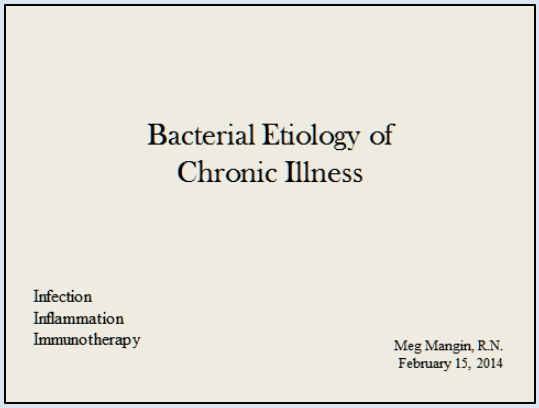
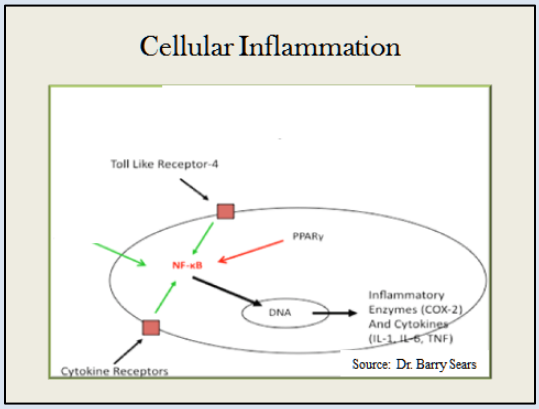
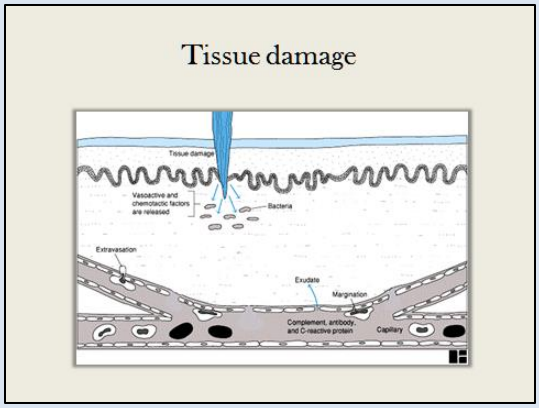


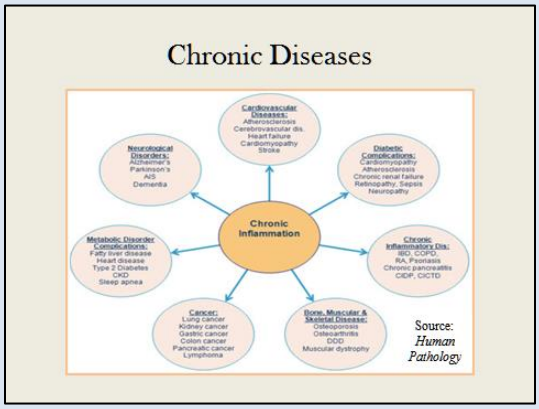
For many decades, atypical bacteria have been suspected of being persistent pleomorphic vehicles contributing to subsequent relapse in infectious diseases and as etiologic agents in chronic inflammatory conditions of unknown origin. Infectious agents give rise to various chronic illnesses, sometimes directly but in other cases by triggering damaging immune responses. While flooding us with interesting and often dramatic reports of so-called emerging infectious diseases, the media have largely ignored a more fundamental change in our appreciation of human-microorganism interactions: the discovery that transmissible agents may play important roles in diseases not suspected of being infectious in origin. Persistent infection may be the underlying cause of many clinical entities presently classified as idiopathic or of uncertain origin.



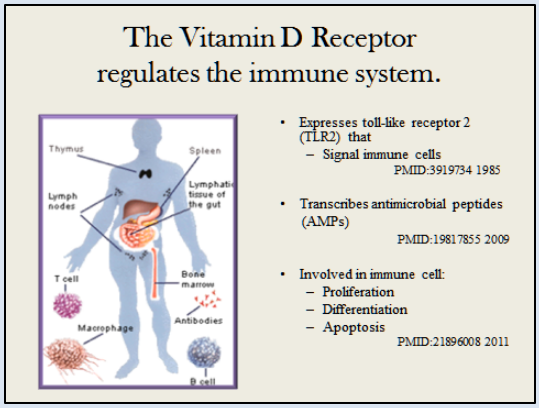
Inflammation is the result of a complex cascade of biochemical events initiated by the immune system in response to harmful stimuli. Classical inflammation is identified by pain (dolor), redness (rubor), heat (calor), and swelling (tumor); and loss of function (torpor). This form of inflammation is typically a short-term response to infection and injury, aimed at removing the infective stimulus and allowing repair of the damaged tissue, ultimately resulting in healing and a return to homeostasis. The usual result of inflammation is protection from the spread of infection, followed by resolution-the restoration of affected tissues to their normal structural and functional state.



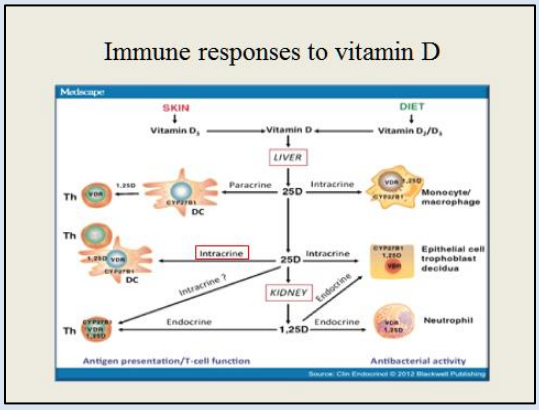
Non-resolving inflammation is a prolonged, dysregulated, and maladaptive inflammatory response associated with many chronic diseases. Chronic inflammation differs from classical inflammation in that it 1) is low-grade, causing only a small rise in immune system markers (i.e., a 4- to 6-fold increase vs a several-hundred-fold increase); 2) is persistent and results in chronic, rather than acute, wear and tear on the body; 3) has systemic rather than local effects; 4) has antigens that are less apparent as foreign agents or microbial pathogens; 5) appears to perpetuate, rather than resolve disease; and 6) is associated with a reduced, rather than increased, metabolic rate. Chronic inflammation eventually results in tissue damage caused by the production of cytokines and microbial factors.



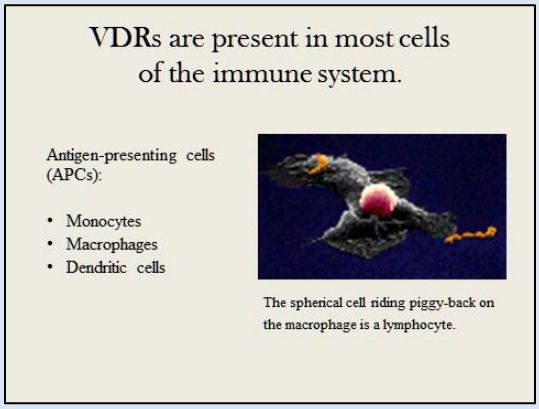
Chronic low-level inflammation that is below the threshold of pain can be termed “silent inflammation”. Since there is no pain associated with this type of inflammation, nothing is done to stop it, and thus it can linger for years, if not decades, causing continual organ damage. As long as appropriate reparative mechanisms and the regenerative/compensatory potential of organs and tissues are maintained, the development of chronic degenerative conditions are prevented or delayed. However, eventually, exhaustion of the reparative/regeneration potential will occur, with subsequent organ damage, loss of function and the onset of overt chronic disease although the initiating pathogenic events may have started decades earlier, triggered by the underlying silent inflammation process.



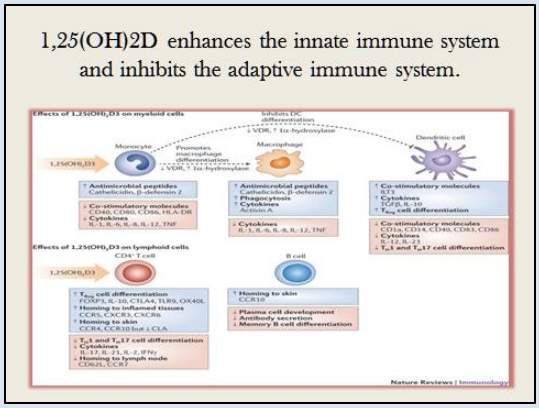
The vitamin D receptor (VDR) regulates the immune system. An effective immune response is heavily dependent on a competent VDR. The influence of the active form of vitamin D-1,25(OH)2D-on the VDR is one of its most important roles. VDR immune system regulation involves cell proliferation, differentiation and apoptosis. In general, the innate system is enhanced and the adaptive system is inhibited by 1,25(OH)2D and it's action on the VDR.



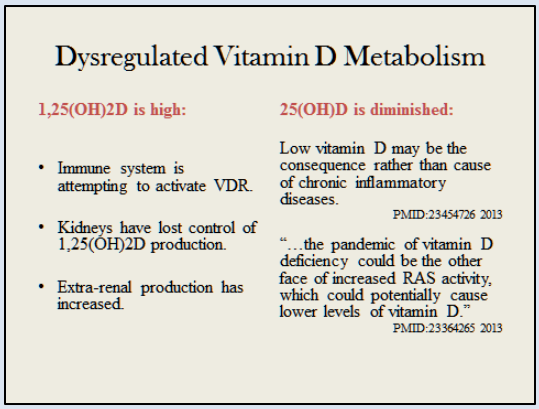
In monocytes and macrophages (innate immune system), synthesis of 1,25(OH)2D from 25(OH)D promotes an antibacterial response to infection. The VDR is expressed in both B and T white blood cells (lymphocytes). 1,25(OH)2D activates the VDR to express antimicrobial peptides (AMPs) such as cathelicidin and beta defensins which attack pathogens. Many cells outside the kidneys also contain VDR and express CYP27B1 (the enzyme that catalyzes 25(OH)D to 1,25(OH)2D).



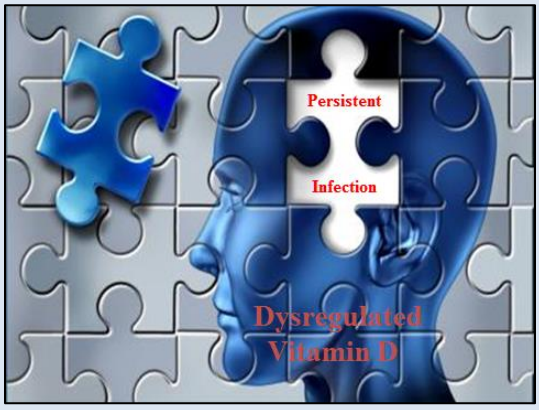
VDRs are present in most cell types of the immune system, particularly in antigen-presenting cells (APCs) such as monocyte, macrophages and dendritic cells. Monocytes sense pathogen-associated molecular patterns (PAMPs) by utilizing pattern-recognition receptors (PRR), such as toll-like receptors (TLRs). Induction of CYP27B1 occurs following PAMP-sensing by TLR2/1. The inflammatory cytokine interferon γ (IFNγ) also stimulates expression of CYP27B1 by macrophages. As a result, 1,25(OH)2D production is increased in response to a pathogen immune challenge.



1,25(OH)2D modulates the adaptive immune system by inhibiting dendritic cell maturation, reducing T helper (Th) cells, and shifting Th_1/Th_{17} cells to the Th_2 and T regulatory pathways. In addition, 1,25(OH)2D inhibits Th_1 cytokines that support cell-mediated immunity and promotes Th_2 cytokines that support humoral immunity (antibodies circulating in bodily fluids). The immune response is heavily dependent on the vitamin D endocrine system, performing a balancing act of inflammation/anti-inflammation.



In the healthy individual, the complex interplay between innate and adaptive immunity cooperates to mount an appropriate response to infection through regulation of the vitamin D endocrine system. In theory, the immune system detects and responds to the presence of CWD bacteria by producing more 1,25(OH)2D to activate the VDR and express the crucial endogenous AMPs which enable the innate immune system to target intracellular pathogens.



Theoretically, persistent intracellular bacterial infection compromises the immune system and causes a chronic inflammatory response. Cell-wall-deficient bacteria parasitize nucleated cells in order to escape host defenses, thus contributing to failures of treatment. The concept that intracellular bacteria are protected from the host's immune response was first proposed by Rous in 1916. In an essay on the renin-angiotensin system and the immune response, Smith postulates that unresolved cellular stress is caused by infectious agents, with the deliberate intent to avoid adaptive immune responses.

Evidence points to persistent pathogens.

- *Outsmarting the host: bacteria modulating the immune response.*

  PMID:18592144 2008

- *Survival of intracellular pathogens within macrophages.*

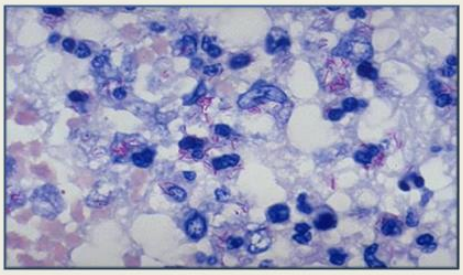
  http://link.springer.com/article/10.1007%2FBF01314950#page-1

- "Unresolved cellular stress may be caused by infectious agents, with the deliberate intent to avoid adaptive immune responses."

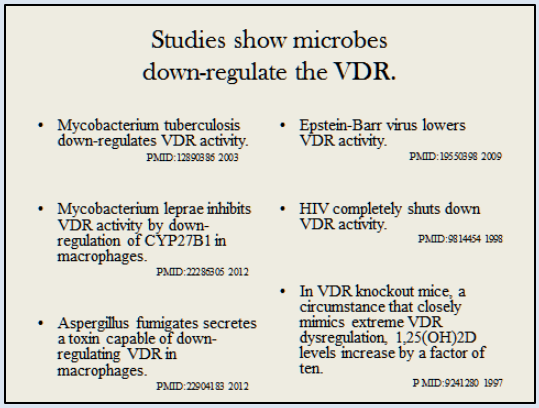
  PMID:23533336 2013

The host immune system has developed many mechanisms to neutralize and remove pathogenic bacteria. In turn, bacteria have developed mechanisms to alter and evade the host immune response. For example, regulation of the vitamin D receptor (VDR) is a common mechanism used in the host defense against pathogens, but certain microbes have been shown to slow innate immune defenses by down-regulating the VDR.

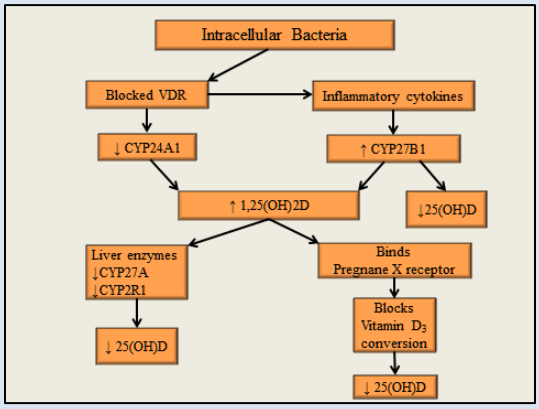
Intracellular Mycobacterium tuberculosis in lung.
Ziehl-Neelsen acid fast stain (CDC)



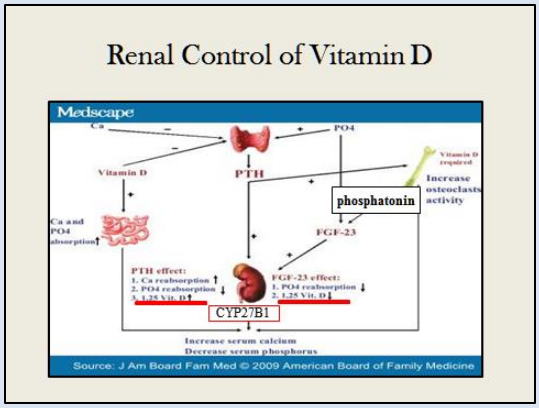
Gabriel Nunez, M.D., Professor of Pathology at the University of Michigan Medical School, was quoted in the university newsletter, "In our study, the presence of bacterial microbes inside the cell is what triggers the immune response." French researchers observe in 2007 that the presence of pathogenic invasive bacteria could be the link between an innate immune response to invasive bacteria and the development of the inflammation. Dr. Siobhan O'Connor, assistant to the director of the National Center for Infectious Diseases, stated, "The epidemiologic, clinical, and pathologic features of many chronic inflammatory diseases are consistent with a microbial cause. Infectious agents likely determine more cancers, immune-mediated syndromes, neurodevelopmental disorders, and other chronic conditions than currently appreciated."



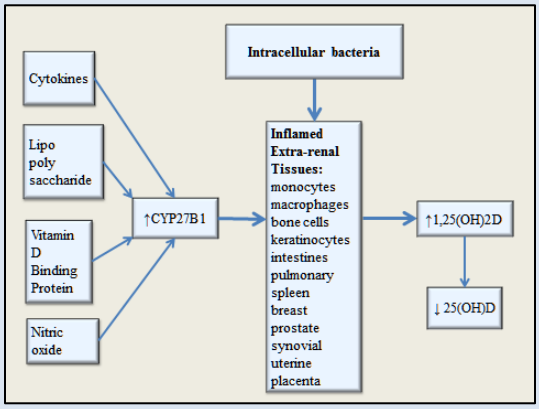
Slowing the ability of the VDR to express elements of innate immune function allows intracellular bacteria to persist in the cytoplasm of nucleated cells and increases susceptibility to co-infections that are commonly found in patients with chronic illnesses (e.g., viruses, fungi, parasites and cell-walled bacteria). 1,25(OH)2D is a marker of vitamin D endocrine function. Down-regulation by bacterial ligands may prevent the VDR from expressing the enzymes necessary to keep 1,25(OH)2D in a normal range. Elevated 1,25(OH)2D also reduces VDR competence, suppresses macrophage function, and inhibits the Nuclear Factor kappa-ß cytokine pathway, thus further compromising the immune system.



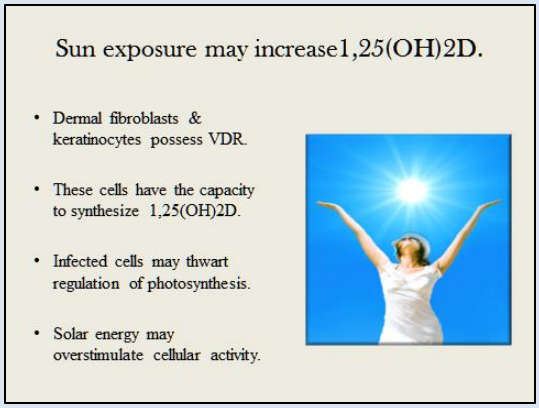
Elevated 1,25(OH)2D further reduces VDR competence, suppresses macrophage function, and blocks the Nuclear Factor kappa-ß pathway; thus inhibiting immune system function. Slowing the ability of VDR to express elements of innate immune function allows intracellular bacteria to persist in the cytoplasm of nucleated cells and also increases susceptibility to co-infections that are commonly found in patients with chronic illnesses (e.g., viruses, fungi, parasites and cell-walled bacteria). In conclusion, high levels of 1,25-D may result when down-regulation of the VDR by bacterial ligands prevents the receptor from expressing enzymes necessary to keep 1,25-D in a normal range. Elevated 1,25(OH)2D appears to be evidence of a disabled immune system's attempt to activate the VDR to combat infection.



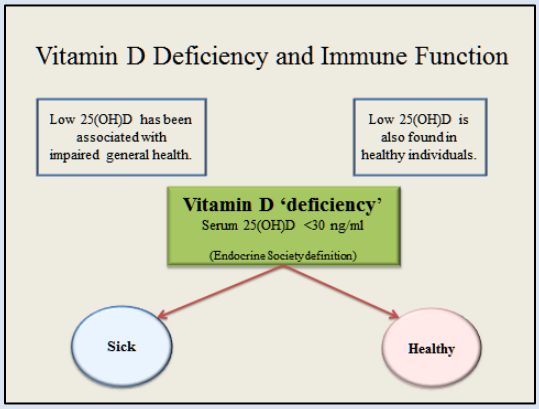
Renal production of 1,25(OH)2D is tightly self-regulated, with the end product down-regulating its own further production. In contrast, extra-renal tissues which produce 1,25(OH)2D are regulated by cytokines, lipopolysaccharide, nitric oxide and intracellular VDBP, which activate the enzyme CYP27B1 to stimulate conversion of 25(OH)D to 1,25(OH)2D. Data suggest that local synthesis of 1,25(OH)(2)D may be a preferred mode of response to antigenic challenge in many tissues and locally synthesized 1,25(OH)2D has the potential to spill-over into the general circulation. This extra-renal production of 1,25(OH)2D in tissues infected with intracellular bacteria can result in an excess production of 1,25(OH)2D which may contribute to depletion and low levels of 25(OH)D.



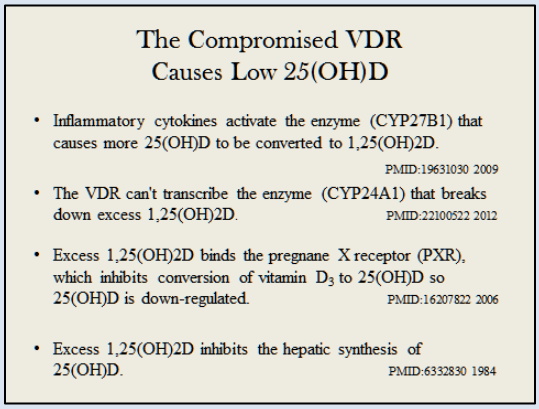
When nucleated cells are parasitized by CWD bacteria, extra-renal production of 1,25(OH)2D increases, the kidneys lose control of 1,25(OH)2D production, and pro-hormone 25(OH)D decreases due to rapid conversion to 1,25(OH)2D. Thus, low 25(OH)D may be a consequence of the inflammatory process. More studies are concluding that suboptimal circulating levels of vitamin D appear to be caused by the disease process.



Sunlight appears to play a part in this process. Vanderschueren et al. observed seasonal variations in 1,25(OH)2D at all levels of 25(OH)D and concluded that sunlight exposure appears to have an influence on 1,25(OH)2D very similar to that of 25(OH)D. The skin (dermal fibroblasts and keratinocytes possess VDR) has the capacity to synthesize 1,25(OH)2D, and represents an important target tissue for 1,25(OH)2D. If keratinocytes in the skin are infected, natural regulation of photosynthesis may be thwarted and solar energy may overstimulate cellular activity, resulting in an increase in cutaneous production of vitamin D_3, 25(OH)D and 1,25(OH)2D following sun exposure.



Low 25(OH)D levels are associated with impaired general health but low 25(OH)D is found in both healthy persons and those with autoimmune or chronic inflammatory diseases. Assessing vitamin D status with the measurement of an additional clinical marker may be helpful.



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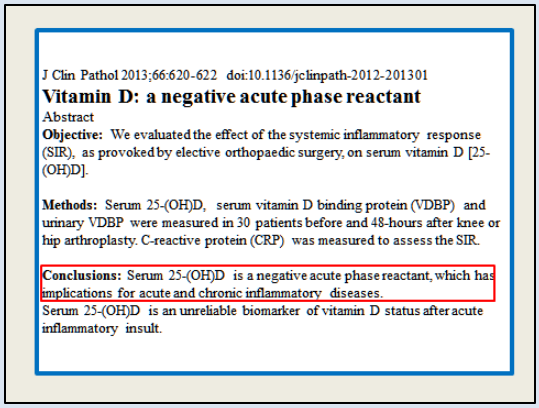


J Clin Pathol 2013;66:620-622 doi:10.1136/jclinpath-2012-201301

Vitamin D: a negative acute phase reactant

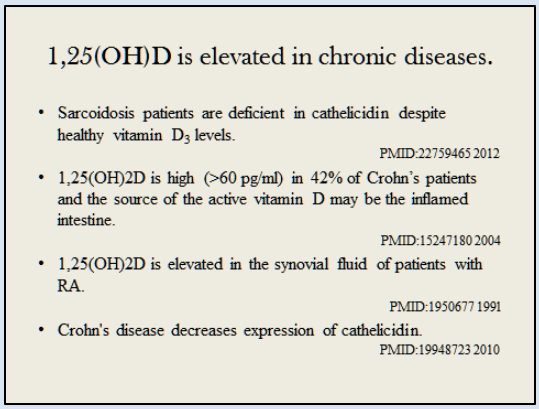
Abstract

Objective: We evaluated the effect of the systemic inflammatory response (SIR), as provoked by elective orthopaedic surgery, on serum vitamin D [25-(OH)D].

Methods: Serum 25-(OH)D, serum vitamin D binding protein (VDBP) and urinary VDBP were measured in 30 patients before and 48-hours after knee or hip arthroplasty. C-reactive protein (CRP) was measured to assess the SIR.

Conclusions: Serum 25-(OH)D is a negative acute phase reactant, which has implications for acute and chronic inflammatory diseases.
Serum 25-(OH)D is an unreliable biomarker of vitamin D status after acute inflammatory insult.

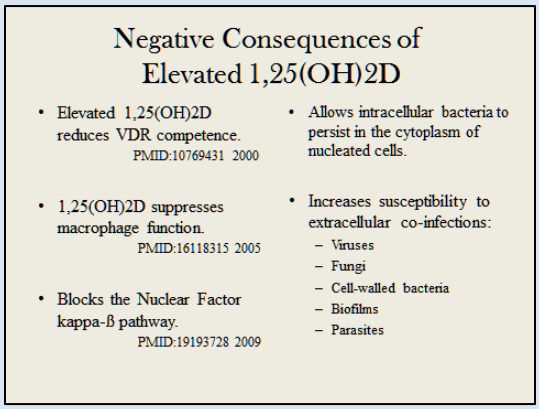
Waldron et. al found serum 25(OH)D was decreased following an acute inflammatory insult (i.e., orthopedic surgery) and concluded hypovitaminosis D may be the consequence rather than cause of chronic inflammatory diseases. Thus, low 25(OH)D may be a consequence of the inflammatory process.



1,25(OH)D is elevated in chronic diseases.

- Sarcoidosis patients are deficient in cathelicidin despite healthy vitamin D_3 levels.
  PMID:22759465 2012
- 1,25(OH)2D is high (>60 pg/ml) in 42% of Crohn's patients and the source of the active vitamin D may be the inflamed intestine.
  PMID:15247180 2004
- 1,25(OH)2D is elevated in the synovial fluid of patients with RA.
  PMID:1950677 1991
- Crohn's disease decreases expression of cathelicidin.
  PMID:19948723 2010

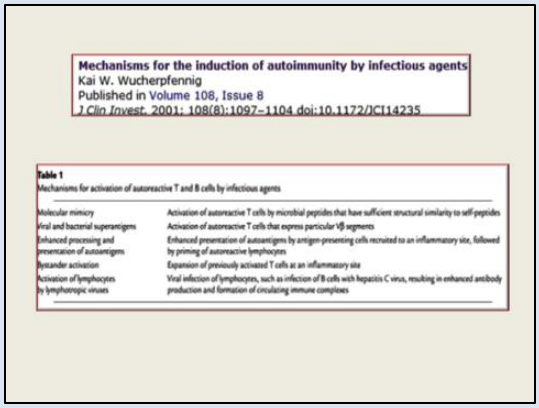
Elevated 1,25(OH)2D is evidence of the dysregulated immune system's attempt to activate the VDR to produce antimicrobial peptides (e.g., cathelicidin) to combat infection. Studies have found elevated 1,25(OH)2D and reduced cathelicidin in chronic diseases.



Negative Consequences of Elevated 1,25(OH)2D

- Elevated 1,25(OH)2D reduces VDR competence.
  PMID:10769431 2000
- 1,25(OH)2D suppresses macrophage function.
  PMID:16118315 2005
- Blocks the Nuclear Factor kappa-ß pathway.
  PMID:19193728 2009
- Allows intracellular bacteria to persist in the cytoplasm of nucleated cells.
- Increases susceptibility to extracellular co-infections:
  - Viruses
  - Fungi
  - Cell-walled bacteria
  - Biofilms
  - Parasites

Regulation of the vitamin D receptor (VDR) is a common mechanism used in the host defense against pathogens, but certain microbes have been shown to slow innate immune defenses by down-regulating the VDR. Slowing the ability of the VDR to express elements of innate immune function allows intracellular bacteria to persist in the cytoplasm of nucleated cells and increases susceptibility to co-infections that are commonly found in patients with chronic illnesses (e.g., viruses, fungi, parasites and cell-walled bacteria).

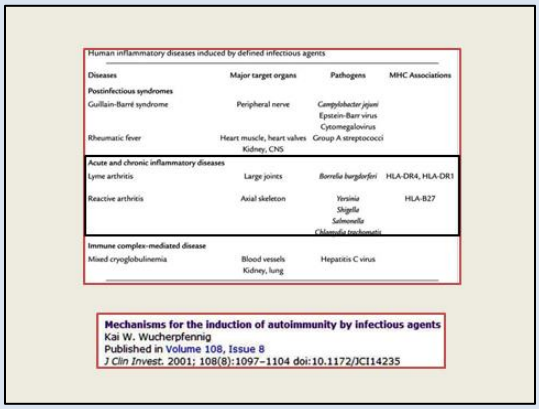


Mechanisms for the induction of autoimmunity by infectious agents
Kai W. Wucherpfennig
Published in Volume 108, Issue 8
J Clin Invest. 2001; 108(8):1097–1104 doi:10.1172/JCI14235

Table 1
Mechanisms for activation of autoreactive T and B cells by infectious agents

Molecular mimicry	Activation of autoreactive T cells by microbial peptides that have sufficient structural similarity to self-peptides
Viral and bacterial superantigens	Activation of autoreactive T cells that express particular Vβ segments
Enhanced processing and presentation of autoantigens	Enhanced presentation of autoantigens by antigen-presenting cells recruited to an inflammatory site, followed by priming of autoreactive lymphocytes
Bystander activation	Expansion of previously activated T cells at an inflammatory site
Activation of lymphocytes by lymphotropic viruses	Viral infection of lymphocytes, such as infection of B cells with hepatitis C virus, resulting in enhanced antibody production and formation of circulating immune complexes

Infections (bacterial, viral and parasitic) are known to induce and exacerbate autoimmune diseases. Numerous examples can be found in which pathogens express antigens that cross-react with host antigens or induce local inflammatory responses that can lead to autoimmune responses through a very complex set of circumstances.



Human inflammatory diseases induced by defined infectious agents

Diseases	Major target organs	Pathogens	MHC Associations
Postinfectious syndromes			
Guillain-Barré syndrome	Peripheral nerve	*Campylobacter jejuni* Epstein-Barr virus Cytomegalovirus	
Rheumatic fever	Heart muscle, heart valves Kidney, CNS	Group A streptococci	
Acute and chronic inflammatory diseases			
Lyme arthritis	Large joints	*Borrelia burgdorferi*	HLA-DR4, HLA-DR1
Reactive arthritis	Axial skeleton	*Yersinia* *Shigella* *Salmonella* *Chlamydia trachomatis*	HLA-B27
Immune complex–mediated disease			
Mixed cryoglobulinemia	Blood vessels Kidney, lung	Hepatitis C virus	

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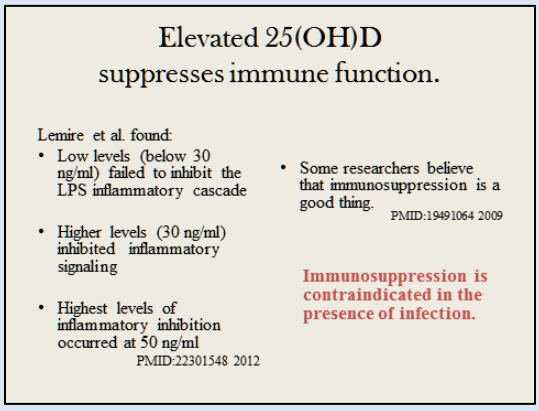
The bacterial pathogenesis theorizes that intracellular bacteria cause abnormal vitamin D endocrine function, resulting in low vitamin D. Specifically, cell wall deficient (CWD) bacteria invade nucleated cells and use strategies to avoid destruction. Excess 1,25(OH)2D is produced in an effort to up-regulate the VDR to transcribe AMPs; and 25(OH)D is rapidly metabolized in the process, resulting in a low serum level. The resulting elevated 1,25(OH)2D causes chronic, systemic inflammation and its accompanying symptoms. Gerald J Domingue, Professor Emeritus of Tulane University School of Medicine commented, “This might translate into an etiology for chronic inflammatory diseases, when the stressed bacteria increase in numbers and overwhelm the normal biological functions of the host.”



Elevated 25(OH)D
suppresses immune function.

Lemire et al. found:

- Low levels (below 30 ng/ml) failed to inhibit the LPS inflammatory cascade
- Higher levels (30 ng/ml) inhibited inflammatory signaling
- Highest levels of inflammatory inhibition occurred at 50 ng/ml

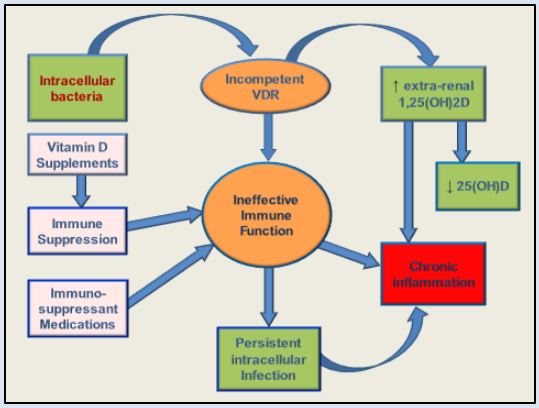
PMID:22301548 2012

- Some researchers believe that immunosuppression is a good thing.

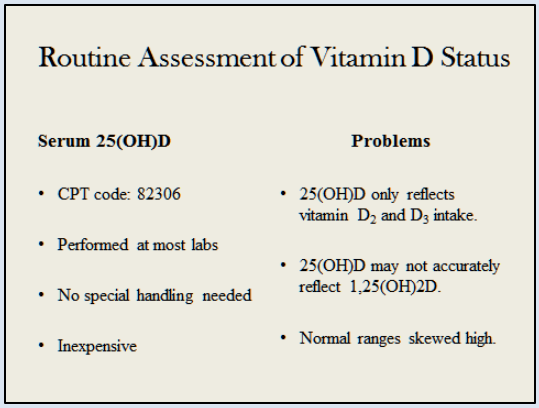
PMID:19491064 2009

Immunosuppression is contraindicated in the presence of infection.

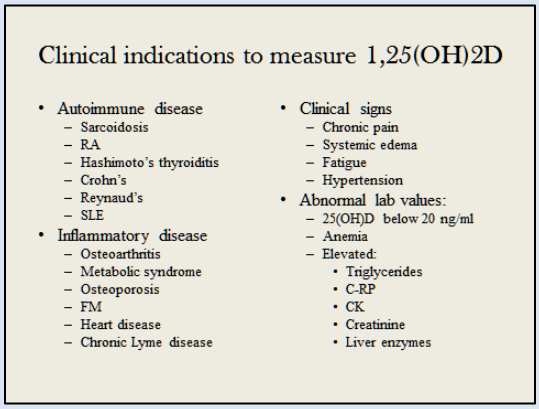
A known effect of 25(OH)D is suppression of the immune system. In a study of a pro-inflammatory molecule, lipopolysaccharide (LPS), Lemire found elevated 25(OH)D reduced the inflammatory cascade. Also, 25(OH)D can be indirectly immunosuppressive by two methods. First, by being converted to excess 1,25(OH)2D. And second, by its effect on the VDBP (Gc protein). Theoretically, immune system suppression allows parasitic microbes to persist and proliferate in host phagocytes, successfully compete for nutritional resources, and displace commensal organisms from their niche.



Chronic inflammation is a sign of immune system dysfunction; a probable cause is found in the ability of cell wall deficient bacteria to invade nucleated cells. These pathogens persist within cellular cytoplasm by using strategies to evade destruction. One of those strategies appears to be down-regulation of the vitamin D receptor (VDR) which is activated by 1,25(OH)2D3 (calcitriol). This is suggested by the presence of elevated calcitriol in many diseases linked to an inflammatory process. Normally, calcitriol production is tightly self-regulated by the kidneys with the end product down-regulating its own further production. In contrast, production of calcitriol in extra-renal tissues is controlled by cytokines. When extra-renal cells are parasitized by bacteria, calcitriol production is stimulated and renal control is lost. Elevated calcitriol indicates the immune system recognizes the presence of parasitic pathogens and is making a futile attempt to combat them by increasing the production of calcitriol in order to up-regulate the VDR and transcribe antimicrobial peptides (AMPs). The result is sustained inflammation, tissue damage and multi-morbidity. The angiotensin receptor blocker olmesartan medoxomil, when used at higher than anti-hypertensive doses, appears to be an agonistic VDR ligand which up-regulates the bacterially-inhibited VDR.



Assessing vitamin D metabolites and diagnosing dysregulated vitamin D metabolism has the potential to guide clinical practice. Vitamin D status is currently determined by measuring the level of 25(OH)D which, presumably, reflects the levels of other vitamin D metabolites (e.g., vitamin D_3, vitamin D_2 and 1,25(OH)2D, etc.). This measurement may not, however, provide enough information to assess vitamin D endocrine function. Although 25(OH)D is the major circulating metabolite of vitamin D and the form most often assessed clinically, it is the active 1,25-dihydroxylated form of the hormone that is responsible for its biological effects.



Measuring 1,25(OH)2D should be considered in patients with low 25(OH)D, abnormal lab results (especially inflammatory markers), a diagnosis of autoimmune disease or other chronic inflammatory illness, or signs of chronic systemic inflammation. For example, elevated 1,25(OH)2D is observed Crohn's disease. In written correspondence (2013), vitamin D researcher Martin Hewison (Professor in Residence at the David Geffen School of Medicine UCLA), stated, "I agree that 1,25(OH)2D is a forgotten component of the vitamin D and human health story - I think measurement of serum 1,25(OH)2D will be more common as LC:MS techniques improve."

Measure 1,25-Dihydroxyvitamin-D

- $1,25(OH)2D_3$
- CPT code: 82652
- ICD-9 codes:
  - 733.00 Osteoporosis, unspecified
  - 733.90 Osteopenia
  - 780.9 Fatigue
- A specialized lab is required
- Freeze for transport to avoid degradation due to agitation
  - A low result may be inaccurate due to sample mishandling
  - A high result is always accurate
- Maximum normal = 45 pg/ml (Merck Manual 2006)

The clinical utility of measuring 1,25(OH)D is not fully understood, but it is clear that associations are being made between this active metabolite of vitamin D and disease states. Measurement of both the active metabolite and its precursor is essential to diagnose dysregulated vitamin D metabolism; assays of 1,25(OH)2D and 25(OH)D provide valuable tools to assess inflammation in chronically ill patients. Vitamin D status encompasses more than vitamin D intake; 1,25(OH)2D formation isn't directly regulated by parental vitamin D and it may be affected by the same factors that cause a decrease in serum 25(OH)D.

Why 1,25(OH)2D isn't measured

- 1,25(OH)2D has a short half-life (hours) and fluctuates rapidly.

However, a high result may be discovered even at trough level.

- 1,25(OH)2D levels are regulated by PTH, calcium, phosphate.

This isn't true in chronic illness when extra-renal production is prevalent.

- 1,25(OH)2D doesn't decrease until 25(OH)D is very low.

A low 25(OH)D may be a sign that 1,25(OH)2D is abnormally high.

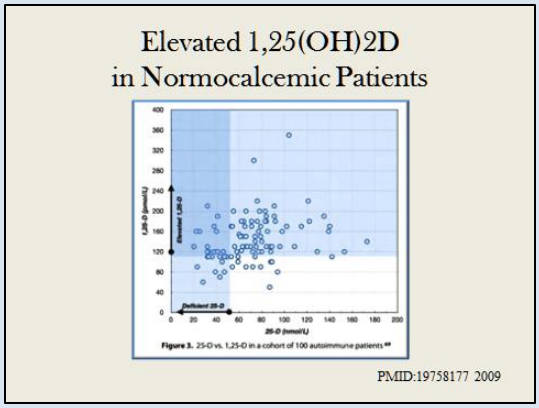
- 1,25(OH)2D is only over-produced in hypercalcemic disease states.

Studies show this isn't true. PMID:19758177 2009

- 1,25(OH)2D may be elevated as a result of up-regulation of the CYP27B1 enzyme.

This begs the question "Why is this enzyme elevated?"

Currently, 1,25(OH)2D is not being used as a measure associated with vitamin D nutritional status or as an intermediate marker related to health outcomes, or even routinely assessed in vitamin D research. In the context of solving the puzzle of low 25(OH)D, the reasons cited for this lapse fail to consider the possibility of abnormal levels in the presence of chronic inflammation.



It is asserted that low levels of 25(OH)D accurately reflect vitamin D status; (i.e., vitamin D storage and VDR-mediated control of calcium metabolism and innate immunity). However, measurement of 1,25(OH)2D often demonstrates a positive correlation of elevated 1,25(OH)2D to inflammatory diseases. Blaney et al. found that serum 25(OH)D is not a sensitive measure of the autoimmune disease state. Their findings support the use of 1,25(OH)2D as a clinical marker in autoimmune conditions. This is illustrated by a study, done in Vancouver, of 100 patients with autoimmune and chronic disease which found 85% of subjects had levels of 1,25(OH)2D higher than 46.2 pg/ml without hypercalcemia. Although this serum level may be considered normal by some, lab ranges for 1,25(OH)2 (e.g., 18-72 pg/ml) may have been skewed high by the presence of patients with unrecognized persistent intracellular infection and, thus, dysregulated vitamin D metabolism.

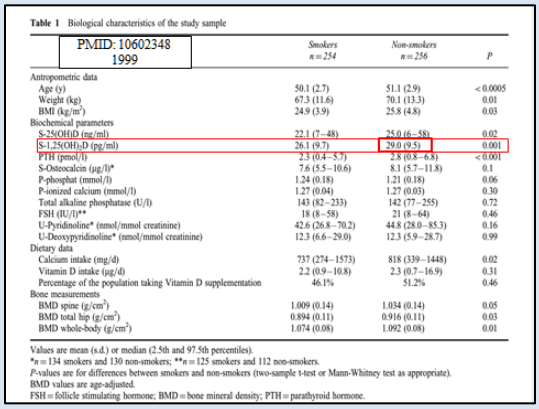


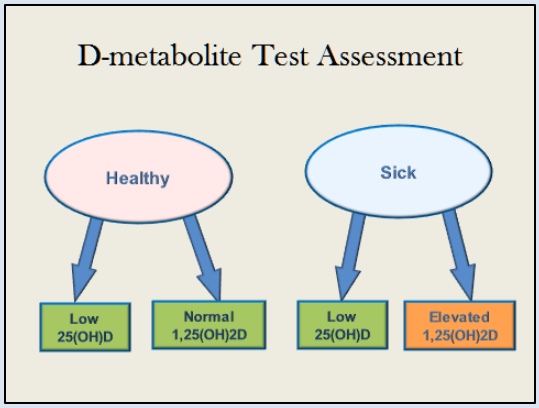
Table 1 Biological characteristics of the study sample

PMID: 10602348
1999

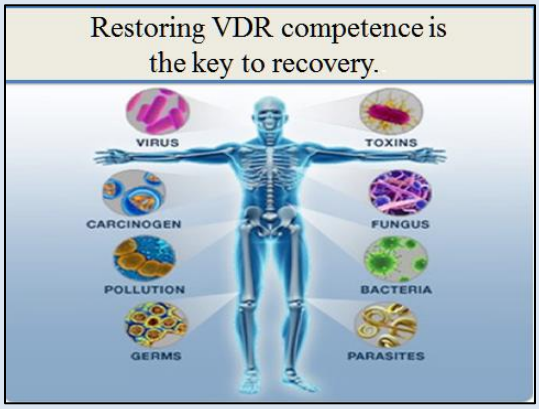
	Smokers n = 254	*Non-smokers n = 256*	*P*
Antropometric data			
Age (y)	50.1 (2.7)	51.1 (2.9)	< 0.0005
Weight (kg)	67.3 (11.6)	70.1 (13.3)	0.01
BMI (kg/m^2)	24.9 (3.9)	25.8 (4.8)	0.03
Biochemical parameters			
S-25(OH)D (ng/ml)	22.1 (7–48)	25.0 (6–58)	0.02
S-1,25$(OH)_2$D (pg/ml)	26.1 (9.7)	29.0 (9.5)	0.001
PTH (pmol/l)	2.3 (0.4–5.7)	2.8 (0.8–6.8)	< 0.001
S-Osteocalcin (µg/l)*	7.6 (5.5–10.6)	8.1 (5.7–11.8)	0.1
P-phosphat (mmol/l)	1.24 (0.18)	1.21 (0.18)	0.06
P-ionized calcium (mmol/l)	1.27 (0.04)	1.27 (0.03)	0.30
Total alkaline phosphatase (U/l)	143 (82–233)	142 (77–255)	0.72
FSH (IU/l)**	18 (8–58)	21 (8–64)	0.46
U-Pyridinoline* (nmol/mmol creatinine)	42.6 (26.8–70.2)	44.8 (28.0–85.3)	0.16
U-Deoxypyridinoline* (nmol/mmol creatinine)	12.3 (6.6–29.0)	12.3 (5.9–28.7)	0.99
Dietary data			
Calcium intake (mg/d)	737 (274–1573)	818 (339–1448)	0.02
Vitamin D intake (µg/d)	2.2 (0.9–10.8)	2.3 (0.7–16.9)	0.31
Percentage of the population taking Vitamin D supplementation	46.1%	51.2%	0.46
Bone measurements			
BMD spine (g/cm^2)	1.009 (0.14)	1.034 (0.14)	0.05
BMD total hip (g/cm^2)	0.894 (0.11)	0.916 (0.11)	0.03
BMD whole-body (g/cm^2)	1.074 (0.08)	1.092 (0.08)	0.01

Values are mean (s.d.) or median (2.5th and 97.5th percentiles).
*$n = 134$ smokers and 130 non-smokers; **$n = 125$ smokers and 112 non-smokers.
P-values are for differences between smokers and non-smokers (two-sample t-test or Mann-Whitney test as appropriate).
BMD values are age-adjusted.
FSH = follicle stimulating hormone; BMD = bone mineral density; PTH = parathyroid hormone.

The Danish 1,25(OH)2D population data (from a large and reliable study done in 1999) provides a more realistic picture of 1,25(OH)2D concentrations. They found the mean value for 1,25(OH)2D in a normal population was 29 pg/ml with a standard deviation of 9.5. More frequent measurement of both D-metabolites in the clinical and research settings, may shed light on the real meaning of low 25(OH)D.



Serum 1,25-D is not an assay that is done routinely; it's usually only performed when endocrine dysfunction is suspected and the meaning of the elevation is often overlooked. For example, in a study of the effect of vitamin D with calcium supplementation on patients with multiple sclerosis, serum 1,25(OH)2D, which was measured coincidentally, revealed high concentrations at baseline and one year later (61 pg/ml ± 22.6 pg/ml and 70.7 pg/ml ± 18 pg/ml respectively). These 1,25(OH)2D concentrations were considered normal and neither calcium or PTH were measured. Measuring both 25(OH)D and 1,25(OH)2D (and PTH, calcium, phosphate when indicated) as clinical markers in chronic disease is more likely to provide a true picture of vitamin D status, than measuring 25(OH)D alone.



The ability to mount an appropriate response to intracellular infection is highly dependent on a competent VDR. When it appears that 1,25(OH)2D is unable to up-regulate the VDR due to microbial activity, another VDR ligand may be able to act as an agonist (an agonist increases the signal transduction activity of a cell when bound to a receptor on that cell) and restore VDR competence.

Ligands may up-regulate the VDR.

Vitamin D VDR ligands

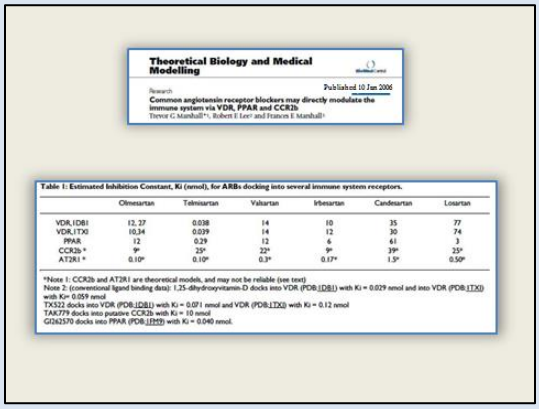
- Endogenous
  - 1,25(OH)2D
  - 25(OH)D
- Synthetic 1,25(OH)D analogs

Non-vitamin D VDR ligands

- Curcumin (turmeric) PMID:20153625 2010
- Omega3/omega6 polyunsaturated fatty acids (PUFAs) PMID:18290715 2007
- Omega-6 fatty acids:
  - Arachidonic acid
  - Linoleic acid

  PMID:18844852 2008
- Lithocolic acid (LCA) derivatives PMID:18844852 2008
- Angiotensin receptor blockers (ARBs) PMID:16403216 2006

Over 3000 synthetic VDR ligands have been identified but most have no clinical use because of their undue disruption to calcium regulation. [2] A number of non-vitamin D VDR ligands have been identified (curcumin, arachidonic acid, linoleic acid), and lithocolic acid but their usefulness is disputed.



Theoretical Biology and Medical Modelling

Published 10 Jan 2006

Research

Common angiotensin receptor blockers may directly modulate the immune system via VDR, PPAR and CCR2b

Trevor G Marshall*†, Robert F Lee† and Frances E Marshall†

Table 1: Estimated Inhibition Constant, Ki (nmol), for ARBs docking into several immune system receptors.

	Olmesartan	Telmisartan	Valsartan	Irbesartan	Candesartan	Losartan
VDR,1DB1	12, 27	0.038	14	10	35	77
VDR,1TX0	10,34	0.039	14	12	30	74
PPAR	12	0.29	12	6	61	3
CCR2b *	9*	25*	22*	9*	39*	25*
AT2R1 *	0.10*	0.10*	0.3*	0.17*	1.5*	0.50*

*Note 1: CCR2b and AT2R1 are theoretical models, and may not be reliable (see text)
Note 2: (conventional ligand binding data): 1,25-dihydroxyvitamin-D docks into VDR (PDB:1DB1) with Ki = 0.029 nmol and into VDR (PDB:1TX0) with Ki= 0.059 nmol
TX522 docks into VDR (PDB:1DB1) with Ki = 0.071 nmol and VDR (PDB:1TX0) with Ki = 0.12 nmol
TAK779 docks into putative CCR2b with Ki = 10 nmol
GI262570 docks into PPAR (PDB:1I7I) with Ki = 0.040 nmol.

Marshall et. al, using computer modeling, found evidence that angiotensin receptor blockers (ARBs) modulate activation of the VDR. In particular, the ARB olmesartan medoxomil (brand name Benicar®) was estimated to have a Ki value in the low nanomolar range, similar to the Ki values of the natural vitamin D ligands.

Olmesartan is a VDR agonist*

*Increases the signal transduction activity of a cell when bound to a receptor on that cell.

- Molecular modeling revealed a high affinity for the VDR PMID:16403216 2006
- Only ARB shown to lower angiotensin II. PMID:11768722 2001
- 1,25(OH)2D has a similar effect by repressing renin genetic expression to down-regulate the RAS. PMID:12122115 2002

The angiotensin receptor blocker olmesartan medoxomil, when used at higher than anti-hypertensive doses, appears to be an agonistic VDR ligand which up-regulates the bacterially-inhibited VDR. Although this use of olmesartan is off-label, its safety profile is well established. The multiple beneficial effects of olmesartan, including the ability to correct imbalance in Th subsets, to treat cardiovascular and kidney disease, prevent migraines, and ameliorate ischemic cerebral brain damage, suggest it could play a key role in the resolution of chronic systemic inflammation.

Olmesartan medoxomil

- US brand name Benicar®.
- Marketed in other countries under different trade names.
- Approved by the FDA to treat hypertension.

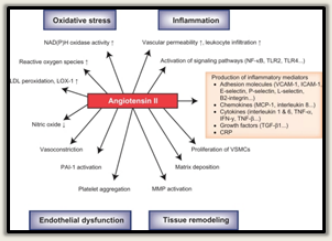
PMID:11967728 2002

- The FDA found doses up to 80mg per day were safe.
- Following a 2011 safety review, the FDA approved continued use of all ARBs.

The angiotensin receptor blocker olmesartan medoxomil, when used at higher than anti-hypertensive doses, appears to be an agonistic VDR ligand which up-regulates the bacterially-inhibited VDR. Although this use of olmesartan is off-label, its safety profile is well established. The multiple beneficial effects of olmesartan, including the ability to correct imbalance in Th subsets, to treat cardiovascular and kidney disease, prevent migraines, and ameliorate ischemic cerebral brain damage, suggest it could play a key role in the resolution of chronic systemic inflammation.

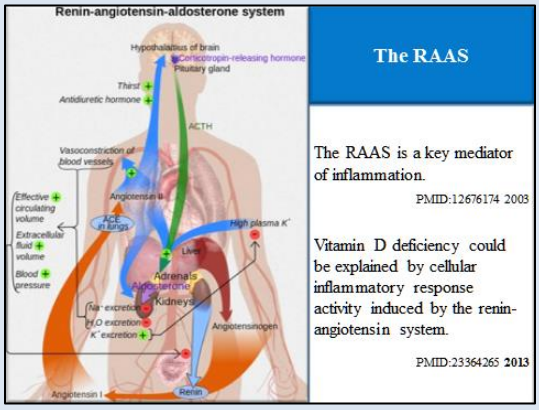
Optimal therapeutic strategy for treating patients with hypertension and atherosclerosis: focus on ***olmesartan medoxomil***

PMID:21796255 2011



Angiotensin II is a peptide that's implicated in the inflammatory process.

All ARBs block angiotensin receptors, which directly causes vasodilation, reduces vasopressin and aldosterone. This usually results is reduction of blood pressure. Olmesartan, however, is the only ARB that also lowers angiotensin II.



The RAAS

The RAAS is a key mediator of inflammation.

PMID:12676174 2003

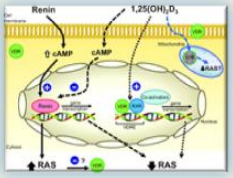
Vitamin D deficiency could be explained by cellular inflammatory response activity induced by the renin-angiotensin system.

PMID:23364265 **2013**

Shao et. al found a link between RAAS activity and activation of the VDR: …the inappropriate stimulation of the RAS has been associated with the pathogenesis of hypertension, heart attack, stroke, and hypertrophy of both the left ventricle and vascular smooth muscle cells.

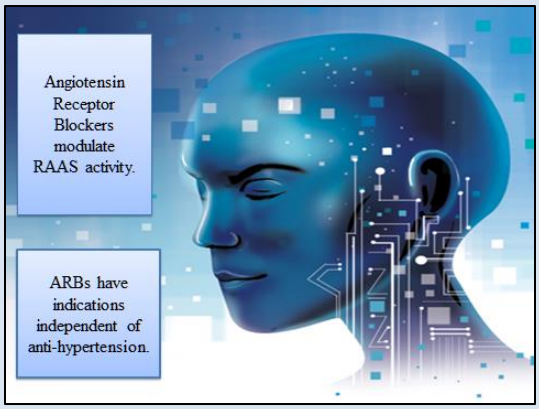
Vitamin D metabolism and the RAAS

- VDR and RAAS receptors are found in the same tissues.
- Both systems regulate inflammatory and immunological mechanisms.
- Changes in RAAS activity and activation of VDR seem to be inversely related.



- Both systems could have a feedback relationship.

Ferder et. al stated: "…there may be a relationship between inflammatory processes induced by chronic overstimulation of the renin angiotensin system (RAS) and the worldwide vitamin D deficiency… In fact, the pandemic of vitamin D deficiency could be the other face of increased RAS activity, which could potentially cause a lower activity or lower levels of Vitamin D."



Changes in RAS activity and activation of VDR seem to be inversely related, making it possible to speculate that both systems could have a feedback relationship. [The researchers conclude] the combination of RAS blockade and VDR stimulation appears to be more effective than each one used individually. This is what olmesartan appears to accomplish (blocking angiotensin II and stimulating the VDR) and is consistent with a theory of VDR incompetence.

Researchers are studying the anti-inflammatory effects of ARBs.

- ARBs may represent a novel class of anti-inflammatory drugs with indications far beyond cardiovascular diseases.
  PMID:16164395 2005
- Modulation of the RAAS with inexpensive, safe pharmaceuticals is an attractive therapeutic strategy for application to autoimmune diseases.
  PMID:19706421 2009
- *Anti-inflammatory properties of drugs acting on the renin-angiotensinsystem.*
  PMID:16341292 2005
- *Anti-inflammatory effects of angiotensin II subtype 1 receptor blockade in hypertensive patients with micro-inflammation.*
  PMID:15313950 2004
- *Protective Effects of Angiotensin II Interruption: Evidence for Antiinflammatory Actions.*
  PMID:16164395 2005

Anti-inflammatory Effects of Olmesartan

After just six weeks, olmesartan significantly reduced serum levels of inflammatory markers:

- HS-CRP
- HS tumor necrosis factor-alpha
- Interleukin-6
- Monocyte chemotactic protein-1.

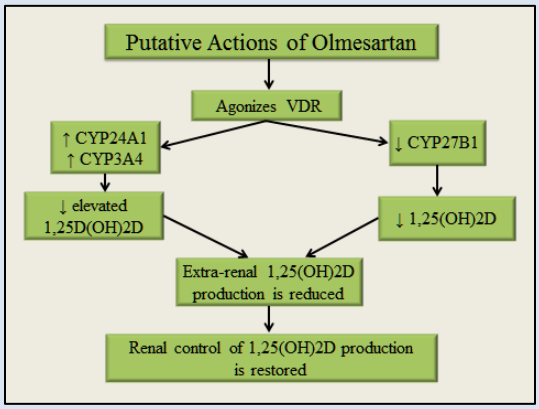
PMID:15313950 2004

Olmesartan is being used off-label by some clinicians to treat dysregulated vitamin D metabolism.

Potential uses of olmesartan:

- CKD — PMID:23154587 2013
- Auto-immune — PMID:15879491 2005
- Cardiac — PMID:24164503 2013
- Atherosclerosis — PMID:17192125 2006
- Migraines — PMID:16618270 2006
- Diabetes — PMID:23303198 2013
- Osteoporosis — PMID:23775504 2013

The multiple beneficial effects of olmesartan, including the ability to correct imbalance in Th subsets, to treat cardiovascular and kidney disease, prevent migraines, and ameliorate ischemic cerebral brain damage, suggest it could play a key role in the resolution of chronic systemic inflammation.



Therapeutic response → Immunopathology*

*Collateral pathological effects on the body caused by the immune system during normal function:

- Hormonal effects
- Endocrine effects
- Cell death
- Blood count changes

Immunopathology suggests bacterial killing due to transcription of AMPs and up-regulation of the VDR.

PMID:19817855 2009

A Jarisch-Herxheimer (JHR) reaction is usually seen following administration of olmesartan. JHR is a cascade of reactions including inflammation, cytokine release, and endotoxin release as part of the immune response to the disintegration of infected cells. These inflammatory symptoms (and inflammatory lab markers) that wax and wane in response to olmesartan administration provide evidence of occult infection.

Phagocytosis leads to bacterial death.

PMID:7619330 1995

Inflammation is increased by:

- Cytokine reaction to:
  - Bacterial endotoxins
  - Cellular debris (dead host & bacteria cells)

- Jarisch-Herxheimer reaction (JHR)
  - TB PMID:18600241 2009
  - Lyme PMID:9846580 1998
  - Syphilis PMID:606336 1977

JHR suggests olmesartan has restored VDR competence.

"A blockade of hijacked receptors may offer promising options to control infection and associated immunopathology."
PMID:21350579 2011

"From a therapeutic point of view, the combination of RAAS blockade and VDR stimulation appears to be more effective than each one used individually."
PMID:23364265 2013

Theoretically, olmesartan restores VDR competence and, thus, phagocytosis leads to bacterial death; consequently, inflammation is increased by cytokine reaction to microbial endotoxins and cellular debris from dead host cells and bacteria. This immunopathology suggests a robust immune response and transcription of AMPs by an activated VDR; and provides additional evidence that olmesartan is a VDR agonist.

Antibacterials are used as an adjunct to olmesartan immunotherapy.

Some researchers recommend antibiotics to treat chronic inflammatory conditions.
PMID:22185451 2012

Some antibiotics provide both antimicrobial and anti-inflammatory effects, which may be key in treating chronic inflammatory disorders.
PMID:23108365 2013

Sub-inhibitory antibiotics are capable of blocking pathogenic mechanisms.
PMID:9421312 1997

Optimal therapy of chronic infections requires the use of antibiotics which can penetrate phagocytes and inactivate intracellular organisms."
PMID:7073264 1982

To help eradicate the intracellular pathogens, select antibiotics are administered which, revealingly, cause an exacerbation of inflammatory symptoms (JHR) with each dose.

Features of Effective Antibiotics

Actions:

- Weaken bacteria ribosomes
- Penetrate cell walls
  PMID:8067738 1994
- Accumulate in phagocytes
  PMID:1864282 1991
- Interfere with folate synthesis
  PMID:7408366 1980
- Immuno-modulatory
  PMID:20021425 2008

- Bacteriostatic
- Good safety profile
- Oral
- Sub-inhibitory
  PMID: 16942902 2006
- Pulsed
- Gradually introduced
- Synergistic combinations

Sub-inhibitory oral antibiotics, are gradually introduced in a pulsed fashion; for their ability to weaken bacterial ribosomes, penetrate cell walls and blood brain barrier, accumulate in phagocytes, or interfere with folate synthesis.

Therapy and Outcomes

Therapy Specifics	Results
• Avoid sources vitamin D • Keep 25(OH)D 10-20ng/ml • Olmesartan q6-8h • Low-dose antibiotics qod • Alternate abx • Symptoms wax/wane • Labs may fluctuate • Treatment may take years	• Elevated 1,25(OH)2D reduces • Symptoms gradually diminish • Labs normalize • Bone density improves • Hormonal imbalances are corrected

A correlating treatment strategy is the avoidance of excessive sunlight exposure, foods high in vitamin D and vitamin D supplements to maintain serum 25(OH)D at a level (20-30 ng/ml) that isn't likely to suppress the immune system and inhibit bacterial elimination. This type of treatment requires several years (to avoid intolerable JHR) and patients must be highly motivated, but dramatic improvement has been seen (e.g., reduction in inflammatory symptoms, decrease in viral and antibody titers, normalization of lab work, improvement in bone density and correction of hormonal imbalances, etc.) in a wide variety of chronic inflammatory and autoimmune diseases.

Immunotherapy reduced ANA titers and symptoms in a patient with RA.

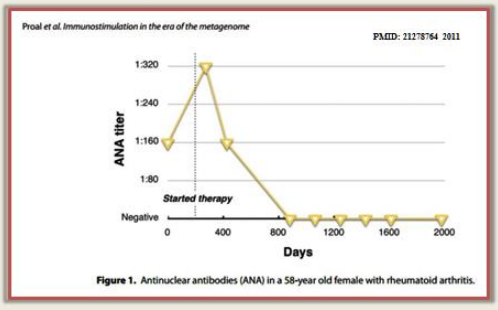


Figure 1. Antinuclear antibodies (ANA) in a 58-year old female with rheumatoid arthritis.

The adaptive immune system may also respond to the presence of fragments of DNA generated by pathogenic and cellular debris, stimulating antibody production in the process. Standard treatments failed to improve this RA patient's condition but Inflammation Therapy gradually reduced ANA titers and symptoms.

Immunotherapy reduced inflammatory markers and symptoms in a patient with ankylosing spondylitis.

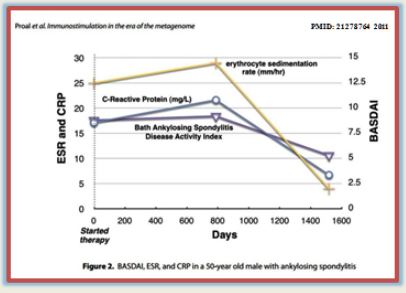
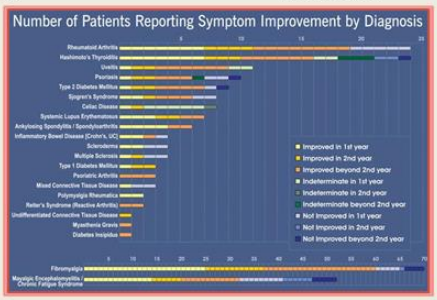


Figure 2. BASDAI, ESR, and CRP in a 50-year old male with ankylosing spondylitis

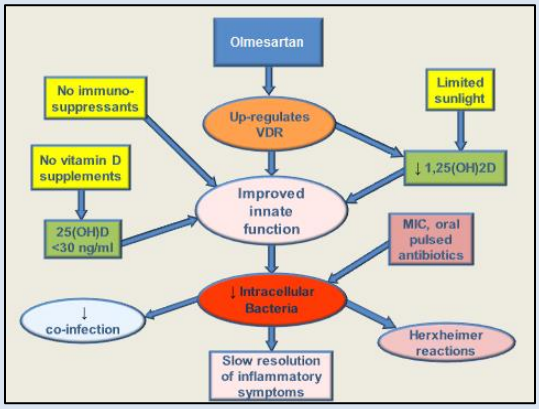
During the first two years of Inflammation Therapy, this patient with ankylosing spondylitis saw inflammatory markers rise before they began to fall. He also reported less depression and fewer symptoms of irritable bowel syndrome (IBS).

Presentation - Bacteria induced vitamin D receptor dysfunction in autoimmune disease: theoretical and practical implications for interpretation of serum vitamin D metabolite levels.

Capt. Tom Perez MPH
6th International Congress on Autoimmunity
Porto, Portugal
September 11, 2008



Accumulating case reports now support the observation that a number of complex, chronic conditions can be improved by restoring VDR function using this type of immunotherapy.



Front Immunol. 2013 Jun 18;4:148. doi: 10.3389/fimmu.2013.00148. eCollection 2013.

The vitamin d receptor and T cell function.

Kongsbak M, Levring TB, Geisler C, von Essen MR.

"It is becoming increasingly clear that microbes slow down immune reactivity by dysregulating the VDR, ultimately to increase their chance of survival.

Immune modulatory therapies that enhance VDR expression and activity should, therefore, be considered in the clinical setting."

Decision to treat

- Individualize decision making to the specific patient
- Take into account:
  - Diagnosis
  - Severity of symptoms
  - History
  - Potential disease course
  - Previous treatments attempted
  - Efficacy of conventional treatments
  - Risk versus benefit of olmesartan immunotherapy

In the absence of evidence based on clinical trials, the determination of when to use off-label olmesartan and an antibiotics protocol should be made on the basis of the doctor's best judgment (using diagnosis, severity of symptoms, history, potential disease course, previous treatments attempted, efficacy of traditional treatments and risk versus potential benefit, etc.) plus consideration of the patient's values.

Prudent Off-label Prescribing

- Based on expertise and on an individual problem-oriented approach to medical practice.
- Severe problems without (or ineffective) standard approaches can justify greater risk taking under informed consent.
- Side effects of drugs can be seen as potentially therapeutic.
- Novel treatments may offer the only chance of survival or an acceptable quality of life.

"Drug repurposing is emerging as a drug development strategy. Familiar drugs that have new uses can improve length and quality of life."

Dr. Bruce Bloom, Cures Within Reach

Evidence-based medicine has limitations.

- EBM is "the conscientious, explicit, and judicious use of current best evidence in making clinical decisions about the care of individual patients" (Sackett et al., 1996)
- Evidence is not always derived from well-executed scientific studies.
- Often, scientific evidence to support EBM has had to be supplemented by professional consensus.
- "Evidence-based medicine is the doctor's judgment, the patient's values, and the evidence. No one of those trumps the others."

David S. Jevsevar, MD, MBA, Chair of the AAOS Committee on Evidence-Based Quality and Value

More research is needed.

- 1,25(OH)2D levels need to be determined in healthy subjects.
- All studies of Vitamin D_3 and 25(OH)D should include assessment of 1,25(OH)2D.
- The effect of olmesartan on D-metabolites and other inflammatory markers needs to be evaluated.
- Long-term clinical trials of olmesartan & antibiotic immunotherapy should be initiated.

Key Points

- Low levels of 25(OH)D are seen in healthy individuals, as well as those with chronic inflammatory conditions.
- 25(OH)D may not always reflect the level of 1,25(OH)2D; accurate assessment of vitamin D status depends on measuring both metabolites.
- Intracellular bacteria may cause dysregulated vitamin D metabolism and impaired immune system function.
- A novel immunotherapy appears to restore VDR competence, correct dysregulated vitamin D metabolism, improve immune system function and reduce inflammatory symptoms.