# An Insight about Neutrophil to Lymphocyte ratio and Vitamin D among Chronic Kidney disease cases

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**Abstract**

**Background:** Neutrophils are responsible for the first line of host immune response against invading pathogens, through different mechanisms, including chemotaxis, phagocytosis, release of reactive oxygen species (ROS), granular proteins and the production and liberation of cytokines. Neutrophils display different phenotypes from the time they leave the bone marrow and enter the circulation (fresh neutrophils) to the time they disappear from the circulation (aged neutrophils). This shift in phenotype is known as aging, since it takes place within a single day, and results in various neutrophils with distinct properties. Neutrophils are the first cell type recruited to sites of inflammation. From there, they can switch phenotypes and generate various subpopulations with different cell functions. Neutrophils can also interact, directly, or via cytokines and chemokines, with other immune cells to modulate both innate and adaptive immune responses. Nonmicrobial inflammation contributes to CKD progression and fibrosis. The neutrophil count reflects inflammation, while the lymphocyte count indicates the status of general stress and nutrition. The neutrophil-to-lymphocyte ratio (NLR) in CKD patients provides information on the inflammation status. Recently, neutrophil-to-lymphocyte ratio (NLR) was reported to be associated with inflammation in ESRD including both hemodialysis (HD) and peritoneal dialysis (PD) patients, and estimate survival in HD patients. Vitamin D plays a crucial role in calcium homeostasis and bone metabolism. With chronic and/or severe vitamin D deficiency, a decline in intestinal calcium and phosphorus absorption leads to hypocalcemia leading to secondary hyperparathyroidism. This secondary hyperparathyroidism then leads to phosphaturia and accelerated bone demineralization.

**Keywords:** NLR, Vitamin D, CKD

## Introduction

Chronic renal failure (CRF) is the most prevalent, worldwide public health problem of the elderly population (1).

Chronic kidney disease (CKD) is a growing, global public health priority that is associated with markedly high morbidity, mortality, and excess healthcare costs **(2).**

As CKD progresses from its early stages to kidney failure (defined as an estimated glomerular filtration rate (eGFR) <15 ml/min/1.73 $m^2$ or treatment by dialysis, morbidity, mortality and health-care costs rise rapidly, and life expectancy is dramatically reduced unless kidney replacement therapy (KRT) is initiated **(3).**

Dialysis is the name for the man-made procedure that involves removing wastes and extra water from the

blood. Disrupted renal function is the major need for receiving dialysis. The requirements for dialysis include the uremic syndrome, hyperkalemia, extracellular volume expansion, acidosis, failure to respond to medical treatment, creatinine clearance of 10 ml/min/1.73 $m^2$, and bleeding diathesis (propensity to hemorrhage because of coagulation abnormalities) **(4).**

According to KDIGO 2012 clinical practice guideline, CKD is classified into five stages considering the GFR level **(5):**

Stage 1: Kidney damage with normal GFR (greater than 90 ml/min)

Stage 2: Mild reduction in GFR (60-89 mL/min)

Stage 3a: Moderate reduction in GFR (45 to 59 mL/min)

Stage 3b: Moderate reduction in GFR (30 to 44 mL/min)

Stage 4: Severe reduction in GFR (15 to 29 mL/min)

Stage 5: Renal failure (GFR less than 15 mL/min)

CKD is expected to be a major $21^{st}$ century medical challenge. In developing nations, the growing prevalence of CKD has severe implications on health and economic output. The rapid rise of common risk factors such as diabetes, hypertension, and obesity, especially among the poor, will result in even greater and more profound burdens that developing nations are not equipped to handle **(2).**

Approximately 30 to 50% of CKD cases have been found to have noticeably raised levels of serum inflammatory biomarkers including C-reactive protein (CRP) and interleukin-6 (IL6). The etiology of inflammation in this case is multifaceted and involves patient-related causes, such as underlying disease, comorbidity, oxidative stress, infections, obesity, genetic or immunologic factors, or on the other hand, hemodialysis-related factors, mainly concerning the dialysis membrane biocompatibility and dialysate quality **(6).**

Neutrophils are responsible for the first line of host immune response against invading pathogens, through different mechanisms, including chemotaxis, phagocytosis, release of reactive oxygen species (ROS), granular proteins and the production and liberation of cytokines **(7).**

Neutrophils also play an important regulatory role in adaptive immunity and are the main effector cells during the systemic inflammatory response (SIRS). As regulators of innate immunity, neutrophils recruit, activate and programme other immune cells, secreting an array of pro-inflammatory and immunomodulatory cytokines and chemokines capable of enhancing the recruitment and effector functions of other immune cells, such as dendritic cells (DCs), B cells, NK cells, CD4, CD8 and γδ T cells, as well as mesenchymal stem cells **(8).**

Neutrophils, also known as polymorphonuclear (PMN) leukocytes, are the most abundant cell type in human blood. They are produced in bone marrow in large numbers, ~$10^{11}$ cells per day. Under homeostatic conditions, neutrophils enter the circulation, migrate to tissues, where they complete their functions, and finally are eliminated by macrophages, all in the lapse of a day **(9).**

Three main antimicrobial functions are recognized for neutrophils: phagocytosis, degranulation, and the release of nuclear material in the form of neutrophil extracellular traps (NETs). These functions were considered, until recently, the only purpose of neutrophils. However, current research by investigators in several fields of neutrophil cell biology has revealed that neutrophils possess a much diverse repertoire of functional responses that go beyond the simple killing of microorganisms. Neutrophils respond to multiple signals and respond by producing several cytokines and other inflammatory factors that influence and regulate inflammation and the immune system **(10).**

The multitude of neutrophil functional responses is induced by transcriptional activation and by changes in expression of surface molecules or activity. These phenotypic changes are usually detected in only a

subset of neutrophils, suggesting that great neutrophil heterogeneity exists **(11).**

Neutrophils display different phenotypes from the time they leave the bone marrow and enter the circulation (fresh neutrophils) to the time they disappear from the circulation (aged neutrophils). This shift in phenotype is known as aging, since it takes place within a single day, and results in various neutrophils with distinct properties **(12).**

- ❖ Neutrophil Life Cycle

Neutrophils represent about 70% of all leukocytes and more than $10^{11}$ cells are produced every day in the bone marrow. From there, neutrophils enter the blood where they circulate until they leave into tissues. Once neutrophils reach the end of their lifespan within tissues, they are cleared mostly by macrophages through the process of phagocytosis **(13).**

Despite this impressive turnover, the number of neutrophils in circulation remains relatively constant thanks to a fine balance between production and elimination. In addition, neutrophils actively change to be able to perform special functions at different times or places **(14).**

- ❖ Neutrophil in Health

It was already mentioned that in fact neutrophils are transcriptionally active cells with the potential to change the expression of several membrane molecules, and to produce cytokines, and consequently neutrophils can perform different cell functions depending on the tissues where they are found **(14).**

- ❖ Neutrophil in Disease

They can display several phenotypes and perform a wide array of cellular functions. Several subsets of neutrophils are found in tissues under homeostatic conditions. In addition, various subsets of neutrophils with distinct properties are also detected in pathological conditions particularly in inflammation and in cancer **(15).**

- ❖ Neutrophil in Inflammation

Neutrophils are the first cell type recruited to sites of inflammation. From there, they can switch phenotypes and generate various subpopulations with different cell functions. Neutrophils can also interact, directly, or via cytokines and chemokines, with other immune cells to modulate both innate and adaptive immune responses **(14).**

In systemic inflammation conditions, another subset of neutrophils is generated with low doses of endotoxin. These cells have a hypersegmented nucleus and display the phenotype CD62 low CD11bhi CD11chi, which is like the one described for murine aged neutrophils **(16).**

Also, they are capable of inhibiting T lymphocytes by direct cell contact involving the integrin Mac1, and by local delivery of reactive oxygen species (ROS) **(16).**

In certain organs such as liver and adipose tissue, few neutrophils are detected in normal homeostatic conditions. However, upon an inflammatory state induced by experimental obesity, neutrophil numbers increase rapidly, and a metabolic imbalance is slowly generated **(16).**

Lymphocyte

Lymphocytes are bone marrow-derived cell lineages arising from a common lymphoid progenitor. In general, lymphocytes can be classified by cell surface receptors and by the specific immune functions attributed to each cell type. Lymphocytes circulate freely throughout the body and perform their functions in concert with other immunologically relevant cells and factors **(17).**

In human adults lymphocytes make up roughly 20 to 40 percent of the total number of white blood cells. They are found in the circulation and are concentrated in central lymphoid organs and tissues, such as the spleen, tonsils, and lymph nodes, where the initial immune response is likely to occur **(18).**

Lymphocytes are a component of complete blood count (CBC) tests that include a white blood cell differential, in which the levels of the major types of white blood cells are measured. Such tests are used to assist in the detection, diagnosis, and monitoring of various medical conditions **(18).**

Lymphocyte counts that are below the reference range, which varies for adults and children, may be indicative of lymphocytopenia (lymphopenia), whereas those above it are a sign of lymphocytosis. Lymphocytopenia is associated with a variety of conditions, ranging from malnutrition to rare inherited disorders such as ataxia-telangiectasia or severe combined immunodeficiency syndrome**(18).**

Lymphocytosis typically is associated with infections, such as mononucleosis or whooping cough, certain cancers of the blood or lymphatic system such as multiple myeloma and chronic lymphocytic leukemia, and autoimmune disorders that cause chronic inflammation, such as inflammatory bowel disease **(19).**

End-stage renal disease (ESRD) has also been associated with lymphopenia, an observation not thought to be exclusively attributable to an effect of dialysis alone. Naive and central memory CD4 and CD8 T cells are significantly reduced in the blood of ESRD patients, apparently because of increased susceptibility of these cells to apoptosis **(19).**

Lymphocyte structure and function Lymphocytes

Lymphocytes are B and T cells, white blood cells that are produced from the stem cells in bone marrow. They provide immunity for future invasions of bacteria, viruses, and parasites by producing antibodies, which have memory and will protect against such antigens **(20).**

Table 1: Lymphocyte Cell Lineages **(17):**

| Natural killer cells | B lymphocytes | T lymphocytes |
|---|---|---|
| Arise from a common lymphoid progenitor in the bone marrow | Arise from a common lymphoid progenitor in the bone marrow | Arise from a common lymphoid progenitor in the bone marrow |
| Non-antigen-specific inhibitory and activating receptors | Antigen-specific receptors recognizing native antigen | Antigen-specific receptors recognizing antigen in association with MHC molecules |
| Important in innate immune responses | Important in adaptive immune responses | Important in adaptive immune responses |

• T cells

These are a type of white blood cell involved in cell-mediated immunity and make up around 80% of circulating white blood cells. T cells originate in the red bone marrow, mature in the tonsils or thymus gland, and have a T cell receptor called αβ T cell receptor on the cell surface, but lack surface antigens. Once released into the blood circulation they have a long-life span **(20).**

T cells enter the blood circulation and the lymphatic system, monitoring for invading pathogens, and produce chemicals that trigger other white blood cells to target invading pathogens: bacteria, viruses, and parasites, as well as cancer **(20).**

• B cells

These are a type of white blood cell involved in humoral immunity. They make up around 20% of white blood cells. B cells originate and mature in the red bone marrow, then after release they concentrate in the lymph nodes, respiratory tract, gastrointestinal tract, and spleen, ready for pathogenic invaders.

Then upon encounter they will begin to produce plasma cells and memory cells **(21).**

Neutrophil-to-lymphocyte ratio Calculation

The NLR is simply the number of neutrophils divided by the number of lymphocytes. Under physiologic stress, the number of neutrophils increases, while the number of lymphocytes decreases. The NLR combines both changes, making it more sensitive than either alone **(22)**.

NLR may be calculated using either absolute cell counts or percentages, as shown here:

Calculation of NLR:

$$NLR = \frac{\text{Absolute \# Neutrophils}}{\text{Absolute \# Lymphocytes}} = \frac{\text{Relative \% Neutrophils}}{\text{Relative \% Lymphocytes}}$$

Interpretation of NLR depends on clinical context. However, to provide some idea of how to interpret this:

- A normal NLR is roughly 1-3.
- An NLR of 6-9 suggests mild stress (e.g. a patient with uncomplicated appendicitis).
- Critically ill patients will often have an NLR of ~9 or higher (occasionally reaching values close to 100) **(22).**

Neutrophil-to-Lymphocyte Ratio and ESRD:

Nonmicrobial inflammation contributes to CKD progression and fibrosis. The neutrophil count reflects inflammation, while the lymphocyte count indicates the status of general stress and nutrition. The neutrophil-to-lymphocyte ratio (NLR) in CKD patients provides information on the inflammation status **(23).**

Recently, neutrophil-to-lymphocyte ratio (NLR) was reported to be associated with inflammation in ESRD including both hemodialysis (HD) and peritoneal dialysis (PD) patients, and estimate survival in HD patients **(8).**

**Vitamin D**

Vitamin D is a group of fat-soluble compounds with a four-ringed cholesterol backbone; it is now recognized as a prohormone. Vitamin D exists in two major forms: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D2 and D3, regardless of the source, are biologically inactive. They are transformed into the biologically active molecule 1,25 dihydroxy vitamin D **(24).**

As a fat-soluble molecule, vitamin D is stored in adipose tissue; however, the exact mechanism by which vitamin D is regulated and mobilized from adipose tissue has not been elucidated at this time. Most vitamin D products are excreted through bile into the gut. Very little is eliminated via kidneys **(24).**

Vitamin D is an essential nutrient and hormone with multiple functions in the body including bone health, regulation of serum calcium and phosphate levels, as well as roles in immune function, cell proliferation, differentiation, and apoptosis. Accordingly, vitamin D deficiency has been associated with a number of health outcomes such as bone disease, diabetes, hypertension, heart disease, cancer, and autoimmune and infectious disease **(24).**

There is no doubt that the kidney is physiologically the overwhelming site of production of calcitriol for circulation, as chronic kidney disease or nephrectomy results in a significant fall in the serum calcitriol level **(25).**

However, there may be other extra-renal 1α-hydroxylation sites that can act as intracrine systems primarily involved in regulation of cell or tissue growth: skin, gastrointestinal tract, or glandular

tissue, such as prostate and breast **(25).**

Furthermore, extra-renal production of calcitriol is clearly found in certain pathological diseases, including granulomatous conditions such as sarcoidosis, lymphoma, and tuberculosis, which can be associated with hypercalcemia. If sarcoidosis is left untreated, the extra-renally produced calcitriol can enter the circulation, resulting in hypercalciuria and eventually hypercalcemia **(25).**

Vitamin D synthesis:

After being synthesized in the skin or absorbed (in chylomicrons) from the gastrointestinal (GI) tract, most vitamin D is bound to specific carrier proteins in the blood (vitamin D–binding protein [DBP] and albumin) and transported to the liver. In the liver, vitamin D is hydroxylated by the enzyme 25-hydroxylase (CYP2R1) to become 25(OH)D. 25(OH)D is the major circulating form of vitamin D **(26).**

From the liver, 25(OH)D is transported to the kidneys via the same carrier proteins. 1,25 dihydroxy vitamin D ($1,25(OH)_2$ D) is formed when 25(OH)D is hydroxylated by the enzyme 1α-hydroxylase (CYP27B1), which is in the mitochondria of proximal tubules of the kidney **(26).**

$1,25(OH)_2$ D is the biologically active form of vitamin D. As a result of 1 and 25 hydroxylation, the prohormone vitamin D has been transformed into an active hormone. $1,25(OH)_2$ D is a steroid like hormone **(26).**

In target cells, such as classic steroid hormones, it binds to a specific cytoplasmic VDR; the vitamin D bound to VDR then translocate to the nucleus, where its effects are initiated at a transcriptional level **(27).**

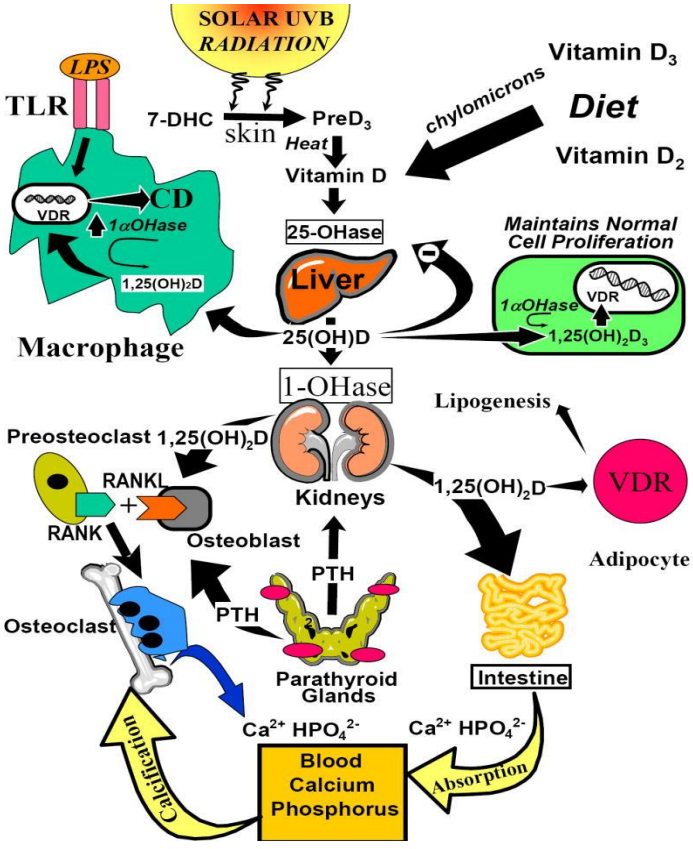


Figure 1: The metabolism and biologic function of vitamin D **(28).**

Functions of Vitamin D:

- ❖ Calcium and Phosphate Homeostasis

The dominant function of vitamin D in its hormonal form (calcitriol or 1,25-dihydroxyvitamin D) is the elevation of plasma calcium and phosphate levels, which are required for mineralization of bone **(29).**

- ❖ Other Actions

It is noteworthy that the VDR is present in the nucleus of many tissues that are not involved in the regulation of calcium and phosphate metabolism. For example, the VDR has been clearly described in epidermal keratinocytes, in activated T cells of the immune system, in antigen-presenting cells, in macrophages and monocytes, and in cytotoxic T cells **(30).**

In mice missing the VDR gene (Vdr-null), calcitriol and the VDR play a role in lactational physiology; there is accelerated mammary development during pregnancy, but delayed involution of the mammary tissue after lactation **(30).**

There is emerging evidence that calcitriol plays a role in the immune system that has not yet been clearly described. Exogenous calcitriol can suppress autoimmune diseases, but with hypercalcemia as an important side effect **(30).**

The local conversion of 25OHD into calcitriol in monocytes or macrophages results in an increase in cellular immunity by stimulating the production of cathelicidin, an anti-microbial peptide capable of killing bacteria, particularly Mycobacterium tuberculosis **(30).**

**Stubbs et al., (31)** showed that renal dialysis patients treated with high-dose vitamin D3 develop a population of immune cells with increased CYP27B1, VDR, and cathelicidin expression, although the role of these cells in vivo is unknown.

Calcitriol has an opposite effect on the adaptive immune (B and T cell function) response. Calcitriol generally inhibits T helper cell proliferation and B cell immunoglobulin production **(32).**

Vitamin D orchestrates cell cycle progression via alterations in key regulators such as cyclin-dependent kinases, retinoblastoma protein phosphorylation, and repression of the proto-oncogene myc as well as by modulating growth factor receptor-mediated signaling pathways **(33).**

Vitamin D Measurement:

There is great confusion about what denotes vitamin D deficiency. Commercial laboratories use two standard deviations below the mean as the lowest limit of the normal range. Thus vitamin D deficiency was designated as a serum 25(OH)D of less than 10 ng/ml **(28).**

Serum 25OHD level is widely considered as a marker of vitamin D nutriture, and consideration of serum 25OHD measures for the purposes of nutrient reference value development has generated notable interest. There is agreement that circulating serum 25OHD levels are currently the best available indicator of the net incoming contributions from cutaneous synthesis and total intake **(33).**

Thus, the serum 25OHD level may function as a biomarker of exposure; it reflects the supply of vitamin D to the body and can be a useful adjunct to examining the intake level of vitamin D if the confounders and the measure's variability depending upon a range of variables are kept in mind **(33).**

Calcitriol, the active hormonal form of the nutrient, has not been used typically as a measure associated with vitamin D nutriture or as an intermediate related to health outcomes. Calcitriol is not useful as such a measure, for several reasons. Its half-life is short (hours), its formation is not directly regulated by vitamin D intake, its levels are regulated by other factors (such as serum PTH), and, even in the presence of severe vitamin D deficiency the calcitriol level may be normal or even elevated because of up-regulation of the 1α-hydroxylase enzyme **(33).**

Vit Deficiency

Vitamin D deficiency can lead to osteomalacia and rickets in children and osteomalacia in adults. The fortification of milk with vitamin D in the 1930s was effective in eradicating rickets in the world. However, subclinical vitamin D deficiency is still widely prevalent in both developed and developing countries with a worldwide prevalence of up to 1 billion **(34).**

This subclinical vitamin-D deficiency is associated with osteoporosis, increased risk of falls and fragility fractures. Many conflicting recent studies are now showing an association between vitamin D deficiency and cancer, cardiovascular disease, diabetes, autoimmune diseases, and depression **(34).**

Epidemiology:

Vitamin D deficiency is a global public health issue. About 1 billion people worldwide have vitamin D deficiency, while 50% of the population has vitamin D insufficiency **(34).**

The prevalence of patients with vitamin D deficiency is highest in the elderly, obese patients, nursing home residents, and hospitalized patients. The prevalence of vitamin D deficiency was 35% higher in obese subjects irrespective of latitude and age **(35).**

A greater prevalence of vitamin D deficiency exists in Middle Eastern countries. A study of 316 young adults aged 30-50 years from the Middle East showed that 72.8% had 25(OH)D values of less than 15 ng/dL (that is, severely deficient). This was significantly more common in women than in men (83.9% vs 48.5%, respectively). The difference between sexes probably reflects the cultural and religious practices leading to less skin exposure in women than in men **(35).**

Etiology:

1. Decreased dietary intake and/or absorption:

Certain malabsorption syndromes such as celiac disease, short bowel syndrome, gastric bypass, inflammatory bowel disease, chronic pancreatic insufficiency, and cystic fibrosis may lead to vitamin D deficiency. Lower vitamin D intake orally is more prevalent in the elderly population **(36).**

2. Decreased sun exposure:

About 50% to 90% of vitamin D is absorbed through the skin via sunlight while the rest comes from the diet. Twenty minutes of sunshine daily with over 40% of skin exposed is required to prevent vitamin D deficiency **(36).**

Cutaneous synthesis of vitamin D declines with aging. Dark-skinned people have less cutaneous vitamin D synthesis. Decreased exposure to the sun as seen in individuals who are institutionalized or have prolonged hospitalizations can also lead to vitamin D deficiency. Effective sun exposure is decreased in individuals who use sunscreens consistently **(36).**

3. Decreased endogenous synthesis:

Individuals with chronic liver disease such as cirrhosis can have defective 25-hydroxylation leading to deficiency of active vitamin D. Defect in 1-alpha 25-hydroxylation can be seen in hyperparathyroidism, renal failure, and 1-alpha hydroxylase deficiency **(37).**

4. Increased hepatic catabolism:

Medications such as phenobarbital, carbamazepine, dexamethasone, nifedipine, spironolactone, clotrimazole, and rifampin induce hepatic p450 enzymes which activate degradation of vitamin D **(37).**

5. End organ resistance.

End organ resistance to vitamin D can be seen in hereditary vitamin D resistant rickets **(37).**

Pathophysiology:

Vitamin D plays a crucial role in calcium homeostasis and bone metabolism. With chronic and/or severe vitamin D deficiency, a decline in intestinal calcium and phosphorus absorption leads to hypocalcemia leading to secondary hyperparathyroidism. This secondary hyperparathyroidism then leads to phosphaturia and accelerated bone demineralization. This can further result in osteomalacia and osteoporosis in adults and osteomalacia and rickets in children **(37).**

Vit D and inflammation

VDR expression has been documented in macrophages, a crucial cell type in the innate immune response. IL-15 links TLR2/1-induced macrophage differentiation to the vitamin D-dependent antimicrobial pathway **(38).**

The increase of CYP27B1 results in the accumulation of $1,25(OH)_2D_3$, which further activates VDR, leading to the target gene transcription via vitamin D response elements located in the regulatory regions of $1,25(OH)_2D_3$ target genes **(38).**

**Chen et al., (39)** found that $1,25(OH)_2D_3$ can regulate TLR signaling via stimulating SOCS1 by downregulating miR-155 in macrophages, which provide a novel negative feedback regulatory mechanism for vitamin D to control innate immunity.

In a recent study, both forms of vitamin D – $1,25(OH)_2D_3$ and $25(OH)D_3$ – dose-dependently inhibited lipopolysaccharide-induced p38 phosphorylation, IL-6, and TNFα production by human monocytes via histone H4 in an acetylation-dependent manner. Moreover, $1,25(OH)_2D_3$ or its analogs have been shown to initiate the differentiation of myeloid progenitors into macrophages, and to reduce MCP-1 and IL-6 expression via inhibiting the activation of NF-κB in macrophages **(40).**

In addition, Vitamin D has been thought to be a natural endoplasmic reticulum stress reliever, and can selectively suppress key effector functions of interferon (IFN)-γ-activated macrophages **(40).**

Interestingly, in the presence of $1,25(OH)_2D_3$, VDR has also been found to repress gene transcription via displacing the deoxyribonucleic acid-bound nuclear factor of activated T-cells, thus repressing inflammatory cytokine expression **(40).**

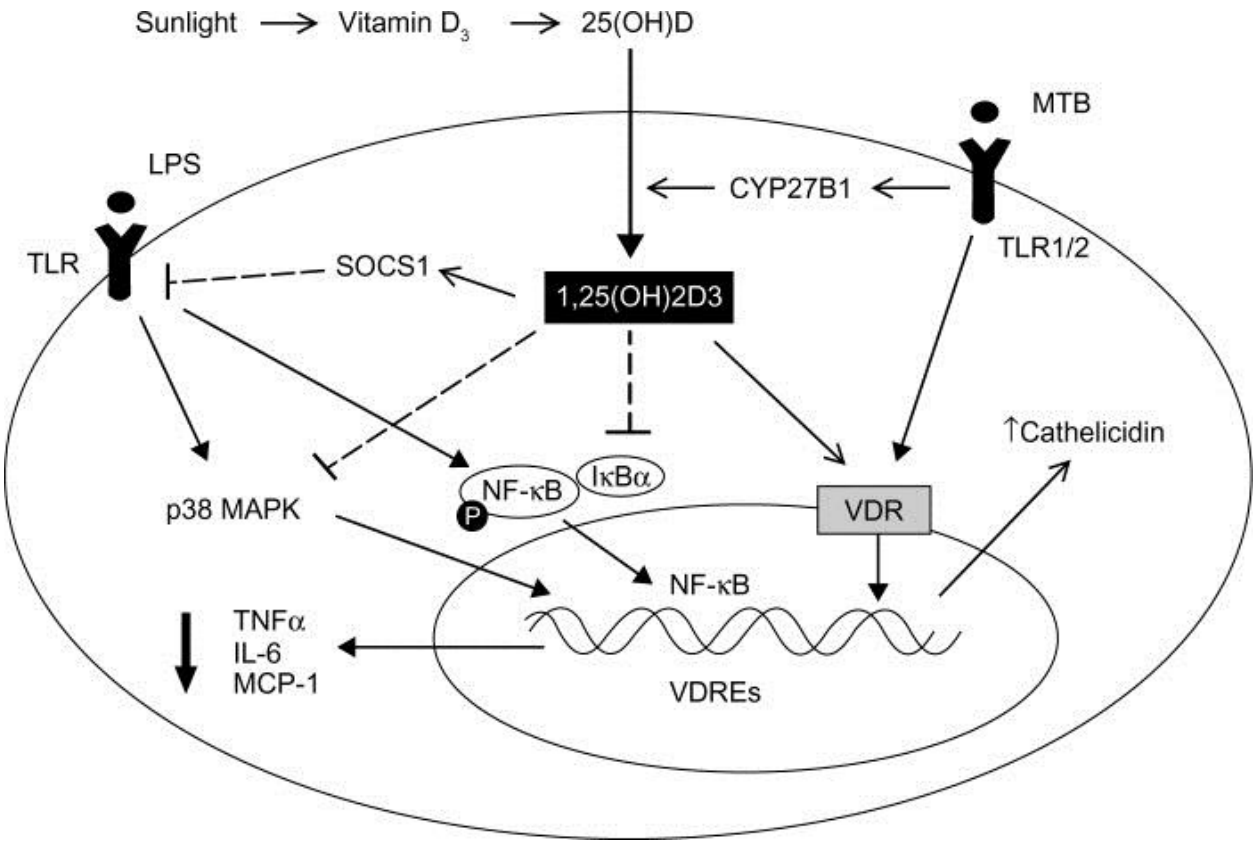


Figure 2: Schematic representation of the primary mechanisms through which vitamin D regulates macrophage-mediated innate immune response **(41).**

Dendritic cells (DCs) are the most potent antigen-presenting cells. A number of studies have shown that $1,25(OH)_2D_3$ inhibits the differentiation, maturation, and immunostimulatory capacity of human DCs, characterized as the tolerogenic properties, in a VDR-dependent manner **(41).**

Molecular mechanisms underlying the modulation of tolerogenic properties of DCs by $1,25(OH)_2D_3$ include decreasing surface expression of major histocompatibility complex II and costimulatory molecules (CD40, CD80, CD86), upregulating inhibitory immunoglobulin-like transcript 3 molecules, and enhancing secretion of chemokine (C–C motif) ligand 22 and IL-1029 (Suuring and Moreau, 2021).

The enhancement of DC tolerogenicity by $1,25(OH)_2D_3$ results in the induction of T-regulatory cells, a critical event for suppressing the inflammatory response of T-effector cells **(3).**

$1,25(OH)_2D_3$ also acts directly with VDR on the T lymphocyte to inhibit its proliferation. Although native T-cells did not express VDR, VDR expression was induced by T-cell antigen-receptor signaling via the alternative p38 MAPK pathway, which is crucial for T-cell antigen-receptor responsiveness in naïve T-cells **(41).**

T-cell cytokines also control vitamin D metabolism in macrophages. For example, IFNγ, a T-helper (Th)-1 cytokine, upregulates the macrophage CYP27B1, leading to enhanced bioconversion of $25(OH)D_3$ to its active metabolite – $1,25(OH)_2D_3$. In contrast, the Th2 cytokine IL-4 induces catabolism of $25(OH)D_3$ to the inactive metabolite $24,25(OH)_2D_3$ suggesting a potential mechanism by which vitamin D metabolism links the cell-mediated immune responses to the innate immune responses **(41).**

Conflict of Interest: None

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