Vitamin D₃ and Vitamin K₂ as Modulators of Adaptive Immunity: Insights from Experimental Models of Lymphocyte Dysfunction

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Abstract

Background: Vitamin D₃ and Vitamin K₂ have emerged as key nutrient regulators of immune balance, particularly within the adaptive immune system where B and T lymphocytes play central roles in antigen-specific responses. Experimental and clinical evidence increasingly suggests that Vitamin D₃ modulates lymphocyte maturation, differentiation, cytokine expression, and tolerance, primarily through binding of its active metabolite calcitriol to the vitamin D receptor (VDR) expressed on immune cells. In parallel, Vitamin K₂ has gained attention for its involvement in regulating inflammatory signaling, sphingolipid metabolism, apoptosis pathways, and cellular energy processes relevant to lymphocyte survival and function. Despite their individual immunomodulatory actions, growing data indicate that these micronutrients may interact synergistically, contributing to balanced adaptive responses while mitigating aberrant immunity characteristic of immunodeficiency, autoimmunity, and chronic inflammation.

The aim of this review is to analyze and synthesize current mechanistic and experimental findings regarding the roles of Vitamin D₃ and Vitamin K₂ in modulating B and T lymphocyte function, with a specific focus on insights derived from preclinical models of lymphocyte dysfunction. This includes evaluation of how these vitamins influence lymphocyte development in primary lymphoid organs, activation pathways during antigen exposure, regulatory versus effector T cell balance, B cell antibody production, memory cell formation, and apoptosis or survival signaling in immunocompromised physiological states. Emphasis is placed on experimental animal models, including immunodeficient rodent studies, which provide controlled mechanistic insights not easily obtained from human research. Additionally, the review highlights the emerging interplay between the vitamins, including complementary actions on gene expression, cell signaling, oxidative stress, and metabolic regulation.

In conclusion, converging evidence demonstrates that Vitamin D₃ and Vitamin K₂ significantly influence adaptive immune function through distinct yet potentially synergistic mechanisms. Their effects on B and T cells appear particularly relevant in conditions of lymphocyte impairment, suggesting therapeutic potential for restoring immune competence. Nonetheless, gaps remain regarding optimal dosing strategies, temporal relationships during immune activation, and the mechanistic basis of their interaction. Future research in targeted experimental models is needed to clarify these relationships and support translational application of combined Vitamin D₃–Vitamin K₂ interventions in immunodeficient states.

Keywords: *Vitamin D₃, Vitamin K₂ , Immunity*

Introduction

Vitamin D_3 and Vitamin K_2 have emerged as important modulators of adaptive immune function due to their direct and indirect influence on lymphocyte development, activation, and regulatory balance. The active metabolite of Vitamin D_3, 1,25-dihydroxyvitamin D_3, exerts immunomodulatory actions through binding to the vitamin D receptor (VDR), a transcription factor expressed on activated T cells, B cells, and antigen-presenting cells, enabling it to influence cytokine transcription and immune tolerance pathways [1]. In contrast, Vitamin K_2, traditionally associated with hepatic coagulation factor activation, has more recently been implicated in the regulation of inflammation and immune cell survival through its effects on mitochondrial electron transport and NF-κB signaling pathways, positioning it as a potential immunomodulatory micronutrient [2].

Experimental models of lymphocyte dysfunction have demonstrated that Vitamin D_3 deficiency disrupts T cell homeostasis, reducing regulatory T cell populations while promoting a shift toward proinflammatory Th1 and Th17 responses, ultimately impairing adaptive immune balance [3]. Vitamin D_3 has also been shown to limit B cell proliferation and immunoglobulin production, suggesting a broader regulatory role in adaptive immunity beyond T cell modulation [4]. Meanwhile, studies examining Vitamin K_2 in immunological contexts indicate that menaquinone isoforms can modulate cytokine production, reduce oxidative stress, and alter apoptosis signaling in immune cells, supporting the hypothesis that Vitamin K_2 contributes to lymphocyte resilience under stress or immunodeficient conditions [5].

Despite these advances, limited research has directly investigated the combined impact of Vitamins D_3 and K_2 on adaptive immune regulation. This presents a critical research gap, as emerging biochemical data suggest potential synergistic interactions, including coordinated regulation of calcium-dependent signaling, gene transcription, and cellular metabolic processes relevant to immune activation and tolerance [6]. The objective of this review is to examine and integrate current experimental findings on the roles of Vitamin D_3 and Vitamin K_2 in modulating B and T lymphocyte function, with specific emphasis on data derived from immunodeficient animal models that allow detailed mechanistic exploration. By synthesizing this evidence, the review aims to clarify their individual and potential synergistic effects, while outlining key questions for future research that may guide therapeutic applications in immunocompromised conditions [7].

Physiological Basis of Vitamin D_3 in Adaptive Immunity

Vitamin D_3 exerts its immunological effects primarily through its active metabolite, 1,25-dihydroxyvitamin D_3, which binds to the vitamin D receptor (VDR) widely expressed in cells of the adaptive immune system. VDR activation in lymphocytes leads to changes in transcription of genes regulating cytokine synthesis, cell proliferation, and differentiation, establishing Vitamin D_3 as a key endocrine regulator of immune homeostasis [8]. The presence of 1α-hydroxylase in dendritic cells and activated T cells allows for local conversion of Vitamin D_3 into its active form, enabling paracrine and autocrine immune modulation within lymphoid tissues [9]. This intracrine pathway provides fine-tuned immunological control, particularly during antigen exposure, by attenuating excessive effector responses while supporting regulatory T cell differentiation. Such physiological mechanisms reinforce Vitamin D_3 as an essential micronutrient for integrity of adaptive immune function [10].

Physiological Basis of Vitamin K_2 in Adaptive Immune Regulation

Vitamin K_2 plays a substantial yet historically underrecognized role in immune physiology, extending beyond its classical function in γ-carboxylation of coagulation factors. In immune cells, Vitamin K_2 influences intracellular signaling pathways that determine inflammatory tone and lymphocyte reactivity. One of its most studied mechanisms involves inhibition of NF-κB activation, a central transcription factor regulating proinflammatory cytokines, T cell activation thresholds, and survival pathways during immune stress. By suppressing NF-κB, Vitamin K_2 reduces transcription of IL-6, TNF-α, and other mediators that drive lymphocyte overactivation and tissue pathology. This anti-inflammatory effect positions Vitamin K_2 as an important micronutrient in maintaining controlled immune activation rather

than the unchecked responses observed in immune dysfunction. [11]

Beyond its effects on inflammatory gene expression, Vitamin K_2 acts as a unique mitochondrial modulator capable of influencing the bioenergetic status of immune cells. During T and B lymphocyte activation, the metabolic shift from oxidative phosphorylation to glycolysis is essential for clonal expansion; however, mitochondrial integrity remains critical for sustaining ATP production and controlling reactive oxygen species (ROS). Vitamin K_2, particularly menaquinone-4 (MK-4), functions as an alternative electron carrier in the mitochondrial electron transport chain. This action preserves mitochondrial membrane potential, limits excessive ROS accumulation, and prevents energy collapse during immune stress. This property is especially relevant in immunodeficiency models where lymphocyte mitochondrial resilience is compromised, leading to impaired activation and accelerated apoptosis. [12]

Recent findings further suggest that Vitamin K_2 participates in regulating sphingolipid metabolism, a pathway central to lymphocyte survival, apoptosis, and membrane signaling. Sphingolipids modulate receptor clustering in lipid rafts, influencing T cell receptor (TCR) signaling, B cell receptor (BCR) activation, and downstream calcium-dependent pathways. Vitamin K_2 appears to alter ceramide and sphingosine-1-phosphate (S1P) dynamics, shifting the balance away from pro-apoptotic signals toward pathways that support lymphocyte maintenance and controlled activation. This aligns with observations from cellular and animal studies demonstrating improved lymphocyte viability and reduced oxidative apoptosis under Vitamin K_2 supplementation. Such findings underscore the multifaceted, non-coagulation roles of Vitamin K_2 in adaptive immunity and highlight its therapeutic potential in states of lymphocyte dysfunction. [13].

Experimental Models of Lymphocyte Dysfunction

Experimental models of lymphocyte dysfunction provide essential insights into how micronutrients such as Vitamin D_3 and Vitamin K_2 influence adaptive immune responses under compromised physiological conditions. Immunodeficient rodent models, including those induced by cyclophosphamide, irradiation, or genetic manipulation, allow controlled evaluation of alterations in T and B cell proliferation, apoptosis, receptor expression, and cytokine dynamics. These models reliably reproduce key features of human immune impairment, such as reduced thymic output, diminished splenic lymphocyte populations, and disrupted antigen-specific responses. By establishing a predictable pattern of immune suppression, these systems enable researchers to assess how nutrient supplementation restores or modulates adaptive immune function in a quantifiable manner. [14]

Cyclophosphamide-induced immunosuppression is one of the most widely used experimental approaches due to its reproducible suppression of both cellular and humoral immunity. Cyclophosphamide selectively targets rapidly dividing lymphocyte populations, leading to depletion of $CD4^+$ and $CD8^+$ T cells, impairment of regulatory T cell subsets, and reduction in antibody-producing B cells. These changes mimic clinical immunodeficiency conditions found in chemotherapy-treated individuals, making this model valuable for studying nutrient-based immune restoration. Studies employing this model demonstrate not only quantitative reductions in lymphocyte subsets but also functional impairments, including reduced cytokine production, weakened antigen-specific proliferation, and disordered lymphoid organ histology. Such features create a rigorous platform for evaluating how Vitamin D_3 or Vitamin K_2 supplementation may reverse or ameliorate impaired immune responses. [15]

Other models, such as high-dose glucocorticoid-induced immunosuppression, provide complementary insights by targeting different immunological pathways. Glucocorticoids induce apoptosis in immature thymocytes, reduce peripheral T cell survival, inhibit B cell antibody synthesis, and broadly suppress cytokine gene transcription. These effects differ mechanistically from cytotoxic immunosuppressants and therefore allow researchers to determine whether Vitamin D_3 or Vitamin K_2 exert generalized lymphocyte-supportive effects or act via pathway-specific mechanisms. Experimental findings have shown that immune suppression through glucocorticoids leads to pronounced reductions in IL-2 and IFN-γ expression, diminished T cell receptor signaling, and altered splenic architecture, all of which can

be carefully quantified in nutrient intervention studies. This diversity of models ensures that conclusions drawn regarding the immunomodulatory roles of Vitamins D₃ and K₂ are not dependent on a single suppression mechanism but instead reflect broader biological relevance. [16]

Vitamin D₃ Effects on T Lymphocyte Function

Vitamin D₃ plays a central role in regulating T lymphocyte development and functional differentiation through its active metabolite, 1,25-dihydroxyvitamin D₃, which binds directly to the vitamin D receptor (VDR) expressed on T cells. Activation of VDR influences transcription of genes involved in cytokine production, cell cycle progression, and T cell signaling. One of the most consistent findings is the shift in T helper cell polarization, where Vitamin D₃ suppresses Th1 and Th17 responses—immune pathways associated with excessive inflammation—and concurrently promotes Th2 and regulatory T cell (Treg) differentiation. This immunological shift contributes to a more controlled and anti-inflammatory T cell environment, reducing pathological immune activation and supporting immune tolerance. These effects highlight Vitamin D₃ as a crucial factor in maintaining balanced T cell responses, especially in states of immune dysfunction. [17]

Vitamin D₃ also affects T cell activation thresholds by modulating antigen-presenting cell interactions and altering the expression of co-stimulatory molecules essential for T cell priming. Dendritic cells exposed to Vitamin D₃ exhibit reduced expression of MHC class II and costimulatory molecules such as CD80 and CD86, resulting in a more tolerogenic phenotype. These tolerogenic dendritic cells produce lower levels of IL-12, a cytokine required for Th1 differentiation, and higher amounts of IL-10, which reinforces regulatory pathways in T cells. Through these changes, Vitamin D₃ indirectly shapes T cell activation outcomes, preventing excessive effector activity while promoting mechanisms that limit immunopathology. This dual mechanism—direct effects on T cells and indirect regulation through antigen-presenting cells—demonstrates the multifaceted role of Vitamin D₃ in T cell immunoregulation. [18]

Moreover, Vitamin D₃ contributes to T cell survival and metabolic fitness, crucial for sustaining effective adaptive immune responses. Upon activation, T cells undergo rapid metabolic reprogramming, increasing glycolysis and mitochondrial respiration to support proliferation and effector functions. Vitamin D₃ helps maintain mitochondrial integrity by enhancing oxidative phosphorylation efficiency and reducing reactive oxygen species (ROS) accumulation, thereby preventing premature T cell exhaustion or apoptosis. In immunodeficiency models, Vitamin D₃ supplementation has been shown to restore T cell metabolic function, increase IL-2 production, and improve effector proliferation, indicating its potential therapeutic value in restoring compromised T cell functionality. These metabolic effects complement its transcriptional regulation, making Vitamin D₃ a key micronutrient for T cell fitness and resilience under immunological stress. [19]

Vitamin D₃ Effects on B Lymphocyte Function

Vitamin D₃ exerts significant regulatory effects on B lymphocytes, influencing their proliferation, differentiation, and antibody-producing capacity. The active metabolite 1,25-dihydroxyvitamin D₃ binds to the vitamin D receptor expressed on activated B cells, leading to modulation of key transcriptional pathways that control immunoglobulin synthesis and plasma cell formation. Research has shown that Vitamin D₃ directly inhibits B cell proliferation and blocks differentiation into plasma cells, resulting in reduced production of immunoglobulins including IgG and IgM. This controlled suppression helps prevent excessive humoral responses, which are characteristic of hyperactive or dysregulated immunity. In models of lymphocyte dysfunction, Vitamin D₃'s ability to limit uncontrolled B cell activation is particularly relevant, as it prevents the expansion of autoreactive clones and excessive antibody production that may contribute to inflammatory tissue damage. [20]

In addition to regulating antibody production, Vitamin D₃ enhances the tolerogenic properties of B cells, promoting a phenotype associated with immune regulation rather than pathogenic activation. One crucial mechanism involves the induction of IL-10–producing regulatory B cells (Bregs), which suppress excessive T cell activation and help maintain immune homeostasis. IL-10 produced by Bregs inhibits inflammatory cytokine production and supports the expansion of regulatory T cells, thereby

strengthening immune tolerance pathways. Vitamin D₃ has been shown to increase IL-10 secretion while reducing proinflammatory cytokines such as IL-6 and TNF-α in activated B cells, thereby shifting the humoral immune environment from a proinflammatory to a regulatory state. This effect is especially beneficial in immunodeficient models where a lack of regulatory control contributes to impaired or unstable immune responses. [21]

Vitamin D₃ also influences B cell survival by modulating apoptosis pathways during immune activation. Activated B cells undergoing rapid proliferation are highly susceptible to oxidative stress and mitochondrial dysfunction. Vitamin D₃ supports mitochondrial stability, reduces reactive oxygen species accumulation, and enhances anti-apoptotic signaling, thereby promoting controlled B cell survival while preventing excessive expansion. In immunodeficient states, this balancing effect helps maintain sufficient B cell numbers to support adaptive immunity without promoting aberrant clonal growth. Furthermore, Vitamin D₃ limits the expression of activation-induced cytidine deaminase (AID), a key enzyme in class-switch recombination and somatic hypermutation, thereby fine-tuning antibody diversity and preventing dysregulated or autoreactive antibody formation. These combined effects underscore the role of Vitamin D₃ as a central regulator of B cell function and humoral immune integrity. [22]

Vitamin K₂ Effects on T Lymphocyte Function

Vitamin K₂ influences T lymphocyte function through its capacity to modulate intracellular signaling pathways that govern T cell activation, cytokine production, and survival. One of the key mechanisms involves suppression of NF-κB activation, a master transcriptional regulator of inflammatory T cell responses. By reducing NF-κB activity, Vitamin K₂ decreases the transcription of cytokines such as IL-2, IL-6, and TNF-α, which are essential for T cell proliferation and effector activity. This dampening of inflammatory cytokine signaling creates a more controlled T cell response, preventing the hyperactivation commonly observed in immune dysregulation or immunodeficiency models. Experimental studies have demonstrated that Vitamin K₂ supplementation reduces inflammatory gene expression in activated immune cells, suggesting that it may create an immunophysiological environment favoring balanced T cell activation rather than excessive effector function. [23]

Beyond its effects on transcriptional control, Vitamin K₂ plays an important role in maintaining mitochondrial homeostasis in T cells, a feature that is essential for sustaining robust immune responses. T lymphocyte activation requires high metabolic reprogramming with increased reliance on oxidative phosphorylation and glycolysis. Vitamin K₂ can act as an electron carrier in the mitochondrial respiratory chain, supporting ATP synthesis and reducing the burden of reactive oxygen species. This function allows T cells to maintain energy production under stressful or immunocompromised conditions, thereby preventing premature exhaustion or apoptosis. In models where mitochondrial dysfunction contributes to impaired T cell immunity, Vitamin K₂ supplementation has been associated with improved mitochondrial efficiency and increased viability of activated T cells. These findings support its role as a metabolic stabilizer during adaptive immune activation. [24]

Vitamin K₂ may also influence T cell survival and apoptosis through modulation of sphingolipid metabolism and cellular redox balance. Sphingolipids regulate key immune processes, including activation-induced cell death, T cell receptor clustering, and calcium-dependent signaling pathways essential for T cell activation. Vitamin K₂ has been shown to modify ceramide and sphingosine-1-phosphate (S1P) signaling, shifting the balance toward cellular survival and reducing susceptibility to activation-induced apoptosis. Additionally, its antioxidant properties contribute to protection against oxidative stress, a major driver of T cell dysfunction in immunodeficient states. Together, these mechanisms highlight Vitamin K₂ as a micronutrient with important regulatory influence on T cell longevity, signaling integrity, and effector capacity, particularly under conditions characterized by lymphocyte vulnerability. [25]

Vitamin K₂ Effects on B Lymphocyte Function

Vitamin K₂ influences B lymphocyte function through mechanisms that support cellular survival, regulate inflammatory signaling, and modulate pathways involved in antibody production. One of its

primary actions is attenuation of NF-κB–mediated transcriptional activity, a pathway essential for B cell activation, class-switch recombination, and proinflammatory cytokine production. By suppressing NF-κB signaling, Vitamin K₂ reduces the expression of IL-6 and TNF-α, cytokines that stimulate B cell proliferation and differentiation into antibody-secreting plasma cells. This reduction in inflammatory drive helps to prevent excessive or aberrant activation of B cells, a process commonly observed in immunodeficiency states where dysregulated cytokine signaling contributes to impaired humoral responses. As a result, Vitamin K₂ may help restore a balanced B cell activation profile conducive to efficient, rather than excessive or dysfunctional, antibody production. [26]

Additionally, Vitamin K₂ contributes to B cell survival by enhancing mitochondrial resilience and reducing oxidative stress, both of which are crucial for sustaining B cell function during immune challenges. Rapidly proliferating B cells generate significant reactive oxygen species (ROS), which can induce apoptosis and compromise antibody production if mitochondrial defenses are insufficient. Vitamin K₂, functioning as an alternative electron carrier within the mitochondrial electron transport chain, improves ATP production while lowering ROS accumulation. This metabolic stabilization supports the survival and proper maturation of B cells, particularly under conditions of immunosuppression where oxidative stress is elevated. Improved mitochondrial function results in enhanced B cell viability and may contribute to more effective restoration of humoral immunity in experimental models. [27]

Emerging evidence also suggests that Vitamin K₂ influences sphingolipid-mediated signaling pathways that regulate B cell apoptosis and receptor-mediated activation. Sphingolipids, especially ceramide and sphingosine-1-phosphate (S1P), are critical modulators of B cell fate, affecting processes such as activation-induced cell death, membrane microdomain formation, and B cell receptor (BCR) signaling. By modulating sphingolipid metabolism, Vitamin K₂ may reduce ceramide-associated apoptosis and promote survival signals mediated by S1P. This modulation supports B cell longevity and capacity to engage in appropriate antibody responses. Such actions provide a mechanistic rationale for the observed protective effects of Vitamin K₂ in models of immune dysfunction and highlight its potential importance in maintaining humoral immunity under stress or immunodeficient conditions. [28]

Combined and Synergistic Effects of Vitamin D₃ and Vitamin K₂ on Adaptive Immunity

The potential synergy between Vitamin D₃ and Vitamin K₂ is increasingly recognized in the context of calcium metabolism and vascular health, and this framework provides a useful model for understanding how these vitamins might jointly influence adaptive immunity. Vitamin D₃ upregulates the synthesis of several vitamin K–dependent proteins, while Vitamin K₂ is required for their γ-carboxylation and functional activation, establishing a coordinated endocrine–nutrient axis. In bone and vasculature, this interaction ensures that calcium is directed toward mineralization sites and away from soft tissues, thereby maintaining structural integrity. Translating this concept to immune organs, the same coordinated regulation of vitamin K–dependent proteins, local calcium fluxes, and membrane signaling may shape lymphocyte activation thresholds, receptor clustering, and survival signaling. Although direct studies on T and B cells are limited, the mechanistic template of Vitamin D–induced protein expression combined with Vitamin K–dependent activation suggests a biologically plausible synergy in adaptive immune regulation. [29]

Clinical and translational studies evaluating combined Vitamin D₃ and Vitamin K₂ supplementation provide additional indirect evidence for systemic immunomodulatory synergy that may secondarily impact adaptive immunity. Trials in patients with coronary artery disease or aortic valve calcification have tested combined Vitamin K₂ and Vitamin D supplementation, demonstrating acceptable safety and suggesting effects on vascular calcification dynamics and markers of inflammation, including IL-6 and TNF-α. Although the primary endpoints in some studies, such as coronary artery calcification progression, have been neutral, subgroup analyses and secondary findings point toward potential anti-inflammatory benefits of combined therapy. Because systemic inflammation strongly shapes T cell polarization, B cell activation, and lymphocyte trafficking, any coordinated reduction in proinflammatory cytokine burden induced by dual Vitamin D₃–K₂ therapy is likely to modulate adaptive

immune function, even if lymphocyte-specific outcomes were not directly measured. [30]

Broader reviews of Vitamin K biology emphasize its roles in oxidative stress control, mitochondrial function, and chronic inflammatory disease, and frequently discuss its interaction with Vitamin D as part of an integrated micronutrient network. These analyses underscore that Vitamin K deficiency often coexists with low Vitamin D status in at-risk populations, and that combined inadequacy is associated with worse clinical outcomes in age-related and cardiometabolic diseases. Such conditions are characterized by low-grade chronic inflammation, altered cytokine profiles, and impaired immune responsiveness, all of which are intimately linked with T and B cell dysfunction. From a physiological standpoint, restoring both Vitamin D_3 and Vitamin K_2 may therefore be necessary to optimize the intracellular environment for lymphocyte survival, metabolic fitness, and balanced effector–regulatory responses. While direct experimental data on combined D_3–K_2 effects in lymphocyte-specific models remain scarce, current mechanistic and clinical evidence justifies targeted studies in immunodeficient animal models to clarify the extent and nature of this proposed synergy. [31]

Insights from Experimental Immunodeficient Rodent Models Supplemented with Vitamin D_3

Immunodeficient rodent models have been crucial in revealing how Vitamin D_3 modulates adaptive immunity under conditions of lymphocyte dysfunction. In Vitamin D receptor (VDR)–deficient mice, researchers have observed thymic atrophy, altered T cell maturation, and a shift toward proinflammatory Th1 and Th17 responses, together with reduced regulatory T cell populations. These findings demonstrate that intact Vitamin D signaling is required for normal T cell development and for maintaining a balanced effector–regulatory profile. In parallel, Vitamin D deficiency or VDR knockout has been associated with increased susceptibility to autoimmune phenomena and exaggerated inflammatory responses to antigenic challenges, emphasizing the role of Vitamin D_3 as a gatekeeper of adaptive immune tolerance in vivo. [32]

Chemically induced immunodeficiency models in rodents further support the immunorestorative potential of Vitamin D_3 in compromised adaptive immunity. In cyclophosphamide-treated rats and mice, which exhibit profound lymphopenia and impaired cellular and humoral responses, administration of active Vitamin D analogs has been shown to enhance T cell proliferative responses, normalize cytokine patterns, and reduce tissue inflammation in models of colitis and other immune-mediated pathology. These experiments indicate that Vitamin D_3 can partially restore immune competence even when lymphocyte populations are numerically and functionally depressed by cytotoxic agents. Such findings are particularly relevant when considering translational applications of Vitamin D_3 supplementation in iatrogenic or disease-related immunodeficiency states. [33]

Murine models of infection have added an additional dimension by linking Vitamin D–dependent innate responses to downstream adaptive lymphocyte function. In experimental tuberculosis and other intracellular infections, Vitamin D_3 supplementation enhances macrophage antimicrobial pathways and modifies cytokine environments, which in turn shape T cell polarization and memory formation. VDR-deficient or Vitamin D–deficient mice display impaired control of infection, altered granuloma structure, and dysregulated T cell responses, underscoring the interconnectedness of innate and adaptive compartments in the context of Vitamin D_3 biology. Together, these rodent models demonstrate that adequate Vitamin D_3 signaling is essential not only for optimal T and B cell function but also for coordinated immune responses during immunological stress, providing a strong experimental basis for further exploration of combined micronutrient strategies involving Vitamins D_3 and K_2. [34]

Experimental and Indirect Evidence for Vitamin K_2 in Immunodeficient or Stress-Related Immune Models

Direct experimental studies evaluating Vitamin K_2 supplementation in classic immunodeficient rodent models are relatively limited, but mechanistic and organ-specific models provide important indirect insight into how Vitamin K_2 may protect lymphocyte function under stress. In inflammatory liver injury models, Vitamin K compounds have been shown to suppress lipopolysaccharide-induced production of IL-6 and other proinflammatory mediators through inhibition of NF-κB signaling. Because IL-6 is a key cytokine driving B cell differentiation and T helper cell skewing toward inflammatory phenotypes, its

attenuation by Vitamin K₂ suggests a capacity to blunt systemic inflammatory cascades that secondarily impair adaptive immune balance. These findings imply that under conditions of immune stress, Vitamin K₂ may contribute to re-establishing a cytokine milieu more favorable to controlled T and B cell responses rather than hyperinflammatory, tissue-damaging activation. [23]

Mitochondrial stress models offer another line of indirect evidence for a protective role of Vitamin K₂ in immune-relevant cellular resilience. In pink1-deficient Drosophila and mammalian systems, Vitamin K₂ has been identified as an alternative mitochondrial electron carrier capable of restoring electron transport and ATP production while reducing reactive oxygen species accumulation. Although these studies have primarily focused on neuronal tissue, the underlying mechanisms are directly applicable to rapidly proliferating immune cells, including activated T and B lymphocytes, which rely heavily on intact mitochondrial function to sustain clonal expansion and effector activity. In immunodeficient states where oxidative stress and mitochondrial dysfunction contribute to lymphocyte apoptosis and functional exhaustion, a Vitamin K₂–mediated improvement in mitochondrial bioenergetics could support better survival and function of adaptive immune cells, even if not yet explicitly tested in classic lymphocyte-deficient models. [24]

Additional experimental systems have highlighted the capacity of Vitamin K₂ to modulate gene expression linked to sphingolipid metabolism, a pathway highly relevant to immune cell fate decisions. Work in neuronal cells has shown that menaquinone-4 upregulates genes involved in sphingolipid synthesis and signaling, which in turn influence apoptosis, membrane microdomain organization, and receptor signaling. Given that ceramide and sphingosine-1-phosphate govern activation-induced cell death and survival in lymphocytes, these findings suggest that Vitamin K₂ may help tilt the balance away from excessive apoptosis toward controlled survival under stress conditions. In combination with its anti-inflammatory and mitochondrial effects, this sphingolipid-related modulation supports the concept that Vitamin K₂ can enhance cellular robustness in environments characterized by oxidative stress, inflammatory cytokine excess, or cytotoxic exposure, conditions that are common in experimental immunodeficiency models. [25]

Comparative Impact of Vitamin D₃ and Vitamin K₂ on T Cell Subset Balance in Dysfunctional States

Vitamin D₃ has a well-characterized influence on T cell subset balance, particularly in skewing responses away from proinflammatory Th1 and Th17 phenotypes toward Th2 and regulatory T cells. Through VDR-mediated transcriptional control in both T cells and antigen-presenting cells, 1,25-dihydroxyvitamin D₃ reduces IL-2, IFN-γ, and IL-17 production while enhancing IL-4 and IL-10, leading to a functional profile that favors tolerance and controlled inflammation. In experimental models of inflammation and autoimmunity, this shift is associated with attenuation of tissue damage and restoration of immune homeostasis. In immunodeficient or lymphocyte-dysfunctional states, such rebalancing is particularly important because exaggerated Th1/Th17 responses can coexist with overall impaired host defense, compounding clinical vulnerability. [1]

In contrast, Vitamin K₂ modulates T cell subset balance more indirectly, primarily by altering inflammatory signaling and cellular stress pathways that influence T helper cell polarization. By dampening NF-κB–driven cytokines such as IL-6 and TNF-α, Vitamin K₂ can reduce the upstream signals that promote Th17 differentiation and sustain chronic Th1 activation. At the same time, its mitochondrial-supporting and antioxidant actions help preserve T cell viability under conditions of oxidative stress, which often preferentially depletes regulatory and naïve T cell pools. The net effect is a milieu with less inflammatory drive and improved survival of T cells able to participate in balanced effector and regulatory responses, even though Vitamin K₂ does not directly engage nuclear receptors in T cells in the same canonical way as Vitamin D₃. [11]

Taken together, Vitamin D₃ and Vitamin K₂ appear to exert complementary influences on T cell subset balance in dysfunctional immune states: Vitamin D₃ acts as a direct transcriptional and developmental regulator of T helper and regulatory lineages, whereas Vitamin K₂ conditions the inflammatory and metabolic environment that constrains or permits these lineage choices. In chronic inflammatory or

immunodeficient conditions characterized by elevated IL-6, TNF-α, oxidative stress, and mitochondrial instability, combined optimization of both vitamins may therefore be required to achieve full restoration of T cell homeostasis. This conceptual model aligns with broader observations that coexisting deficiencies of Vitamins D and K are associated with worse inflammatory and age-related outcomes, and it provides a rationale for exploring dual supplementation strategies in experimental models of lymphocyte dysfunction. [31]

Comparative Effects of Vitamin D₃ and Vitamin K₂ on B Cell Responses and Antibody Production in Dysfunctional States

Vitamin D₃ has a predominantly inhibitory and regulatory influence on B cell responses, while Vitamin K₂ appears to act more as a protector of B cell viability and a modulator of the inflammatory environment in which B cells function. Vitamin D₃, via VDR signaling in activated B cells, reduces proliferation, blocks differentiation into plasma cells, and lowers immunoglobulin secretion, thereby limiting excessive or autoreactive antibody production. This is particularly important in dysfunctional immune states where dysregulated humoral responses can coexist with global immunodeficiency, contributing to tissue damage and ineffective host defense. In contrast, Vitamin K₂ does not directly suppress B cell differentiation but instead attenuates NF-κB–driven cytokine signals such as IL-6 that drive pathologic B cell activation, while preserving cellular energy supply and reducing oxidative stress through its mitochondrial effects. Together, these complementary actions suggest that Vitamin D₃ primarily restrains overactive humoral immunity, whereas Vitamin K₂ secures the metabolic and signaling conditions required for stable, non-pathologic B cell function. [20]

Under conditions of lymphocyte dysfunction, such as chemotherapy-induced immunosuppression, chronic inflammation, or metabolic disease, the combined optimization of Vitamin D₃ and Vitamin K₂ may be particularly relevant for restoring effective yet controlled antibody responses. Low Vitamin D is associated with impaired regulatory B cell (Breg) function and reduced IL-10 production, while Vitamin K deficiency has been linked to heightened inflammatory burden and worsened outcomes in age-related and cardiometabolic disorders. Correcting Vitamin D₃ status can promote Breg development and dampen inappropriate antibody production, whereas adequate Vitamin K₂ may reduce IL-6–driven plasmablast expansion and protect B cells from oxidative apoptosis, thereby maintaining a pool of competent antigen-responsive cells. This integrated view supports the concept that, in dysfunctional immune states, Vitamin D₃ and Vitamin K₂ act on distinct but converging aspects of B cell biology—transcriptional control and differentiation on one side, and inflammatory and mitochondrial homeostasis on the other—together favoring a humoral response that is both quantitatively sufficient and qualitatively regulated. [31]

Conclusion

Vitamin D₃ and Vitamin K₂ represent two micronutrients with distinct yet complementary roles in shaping adaptive immune responses, particularly under conditions of lymphocyte dysfunction. Across experimental models, Vitamin D₃ consistently emerges as a direct regulator of T and B cell development, differentiation, and cytokine programming through VDR-mediated transcriptional pathways. It promotes regulatory phenotypes, suppresses excessive effector responses, and stabilizes immune tolerance mechanisms that are often disrupted in immunodeficient states. Vitamin K₂, while acting through different molecular mechanisms, contributes critically to the inflammatory and metabolic environment in which lymphocytes operate. By modulating NF-κB–driven cytokine expression, enhancing mitochondrial electron transport, and influencing sphingolipid-mediated survival pathways, Vitamin K₂ supports the resilience and controlled activation of both T and B cells during immune stress. When viewed together, the evidence suggests that Vitamins D₃ and K₂ are not merely parallel modulators but potentially synergistic supporters of adaptive immunity. Vitamin D₃ shapes the transcriptional and developmental landscape of lymphocytes, whereas Vitamin K₂ maintains the metabolic integrity and inflammatory balance required for these cells to function effectively. This complementary relationship becomes especially relevant in immunodeficient rodent models, where dysregulated cytokine environments, mitochondrial instability, and imbalanced T and B cell responses underscore the need for

multifaceted regulatory support. Although direct experimental studies assessing their combined effects remain limited, the mechanistic coherence and indirect evidence strongly indicate potential benefit from coordinated optimization of both vitamins.

The integration of findings from diverse experimental systems reveals that nutritional status—in particular the sufficiency of Vitamins D_3 and K_2—plays a more central role in immune health than previously appreciated. Restoring these micronutrients may not only improve lymphocyte function but also enhance overall immune homeostasis, reduce inflammation, and support recovery in immunocompromised conditions. Future research should prioritize targeted studies assessing combined supplementation in immunodeficient models, with attention to dose-response relationships, mechanistic synergism, and long-term functional outcomes on adaptive immunity. This growing field holds promise for developing nutritional strategies that augment immune resilience and improve the management of immune dysfunction

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