction	1. Low 25-D and high 1,25-D serum levels, resulting from high 1,25-D inhibiting conversion of vitamin D to 25-D, will be useful for diagnosis of chronic inflammatory illnesses.
	2. Increasing vitamin D metabolites to high levels will be palliative in the short term and harmful in the long term.
	3. Improved immune function with treatment will aid in the control of many bacterial and nonbacterial pathogens.

25-D), actually contributes to VDR blockage and a decrease in the innate immune response (Table 1).[5]

In addition, supplemental vitamin D, by increasing levels of 25-D and 1,25-D, can affect the activation of other receptors.[2] Molecular modeling indicates that at high levels 25-D and 1,25-D can displace the natural ligands from nuclear receptors, such as the thyroid-α-1, adrenal, and glucocorticoid receptors. This displacement appears to have the potential to disrupt the endocrine system.[2] It could also lead to immunosuppression by reducing the ability of these receptors to induce production of antimicrobial peptides.[2]

Thus, at least two mechanisms exist by which vitamin D supplementation could suppress innate immune system function. This suppression would lead to short-term improvements in patients taking higher levels of vitamin D by slowing bacterial death and subsequent immunopathological reactions. Therefore, studies that evaluate the role of vitamin D supplementation in chronic inflammatory diseases should take into account the potential for short-term symptom reduction as well as exacerbation of the disease process over the long term from pathogen increase.

Short-term palliation generated by increased production of vitamin D in the skin could account for some reports of seasonal improvement in autoimmune disease symptoms.[25] It is also consistent with observations of recovery being hampered in the long term by high serum 25-D and 1,25-D levels.[7]

Evidence supporting this negative effect of higher 25-D and 1,25-D levels can be found in a number of studies in diverse fields. Peacock *et al.*[26] found higher levels of 25-D blunted the favorable effect of calcium on bone density. Some recent studies are finding higher levels of cancer when 25-D is raised[27,28] or when there is evidence of higher amounts of long-term sun exposure.[29] Freedman *et al.*[30] found higher overall cancer mortality at the two highest 25-D quintiles in a large, moderately long-term (median 8.9 years), prospective study, although it did not reach statistical significance. Ramos-Remus *et al.*[31] found a 12-year earlier average age of onset of rheumatoid arthritis in Mexico as compared to the relatively light-deprived Canada. Payne *et al.*,[32] using MRI, found a higher volume of brain lesions was associated with greater vitamin D intake. In the multivariable regression model, vitamin D intake retained its highly significant correlation with

brain lesion volume ($P = 0.007$) even after the effect of calcium consumption was statistically removed. These lesions have been found to reflect gray and white matter damage and are associated with dementia, depression, stroke, and other impairments.[32]

Marshall[2] reviewed recent large meta-analyses looking at vitamin D and autoimmune disease, overall mortality, cancer mortality, and bone density and found that, in general, they did not show a clear benefit for vitamin D supplementation. Problems with many studies on vitamin D include failure to adequately consider confounding factors, failure to measure the active metabolite (1,25-D), inadequate study lengths, over reliance on *in vitro* and animal studies, lack of randomization,[7] and a failure to consider the alternative hypothesis for low vitamin D levels.

As mentioned previously, the majority of studies inferring vitamin D deficiency plays a role in causing disease are epidemiological studies that are just as consistent with the alternative hypothesis that low vitamin D levels are a result of the disease process rather than a cause. One exception is in diabetes mellitus type I where there have been several studies that found vitamin D supplementation to be associated with lower diabetes rates.[1] These studies, however, were not randomized and thus could easily be biased by confounding factors affecting who decides to give supplemental vitamin D to their infants and small children. And even if a preventive benefit were confirmed in further studies, it would not follow that vitamin D supplementation would be beneficial once disease is established or when subjects are older; nor would it follow that it would be the safest or most effective means of prevention.

Although there is some evidence that raising 25-D levels fuels innate immune function by increasing the availability of precursor 25-D to be converted to 1,25-D,[10,33] the results are not consistent,[34] and further studies are needed. Even if this were to be confirmed in the healthy general population, however, it would not necessarily be true for those who already have bacteria-induced VDR dysfunction. A divergence in response in different populations might even be an explanation for the mixed inconclusive results in vitamin D studies.

On the whole, we see increasing support for the alternate model in which bacteria-induced VDR dysfunction is the cause of many autoimmune diseases. Vitamin D dysregulation would be expected to precede the onset of symptoms and increase with advancing illness. It would also account for the lower average 25-D levels observed in a wide range of chronic diseases.

Practical Implications of the Model for Diagnostic Purposes

Acceptance of the bacteria-induced VDR dysfunction model has a number of implications for diagnosis, identification of at-risk family members, assessment of disease severity, and treatment decisions in autoimmune disease. A high 1,25-D, a low 25-D, or a high D ratio (1,25-D/25-D) can all be used as signs of vitamin D dysregulation and suggest inflammatory disease involving VDR dysfunction (Table 1). Use of serum 25-D and 1,25-D levels as markers of inflammation requires first ruling out secondary hyperparathyroidism as a result of low calcium intake or elevated phosphorus from kidney disease.

Marshall *et al.*[3] found an elevated D ratio ranging from 2.0 to more than 4.5 in sarcoidosis. An abnormal ratio has also been observed in a number of different autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, and Sjogren's syndrome.[3,7]

Although vitamin D metabolite levels can indicate whether a patient has one of a number of inflammatory or autoimmune diseases related to vitamin D dysregulation, they do not allow for determination of a specific diagnosis.[7] For the latter goal, other tests would be needed. However, a very high 1,25-D (e.g., >80 pg/mL or 200 nmol/L) suggests involvement of one or more highly perfused tissues, such as the lungs, heart, or gastrointestinal tract.[7]

Because the active form of vitamin D (1,25-D) tends to degrade easily, the serum sample must be kept frozen for transport and analyzed fairly soon after it is received.[7] While the largest owner of laboratories in the United States, Quest Diagnostics, requires this level of rigor, many others do not.

Evidence indicates that the normal reference ranges used by most laboratories are too broad.[7,22] One of the reasons for this is the supplementation of the food supply in some countries. Also, there are likely to be individuals assumed to be "normal" who have early, relatively asymptomatic, undiagnosed illness resulting in high 1,25-D levels. These and other factors make it hard to determine the most appropriate normal range for vitamin D metabolites.

As with nearly all diagnostic tests, serum vitamin D tests can give false negatives. For instance, VDR dysfunction may be so high that bacterial killing is minimized and inflammation is relatively low, leading to lower 1,25-D than expected. Alternatively, regulation of serum 1,25-D by the kidneys may be able to compensate for the production by activated macrophages. Thus, in some tissues, 1,25-D may reach high levels locally, but there may be little or no elevation in serum levels.[7]

In many cases low 25-D may be a good indicator of disease state because of the feedback mechanism discussed earlier in which blockage of the VDR downregulates the conversion of vitamin D to the 25-D form. In other cases, 25-D is likely to be less diagnostic because of particularly high supplemental/dietary vitamin D ingestion and/or high sun exposure.

In cases where 25-D levels are normal and disease is suspected, a therapeutic probe can be used to aid in diagnosis. This involves administering the VDR agonist olmesartan, followed by pulsed low dosages of minocycline.[a] Typically, systemic immunopathological reactions that wax and wane with each dose of antibiotic reveal the presence of bacteria.[7,8,22] The enhancement of the response by the VDR agonist olmesartan supports the importance of VDR dysfunction. In some patients with high 25-D, we have observed the response to the therapeutic probe to be delayed or reduced until their high 25-D levels decline through reducing ingested vitamin D and sun exposure. This further supports the contention that high 25-D levels have the ability to slow the innate immune response in chronically ill patients.

Reversal of Autoimmune Diseases through Restoring VDR Function

Evidence indicates that remission can be induced in a wide range of autoimmune diseases with an approach that restores innate immune function through restoring VDR function.[7,8] Upon administration of the VDR agonist olmesartan, most patients with autoimmune diseases experience a significant change in their symptom level as a result of a combination of olmesartan's effect on VDR activation and its palliative effects on other receptors.[22]

In silico data have shown that olmesartan has a high affinity for the VDR (Fig. 1).[5] The high concentrations of olmesartan used in this treatment protocol allow it to activate the VDR at an effective level for the activation of the innate immune response, despite the bacteria-induced VDR dysfunction.

The use of pulsed, subinhibitory, bacteriostatic antibiotics is an important part of this approach. Research indicates that most standard antibiotic protocols used so far to treat chronic inflammatory diseases are ineffective[35] and so new ways of using antibiotics are called for to deal with the relative treatment resistance of the bacteria involved. For instance, many antibiotics will not be effective because they target cell walls and this actually promotes the production of cell wall-deficient forms of bacteria.[18] Furthermore, when taken at high dosages, many antibiotics are able to cause potentially significant inhibition of phagocytic functioning.[36]

[a] For guidelines detailing the antibiotics, dosages and pulsing schedules used, contact Foundation@AutoimmunityResearch.org.

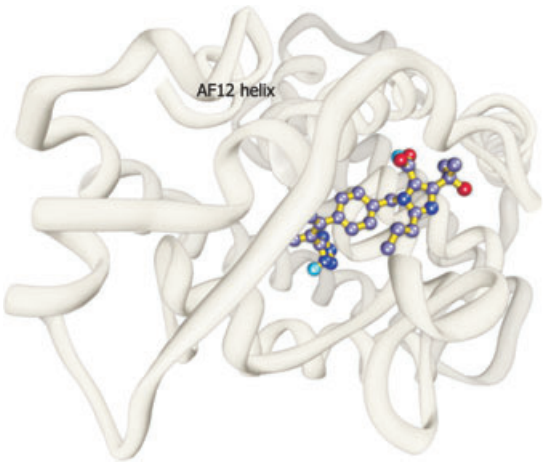

Figure 1. Olmesartan in the ligand binding pocket of the vitamin D receptor (VDR). The AF12 helix is identified to indicate the position of olmesartan within the receptor. Marshall[2,5] used molecular dynamics modeling, confirmed with clinical data, to demonstrate that olmesartan is an agonist for the human VDR.

The survival of "persister" cells when constant dosages are used means that pulsed antibiotics are likely to be more effective.[18] The ability of bacteriostatic antibiotics to be effective at low dosage levels has been documented.[37] The existence of communities of multiple bacterial species, like those occurring in biofilms, means that combinations of antibiotics are likely to be necessary to fully target all species. Thus, there is increasing support for the use of pulsed low dosages of combinations of bacteriostatic antibiotics.

The immunopathological reactions elicited by the VDR agonist olmesartan support the presence of VDR dysfunction. The waxing and waning of symptoms that typically follows each of the pulsed antibiotic doses supports the model's contention that bacteria are involved in autoimmune disease. The fact that these immunopathological responses decline over time, as the patient improves during treatment, provides evidence of disease reversal.

Diseases responding to treatment include systemic lupus erythematosus, rheumatoid arthritis, scleroderma, Sjogren's syndrome, autoimmune thyroid disease, psoriasis, ankylosing spondylitis, Reiter's syndrome, type I and II diabetes mellitus, and uveitis,[8] and sarcoidosis.[3] Fibromyalgia, although not generally established as an autoimmune disease, has also responded favorably to this approach.[5,7]

Bone density data gathered so far indicate that as long as patients ingest adequate calcium there is typically either improvement in bone density or, at least, a reduced rate of decline in bone density over the course of this treatment. This is consistent with the model's prediction that use of the VDR agonist olmesartan and elimination of the intraphagocytic bacteria will improve VDR function and allow for sufficient calcium absorption without supplemental vitamin D.

It should be noted that the immunopathological reactions resulting from this approach are sometimes strong enough to be life threatening in patients with more advanced disease.[3] These reactions are consistent with a high accumulation of chronic pathogens not previously controlled adequately by the immune system.

The intensity of the immunopathological reactions is a potentially limiting factor when it comes to treating patients with advanced disease. The identification or development of palliative medications that can reduce damage from immunopathological reactions but do not also cause VDR dysfunction or immune suppression to a damaging degree could improve prospects for such patients and marks one of our ongoing areas of investigation.

The choice of a VDR agonist is very important so as to achieve the right level of VDR activation without negatively impacting the activity of other nuclear receptors also involved in the immune response.[2] So far, olmesartan is the only agent that seems to satisfy these requirements. In addition, careful control of antibiotic timing and dosage by a schedule determined by experience and modified according to individual reactions[a] is required in order to reduce immunosuppressive effects of the antibiotics and maximize their ability to target these treatment-resistant bacteria.[8,22]

Conclusions

The model presented here describes a method of eliminating chronic infectious agents that are proposed to be the cause of many autoimmune diseases (Table 2). The ability of certain intraphagocytic bacteria to cause VDR dysfunction is believed to be key. Increasing evidence indicates that vitamin D supplementation can contribute to bacteria-induced dysfunction of the VDR. This VDR dysfunction leads to immunosuppression that, while palliative in the short term, is counterproductive for long-term healing. The VDR agonist olmesartan, however, has been found to accomplish the necessary degree of VDR activation without negatively affecting other receptors involved in the innate immune response.

Among patients in which VDR activation is accomplished with olmesartan, the innate immune system, with the help of pulsed, low-dosage, broad-spectrum, bacteriostatic antibiotics,[a] appears to have reversed the disease process in a number of autoimmune diseases.[5,8,22] The immunopathological response typically observed after each pulse of antibiotics, along with subsequent long-term improvement, provides evidence for the validity of this approach and the causal role of bacteria in many autoimmune diseases.[3,5,7,8]

Thus, an "alternate" model exists that is consistent with a variety of observations regarding vitamin D metabolites and autoimmune diseases and is based on bacterial blockage of the VDR. A treatment that targets the source of VDR dysfunction is currently showing great potential for reversing many autoimmune diseases.[3,7,8] Further research is needed on various aspects of the model and antibacterial protocol. However, it appears that this approach could provide a potentially curative treatment for many chronic debilitating diseases.

Because of the length of this chapter, we are unable to summarize all of the cutting-edge issues that surround this research. For this reason, we refer to the following recent literature on this subject.[38–42]

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Conflicts of Interest

The authors declare no conflicts of interest.

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