frontiers in Immunology

TYPE Review
PUBLISHED 04 November 2026
DOI 10.3389/fimmu.2026.2018897

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OPEN ACCESS

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RECEIVED 04 October 2026
REVISED 20 October 2026
ACCEPTED 22 October 2026
PUBLISHED 04 November 2026

CITATION
Jaroslawska J and Carlberg C (2026) Context-dependent vitamin D immunoregulation: how a shared VDR-centered pathway generates distinct human immune outcomes.
Front. Immunol. 17:2018897.
doi: 10.3389/fimmu.2026.2018897

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Context-dependent vitamin D immunoregulation: how a shared VDR-centered pathway generates distinct human immune outcomes

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Vitamin D exerts antimicrobial, inflammatory, adaptive, and regulatory effects in human immunity, yet these actions can be perceived as contradictory. This review examines how this heterogeneity reflects context-dependent interpretation of a shared vitamin D receptor (VDR)-centered signaling pathway. The response depends on ligand availability and intracellular metabolism, the identity and state of the responding cell, its inflammatory and regulatory environment, exposure history, and host biology. Exposure history and concurrent immune signals can change the identity and direction of vitamin D-responsive genes, as well as response magnitude. These mechanisms provide experimentally testable links between the shared VDR pathway and the engagement of different immune programs. Antimicrobial defense shows the clearest directional coherence, whereas inflammatory responses are more often selectively modified than uniformly suppressed. Adaptive responses are remodeled according to cell state, and regulatory or tolerogenic properties recur across several cellular systems, although changes in phenotype do not necessarily demonstrate functional tolerance. Transcriptional, chromatin, and metabolic adaptation contribute to these context-dependent responses. This framework also helps explain why robust molecular or cellular responses do not necessarily translate into clinical benefit: target engagement must alter an immune function that is relevant to the disease process and sufficiently important to influence outcome. Future human studies should determine which VDR-dependent program is engaged, under what biological conditions, and with what functional and clinical consequences.

KEYWORDS
antimicrobial defense, context-dependent immunoregulation, epigenetic regulation, immune tolerance, immunometabolism, inter-individual responsiveness, vitamin D, vitamin D receptor

Introduction

Vitamin D is among the most extensively studied immunomodulators, yet its effects on the immune system have been described in markedly different terms: antimicrobial, anti-inflammatory, immunoregulatory, tolerogenic and, in some settings, immunostimulatory.

Human mechanistic studies support these activities, including a vitamin D-mediated antimicrobial response initiated by Toll-like receptor (TLR) activation (1), regulation of dendritic cell (DC) function (2), and modulation of adaptive immune responses (3). TLRs are pattern-recognition receptors (PRRs) that detect conserved microbial components and initiate innate immune signaling. This range of effects does not fit easily with the idea that vitamin D has one dominant immune action. The human evidence suggests that biological context is part of the mechanism that determines the response. Cellular access to active hormone, immune cell identity and activation state, inflammatory and tissue conditions, chromatin and genetic context, exposure history, and inter-individual biology can influence both the effective VDR signal and the regulatory environment in which it acts. The central question is how a shared VDR-centered signaling system generates different immune outcomes under these changing conditions.

Vitamin D does not simply stimulate or suppress immune responses. Antimicrobial defense, inflammatory modulation, adaptive immune remodeling, and immune regulation are distinct but overlapping functional programs that can be engaged in different combinations according to biological context. An antimicrobial response, for example, can coexist with attenuation of selected inflammatory pathways, while regulatory features can emerge without generalized immune suppression. The relevant question is which components of the immune response are modified, in which cells and conditions, and with what functional consequence. The framework builds on earlier evidence for dynamic VDR-associated chromatin regulation (4) and inter-individual variation in molecular responsiveness (5).

Treatment-sequence experiments in primary peripheral blood mononuclear cells (PBMCs) (6) and combined chromatin and transcriptomic profiling in THP-1 cells (a human monocytic leukemia cell line) (7) further suggest that previous exposure and concurrent immune signals can change the response available to the cell. We therefore consider exposure history and signal integration as mechanisms shaping the engagement of different immune programs. This framework also distinguishes molecular responsiveness, altered immune function, and clinical benefit as related but non-equivalent levels of response.

This narrative review draws on literature assembled through ongoing reading, targeted retrieval of relevant PubMed-indexed reports and reference list checking. The included publications span 2000-2026; this range describes the literature included rather than a predefined search window. Selection was purposive rather than systematic or exhaustive and was not based on a fixed search string or a database search conducted on a single date.

Original studies were selected for their relevance to at least one of the six contextual domains or four immune programs addressed in the review. The contextual domains comprised i) vitamin D availability and metabolism, ii) immune-cell identity and activation state, iii) inflammatory and tissue context, iv) chromatin and genetic context, v) exposure timing, and vi) inter-individual or disease-related responsiveness. Eligible studies examined molecular, cellular, functional or clinical outcomes and included mechanistic experiments, observational studies and interventions. Original studies involving primary human cells, human tissues or participants formed the basis of the human evidence counts, according to the figure-specific eligibility criteria. Cell-line studies informed mechanistic interpretation but did not qualify as primary-human evidence. Reviews and meta-analyses informed the synthesis without contributing additional independent primary-human evidence units.

Publications were linked to their underlying experimental datasets and, where applicable, participant cohorts. Secondary analyses remained linked to the source experimental dataset and did not contribute an additional independent dataset, including when they informed a different contextual domain. New experimental measurements in previously studied participants could constitute a distinct dataset but did not represent independently recruited cohorts. We grouped eligible primary-human evidence units into related study programs and counted distinct experimental datasets separately within each contextual domain and evidence dimension. A dataset may contribute to several domains but is counted only once within each domain and dimension. Research-group families were identified using the canonical mapping documented in Supplementary Table S1.

Responses were classified according to the measured endpoints and their predominant direction within each immune outcome domain. Functional evidence required a directly measured immune function; transcriptional, epigenetic or phenotypic changes alone were insufficient. Supplementary Table S1 documents study characteristics, links among publications, datasets and reused cohorts, study-program assignments, figure eligibility and domain-specific counting decisions. The counts describe the breadth of the selected evidence; they do not constitute formal assessments of study quality, effect size or certainty and are not a census of vitamin D research in human immunity. Evidence originating from research programs led by the authors' group accounted for 4 of 37 eligible primary-human evidence units and 13 of 50 unique datasets. Each dataset was counted once across domains for this proportion; collaborative authorship alone did not determine group origin.

A shared VDR-centered pathway provides the common molecular substrate

Divergent immune outcomes do not require distinct vitamin D signaling pathways. Canonical vitamin D signaling is centered on ligand-dependent activation of the transcription factor VDR, heterodimerization with a member of the retinoid X receptor (RXR) family, comprising RXRα, RXRβ and RXRγ, and interaction with regulatory elements in the genome together with transcriptional and chromatin-regulatory machinery (4, 8) (Figure 1). The latter includes co-activators and co-repressors that mediate the contact with the basal transcriptional machinery including RNA polymerase II as well as pioneer transcription factors and chromatin-modifying enzymes that facilitate the access of the VDR-RXR complex to enhancer regions.

The receptor pathway is shared across immune settings, although the strength, timing, and regulatory consequences of its activation can differ. Immune cells can also modify the signal

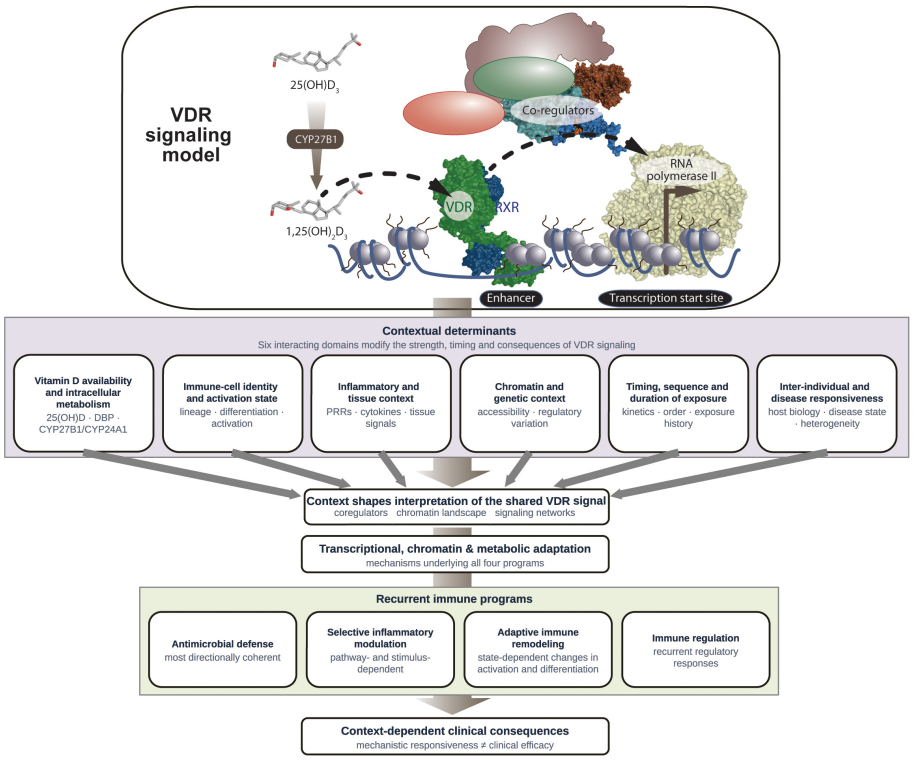


FIGURE 1
A framework for context-dependent human vitamin D immunoregulation. Vitamin D activates a shared VDR-centered signaling pathway, but the resulting immune response is not predetermined. Biological context shapes how the common upstream signal is translated into downstream immune responses through the combined influence of host, environmental, and experimental or clinical determinants. Context-dependent VDR signaling can engage antimicrobial defense, selective inflammatory modulation, adaptive immune remodeling, and immune regulation, supported by transcriptional, chromatin, and metabolic adaptation and potentially linked to different clinical outcomes. The framework summarizes how apparently divergent actions of vitamin D arise from the way a shared VDR-centered pathway operates in different biological contexts rather than from separate signaling pathways. Gray arrows indicate the influence of contextual determinants on VDR signal interpretation; the upper schematic illustrates ligand activation and VDR-associated transcriptional regulation.

reaching VDR through local vitamin D metabolism; human monocyte/macrophage systems provide a well-established example (1, 9). Variation in vitamin D responsiveness can therefore arise from two sources: i) the signal reaching VDR and ii) the regulatory state of the responding cell. These sources are dynamically linked: prior VDR signaling may modify the regulatory state in which subsequent signals are interpreted. Consequently, a vitamin D-responsive gene repertoire is conditional on cell state, accompanying stimuli and sampling time. Responsiveness under these conditions does not, by itself, establish direct regulation through VDR binding.

Biological context shapes how a shared VDR signal is interpreted

Six interacting domains capture the main sources of biological context considered here: i) vitamin D availability and intracellular metabolism, ii) immune cell identity and activation state, iii) inflammatory and tissue context, iv) chromatin and genetic context, v) timing, sequence, and duration of exposure, and vi) inter-individual and disease-related responsiveness (Figure 1). They influence ligand availability, receptor engagement, the regulatory state in which VDR acts, and the functional consequences of the response, while remaining biologically interdependent. Representative human evidence is summarized in Table 1.

Vitamin D availability and intracellular metabolism

At the most proximal level, vitamin D responsiveness depends on whether a cell has access to sufficient active ligand. Circulating 25-hydroxyvitamin D_3 ($25(OH)D_3$) is the principal substrate for immune cell production of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), but total serum concentration does not define cellular exposure. CYP27B1 is the 25-hydroxyvitamin D 1α-hydroxylase that converts $25(OH)D_3$ to active $1,25(OH)_2D_3$. CYP24A1 initiates the catabolism of vitamin D metabolites. Together with substrate bioavailability and uptake, these processes determine how much active ligand is available to VDR in immune cells. Human monocytes and macrophages illustrate this local control particularly clearly: TLR1/TLR2 activation induces VDR and CYP27B1, coupling pathogen recognition to local conversion of $25(OH)D_3$ and VDR-dependent induction of the antimicrobial protein cathelicidin. In this case the magnitude of the response depends on substrate availability (1).

TABLE 1 Representative evidence from human studies and complementary experimental models supporting the context-dependent vitamin D immunoregulation framework.

Reference	Human system/ cohort	Study design	Contextual determinant or immune program	Contribution to the framework	Key limitation
(28)	33,996 participants across discovery and replication cohorts	Genome-wide association study	Vitamin D availability; inter-individual heterogeneity	Large independent human evidence that inherited biology constrains vitamin D availability.	Explains substrate variation, not immune cell response or clinical benefit; Environmental and ancestry interactions remain important.
(13)	Primary human resting and T cell receptor-activated T cells; Donor number not reported	*Ex vivo* primary-human study	Immune cell identity and activation state	Direct demonstration that T cell activation state changes VDR-dependent signaling competence.	Controlled *in vitro* setting and donor metadata incomplete; Early activation mechanism does not define later effector outcomes.
(1)	Primary human monocytes/macrophages in TLR1/2 and mycobacterial context; Exact donor number not reported	*Ex vivo* primary-human mechanistic study	Intracrine metabolism; inflammatory/stimulus context; antimicrobial defense	Functional human evidence linking intracrine metabolism to antimicrobial defense.	Centered on monocyte/ macrophage and mycobacterial context; Not all inflammatory stimuli or pathogens are represented; Pathogen-specific context.
(21)	Primary human monocytes plus wild-type and *Dusp1*-deficient murine macrophages; No unique human donor total reported	*Ex vivo* primary-human study with supporting murine experiments	Inflammatory context; selective inflammatory modulation	Human monocyte evidence, supported by murine experiments, for selective inflammatory attenuation through DUSP1.	Centered on acute LPS/ p38 MAPK signaling; other ligands and immune functions were not tested.
(26)	Primary human monocyte-derived DCs, with blood $CD1c^+$ DC validation and allogeneic $CD8^+$ T cell co-cultures; unique donor total not reported	*Ex vivo* mechanistic and functional study combining DNA methylation profiling, VDR ChIP-seq, protein-interaction analysis and JAK2 inhibition	Chromatin context; cytokine signaling; immune regulation	Connects VDR-associated DNA demethylation and JAK2-STAT3 signaling with directly demonstrated suppression of $CD8^+$ T cell proliferation	Cultured-cell model and pharmacological perturbation; causal roles of individual demethylated loci unresolved; no human intervention or clinical efficacy endpoint
(49)	Human monocyte-derived DCs co-cultured with T cells; No unique donor total reported	*Ex vivo* primary-human DC/T cell study	Metabolic adaptation; immune regulation	Functional link between glucose metabolism, tolerogenic DCs and regulatory T cells.	Monocyte-derived DC model and pharmacological perturbation limit *in vivo* generalization; Evidence is concentrated in one cell model.
(6)	PBMCs from one donor challenged with LPS or β-glucan and treated before, during or after challenge; one donor with replicate cultures	*Ex vivo* primary-human transcriptomic study	Treatment sequence and immune-challenge identity	Primary-PBMC evidence that the transcriptional response depends jointly on $1,25(OH)_2D_3$ treatment sequence and the identity of the immune challenge.	One donor with repeated cultures; mixed PBMCs; simultaneous and sequential models differ in duration; no direct immune-function endpoint.
(7)	THP-1 cells	RNA-seq and ATAC-seq; 10 nM $1,25(OH)_2D_3$ and 100 ng/mL LPS; 24 hour priming followed by 24 hour stimulation	Inflammatory context; treatment sequence; chromatin and transcriptional adaptation	Distinct co-stimulation-associated chromatin and transcriptional responses; priming-dependent non-additive gene regulation; AP1-related motif enrichment	Cell-line model; AP1 causality and immune function untested; primary-PBMC comparison reuses the Malmberg dataset
(4)	THP-1 monocytic leukemia cells in independent time-course experiments; 8,979 accessibility loci; 1,284 regulated genes	*In vitro* THP-1 genomic time-course	Chromatin context; temporal dynamics	Genome-wide time-resolved chromatin–transcriptome evidence.	THP-1 and 100 nM $1,25(OH)_2D_3$ may exaggerate scale; Cell-line model and nonphysiological dose; Independent primary-human replication remains limited.

(Continued)

TABLE 1 Continued

Reference	Human system/ cohort	Study design	Contextual determinant or immune program	Contribution to the framework	Key limitation
(38)	100 healthy Saudi adults receiving an 80,000 IU vitamin D_3 bolus	Longitudinal intervention-cohort analysis	Inter-individual responsiveness; responder classification	Assessment of molecular and cytokine-based response classifications in a Saudi intervention cohort.	Classification remains biomarker- and timing-dependent; No clinical endpoint validation.
(27)	One-person discovery and 13 selected participants from existing cohorts; no new recruitment or independently recruited replication	Longitudinal N-of-1 multi-omics with validation subset	Chromatin context; temporal dynamics; inter-individual responsiveness	Longitudinal *in vivo* RNA-seq/ATAC-seq evidence linking chromatin accessibility and transcription over time.	One-person discovery and selected validation subset; Discovery is N-of-1; Validation subset is selected and small.
(30)	Adults with smear-positive pulmonary tuberculosis receiving adjunctive vitamin D_3 or placebo; 146 randomized, 126 included in the primary efficacy analysis	Randomized controlled trial with linked biomarker analyses	Clinical translation; inflammatory context; inter-individual responsiveness	Illustrates divergence between mechanistic inflammatory effects and the primary clinical endpoint.	Linked biomarker analyses share the randomized trial cohort and are not independent; genotype subgroup findings require replication.
(48)	48 patients with inflammatory bowel disease: 27 with ulcerative colitis and 21 with Crohn disease; paired blood and stool samples; supporting co-cultures from 6–12 participants from the same cohort	Prospective, uncontrolled 12-week vitamin D_2 intervention (50,000 IU/ week), combining microbiota profiling, single-cell transcriptomics, immune-repertoire sequencing and primary-cell co-cultures	Inflammatory and tissue context; immune-cell interactions; adaptive immune remodeling and immune regulation	Links supplementation-associated changes in antibody binding to gut bacteria with lymphocyte repertoire remodeling and regulatory-associated cell states	Uncontrolled intervention with concurrent IBD therapy; BAFF signaling inferred computationally; regulatory phenotypes do not establish suppressive function or durable tolerance
(58)	46 randomized trials with aggregate/stratified data; 64,086 participants	Stratified aggregate-data meta-analysis	Clinical translation; population and regimen heterogeneity	Large recent clinical synthesis showing trial heterogeneity and no statistically significant overall preventive effect.	Heterogeneous trials and incomplete stratified data remain; Subgroup analyses remain less certain than the overall estimate.
(39)	17 participants with relapsing-remitting multiple sclerosis; 42 person VitDPAS comparator cohort	Longitudinal observational multi-omics	Disease-related responsiveness; chromatin/ transcriptional context	Disease-context multi-omics evidence with clear limits on causal interpretation.	Non-randomized groups and confounding limit causal interpretation; Small sample and limited confounder adjustment; Nominal candidate-level findings require replication.
(19)	Primary human B cells from healthy adult donors; separate observational arm comprising participants with systemic lupus erythematosus, rheumatoid arthritis or neither disease	*Ex vivo* primary-human B cell study with cross-sectional clinical cohort	Immune cell identity and activation; adaptive immune remodeling; vitamin D metabolism	Shows that activated human B cells are direct vitamin D targets: $1,25(OH)_2D_3$ restrains ongoing proliferation and plasma-/ postswitch-memory cell differentiation while B cells express vitamin D metabolic machinery.	Healthy-donor total was not reported; the systemic lupus erythematosus arm is observational and does not establish treatment efficacy.
(18)	Primary $CD19^+$ B cells from healthy adult donors	*Ex vivo* primary human B cell study	Immune cell identity and activation; vitamin D metabolism; immune regulation	Shows that B cell activation induces VDR/CYP27B1 competence and that $1,25(OH)_2D_3$ enhances IL10 through direct VDR association with the *IL10* promoter and calcium-dependent signaling.	Donor total was not reported and assay-level replication was modest; IL10 induction alone does not establish durable tolerance *in vivo*.

(Continued)

TABLE 1 Continued

Reference	Human system/ cohort	Study design	Contextual determinant or immune program	Contribution to the framework	Key limitation
(43)	192 healthy adult tuberculosis contacts randomized; primary analysis n = 131	Double-blind randomized controlled trial with functional whole-blood assay	Vitamin D availability; antimicrobial defense; clinical translation	Provides randomized human evidence that a single vitamin D_2 dose can improve a 24 h whole-blood antimycobacterial functional readout while increasing serum $25(OH)D_2$.	The effect was detected in the 24-hour assay ($p = 0.03$), was absent at 96 hours, and did not establish a clinical tuberculosis benefit.

Selected studies illustrate complementary evidence for the major contextual determinants and immune programs discussed in this review. Studies were chosen to represent distinct mechanistic, cellular, functional, longitudinal, and translational contributions rather than to provide a comprehensive catalog of the literature. Where multiple publications derive from the same cohort or experimental program, reused datasets are not considered independent replication. The table distinguishes molecular or cellular responsiveness to vitamin D from experimentally demonstrated immune function and from clinical efficacy. Vitamin D exposures include vitamin D_2, vitamin D_3, $25(OH)D_3$, and $1,25(OH)_2D_3$, as indicated for each study.

Adequate $25(OH)D_3$ is likewise required for interferon gamma (IFNγ)-driven antimicrobial peptide expression, autophagy, phagolysosomal maturation, and intracellular *M. tuberculosis* killing (10). Cellular exposure also depends on extracellular transport and inflammatory signals: vitamin D-binding protein (DBP) concentration and binding characteristics alter responses to vitamin D metabolites (11). IFNγ favors CYP27B1-dependent activation and antimicrobial responses. In contrast, interleukin 4 (IL4) promotes catabolism and reduced antimicrobial responsiveness (12). These *ex vivo* observations show why serum substrate availability alone does not define immune cell exposure.

The capacity to generate and respond to $1,25(OH)_2D_3$ also changes as immune cells differentiate. Human monocyte-derived DCs alter vitamin D metabolism and VDR-linked responses during differentiation, affecting both active-ligand generation and downstream function (9). Circulating vitamin D status is therefore an incomplete measure of immune exposure: serum $25(OH)D_3$ provides the substrate for intracrine signaling, but it does not indicate how much active ligand an immune cell will generate or how strongly the VDR pathway will be engaged.

Immune cell identity and activation state

Vitamin D responsiveness is not fixed within an immune cell lineage. It changes with activation and differentiation as VDR expression, vitamin D metabolism, and the regulatory networks that shape VDR activity also change. In T cells, T cell receptor activation can establish responsiveness to $1,25(OH)_2D_3$, with subsequent effects depending on activation state and cytokine environment (3, 13). A similar pattern is seen during myeloid differentiation: IL15-dependent differentiation induces CYP27B1, VDR, and cathelicidin and permits $25(OH)D_3$-dependent antimycobacterial activity, whereas alternative differentiation conditions do not generate the same program (14). Activation and differentiation can thus determine whether the VDR pathway is functionally available before shaping the downstream response.

Once vitamin D responsiveness is established, the outcome still depends on the regulatory program of the responding cell. Vitamin D alters phenotype, cytokine production, and T cell stimulatory capacity in monocyte-derived DCs, yet human myeloid and plasmacytoid DCs differ markedly in their susceptibility to tolerogenic modulation (2, 15, 16). Cell identity does not define a fixed response: activated cluster of differentiation 4 $(CD4)^+$ T cells can acquire regulatory characteristics in response to $1,25(OH)_2D_3$, and differentiated C-C chemokine receptor type 6 $(CCR6)^+$ memory T cell populations retain substantial plasticity, for example, in the context of rheumatoid arthritis (3, 17). B cells show the same dependence on activation. Activation increases VDR and CYP27B1 expression; $1,25(OH)_2D_3$ can inhibit the generation of plasma cells and post switch memory B cells, while activated B cells can develop a VDR-dependent IL10 response (18, 19). Cell identity and activation state influence both whether a cell responds effectively and which functional responses are available once VDR signaling is engaged.

Inflammatory and tissue context

Inflammatory context can influence both the generation of the VDR signal and the way that signal is interpreted. PRRs like TLRs, together with cytokines, microbial products, and tissue-derived signals, establish transcriptional and metabolic states that modify active-ligand generation, pathway crosstalk, and downstream VDR output. One consequence is selective attenuation rather than generalized suppression. In primary human monocytes, $1,25(OH)_2D_3$ reduces TLR2 and TLR4 expression and downstream inflammatory signaling in a time- and dose-dependent manner (20). Lipopolysaccharide (LPS), a component of the Gram-negative bacterial outer membrane, triggers inflammatory signaling through the TLR4 receptor complex. Vitamin D can induce dual-specificity phosphatase 1 (DUSP1), thereby attenuating LPS-induced p38 mitogen-activated protein kinase (MAPK) activation and the production of selected inflammatory cytokines (21). These findings fit pathway-specific negative feedback better than a universal anti-inflammatory state.

Sterile inflammatory settings provide a further dimension of context. The "reverse hygiene hypothesis" emphasizes environmental and lifestyle pressures that may promote chronic immune dysregulation, highlighting mitochondrial damage and cellular senescence as sources of endogenous inflammatory signals (22).

This broadens the concept of immune challenge beyond microbial stimuli to include metabolic stress and tissue damage. Within the present framework, it raises the question of whether these conditions alter the VDR-responsive gene repertoire and the functional consequences of vitamin D signaling.

The same VDR pathway can support other functions in different inflammatory and tissue settings. Opposing IFNγ and IL4 effects on vitamin D metabolism alter antimicrobial competence in human monocytes (12). In wounded human skin, CYP27B1, TLR2, CD14, and cathelicidin participate in a local vitamin D-dependent defense program (23). CD14 is a microbial-product co-receptor best characterized for facilitating LPS recognition by the TLR4 receptor complex. In contrast, vitamin D-enhanced IL1β from infected macrophages can signal to neighboring airway epithelial cells, inducing defensin beta 4 (DEFB4) and improving antimycobacterial activity in co-culture (24). VDR signaling can attenuate selected cytokine pathways while preserving defense-associated inflammatory signals or coordinating communication between immune and structural cells; which component changes depends on the immune program active in that setting.

In $1,25(OH)_2D_3$-primed THP-1 cells, 331 genes with reported non-additive responses to $1,25(OH)_2D_3$ and LPS were identified; 264 were not responsive to $1,25(OH)_2D_3$ alone under the tested conditions (7). Examples included *CEBPD* (CCAAT enhancer binding protein delta) and *PTGS2* (prostaglandin-endoperoxide synthase 2), which were downregulated by $1,25(OH)_2D_3$ alone but upregulated during combined stimulation. These findings support context-dependent changes in the repertoire and direction of responsive genes. The experiment used a transformed cell line. Independent primary-human replication and consequences for immune cell differentiation and function remain to be established experimentally.

Complementary evidence comes from primary human neutrophils. LPS strongly reduced $1,25(OH)_2D_3$-induced *CYP24A1* expression, with reduced VDR occupancy and increased recruitment of the transcriptional repressors MAFF and BACH1 at a *CYP24A1* enhancer (25). This suggests attenuated catabolic feedback, although prolonged ligand availability was not directly established. Vitamin D also reduced selected LPS-associated inflammatory transcriptional responses while enhancing antibacterial activity in neutrophil-conditioned medium. An antibody against LL-37, the active cathelicidin peptide, abolished the vitamin D-associated increase in antibacterial activity in the conditioned-medium assay. These results illustrate how inflammatory context can modify vitamin D metabolism and distinguish inflammatory from antimicrobial outputs within the same cell type.

Chromatin and genetic context

VDR acts within a regulatory genome whose accessibility depends on cell state and history. The transcriptional consequences of ligand-bound VDR depend on chromatin organization, cooperating transcription factors, and the accessibility of enhancer regions containing VDR-binding motifs in the responding cell. Time-resolved studies show that VDR occupancy and chromatin accessibility change after vitamin D exposure (4). Chromatin regions responsive to combined $1,25(OH)_2D_3$ and LPS stimulation were enriched for binding motifs of AP1-family transcription factors, including JUN/FOS proteins (7). Together with the distinct transcriptional response, this nominates cooperation between vitamin D signaling and stimulus-responsive transcription factor networks as a candidate mechanism of context dependence.

Motif enrichment, however, does not establish factor occupancy, physical interaction with VDR or causal control of the affected genes. A distinct example of cooperation between VDR and other regulatory factors comes from human monocyte-derived DCs. During $1,25(OH)_2D_3$-induced tolerogenic differentiation, DNA demethylation occurred at VDR binding regions, and co-immunoprecipitation demonstrated associations among VDR, phosphorylated STAT3 and the methylcytosine dioxygenase TET2. Pharmacological inhibition of JAK2 reduced IL10 secretion and abolished the ability of these DCs to suppress $CD8^+$ T cell proliferation, without clearly reversing DNA demethylation at the examined loci (26). These findings connect the signaling program to measured suppressive function while showing that the assessed methylation patterns alone do not establish that function. The causal contribution of individual demethylated regions and the generality of these regulatory interactions across other primary human immune populations remain unresolved. Longitudinal transcriptomic and chromatin-accessibility profiling of PBMCs after *in vivo* vitamin D exposure found temporally distinct regulatory responses and substantial between-person variation in responsive chromatin regions (27). The discovery analysis involved one selected high responder, with validation in a small, selected subset of an existing cohort. These regulatory associations need confirmation in independently recruited cohorts and other primary immune cell populations.

Inherited variation represents a different type of context. Common variants in genes involved in vitamin D synthesis, transport, and metabolism contribute to circulating $25(OH)D_3$ concentrations (28, 29), modifying the host background in which immune VDR signaling occurs. Direct evidence that inherited variation predicts immune VDR responsiveness is much weaker. In a randomized pulmonary tuberculosis trial, an interaction between vitamin D treatment and a variation of the *VDR* gene, rs731236 (TaqI), was reported for sputum-culture conversion, whereas no comparable interaction was seen for the VDR variation rs2228570 (FokI) (30). Chromatin state and inherited variation should remain conceptually distinct: chromatin is dynamic and state-dependent, whereas genetic variation provides a more stable background that may influence vitamin D availability or the conditions under which a response occurs.

Timing, sequence and duration of exposure

Timing matters because VDR binding, chromatin regulation, and transcription change on different timescales. Time-resolved genomic studies show progressive reorganization of VDR-dependent regulatory activity (4). Recent VDR cistrome mapping in THP-1 cells similarly showed expansion of ligand-responsive VDR occupancy from early to late time points, accompanied by a shift

from a restricted early transcriptome toward a broader delayed program (31). Treatment sequence introduces an additional dimension of context. When comparing $1,25(OH)_2D_3$ exposure before, during or after LPS or β-glucan challenge in freshly isolated human PBMCs, the resulting transcriptomes depended on both treatment sequence and challenge identity, with stronger sequence dependence for the combined LPS response than for the β-glucan response (6). Thus, an effect observed during simultaneous stimulation cannot automatically be extrapolated to pretreatment or treatment after immune activation. The study provides primary cell evidence for this principle, although it involved one donor with repeated cultures. Comparisons between simultaneous and sequential conditions also involved different total incubation times, requiring further experiments to separate treatment order from exposure duration. Functional responses can also change direction over time. In activated primary human memory $CD4^+$ T cells, $1,25(OH)_2D_3$ enhanced proliferation at day 4 but reduced it at day 7 under the tested culture conditions, with glutamine metabolism supporting the early expansion (32).

An intrinsic vitamin D pathway can also connect immune activation to response contraction. In human $CD4^+$ T cells, complement signaling induced VDR and CYP27B1, enabling a transition from IFNγ-producing type 1 helper T (T_H1) cells toward an IL10-producing regulatory state. This transition involved epigenetic remodeling and cooperation among VDR and the transcription factors JUN, STAT3 and BACH2. These findings illustrate how vitamin D responsiveness can emerge during an immune response and contribute to its resolution (33).

Exposure duration also matters. Acute pharmacological treatment with $1,25(OH)_2D_3$ in cultured cells does not necessarily reproduce the consequences of sustained changes in circulating $25(OH)D_3$ after vitamin D_3 supplementation *in vivo*, because different regulatory processes may emerge over different timescales. At longer timescales, seasonal variation in vitamin D status coincides with changes in immune state. A secondary analysis found that 87 vitamin D-responsive genes from the vitamin D intervention study VitDHiD overlapped with genes showing circadian expression in an independent human whole-blood dataset (34). This links circadian organization to the vitamin D-responsive transcriptome but does not show that the magnitude or functional consequences of immune vitamin D responsiveness vary according to time of day. A single time point or exposure sequence captures only one stage of a changing regulatory response. Whether the effects of acute exposure and priming persist should be tested after stimulus removal and a defined resting interval, followed by functional rechallenge (6, 7). These experiments would distinguish transient modulation from persistent innate immune reprogramming. The existing studies also do not establish that a particular oral vitamin D_3 supplementation schedule improves infection outcomes.

Inter-individual and disease-related responsiveness

Individuals differ not only in vitamin D status but also in the molecular response generated when the VDR-centered pathway is engaged. Such variation is apparent even under controlled conditions: PBMCs exposed *ex vivo* to the same concentration of $1,25(OH)_2D_3$ show large donor-to-donor differences, including for canonical targets (5), while intervention studies show variable biochemical, chromatin, and transcriptional responses to vitamin D_3 exposure (35, 36). Recent data support continuous variation in response magnitude more strongly than fixed responder categories. In the Polish vitamin D intervention cohort VitDPAS, high-, mid-, and low-response classifications changed when different gene sets were used (37), and a pooled analysis of two Saudi intervention cohorts found that classifications depended on whether circulating cytokines or selected vitamin D-responsive genes were used (38). At present, these labels describe the measured response rather than a stable biological type. Comparison of the studies VitDPAS and VitDHiD identified 26 shared genes at FDR < 0.05 whose annotations involve inflammatory signaling, migration, survival and metabolic regulation (37) (Figure 2A). These shared genes indicate a common annotated component of the transcriptional response, without by themselves establishing shared immune function. Establishing that pathways are more reproducible than individual genes requires direct comparison of pathway-level responses using harmonized analyses.

Repeated within-person profiling shows a complementary pattern. Longitudinal transcriptomic and chromatin-accessibility measurements can show reproducible temporal features within an individual while revealing substantially less overlap between individuals (27). In bulk PBMC profiles, apparent differences in responsiveness may reflect both changes within cells and differences in the proportions of responding cell populations. Paired immunophenotyping, sorted-cell analyses or single-cell measurements are therefore needed to distinguish regulation within a cell type from changes in cellular composition.

Disease can further change the cellular and regulatory state in which VDR signaling occurs. Regulatory responses remain inducible in inflammatory T cell populations from rheumatoid arthritis (17), while longitudinal multi-omics profiling in multiple sclerosis suggests selective supplementation-associated immune-regulatory changes against a disease-specific molecular background (39). These studies support disease state as context but do not isolate disease from differences in cohort composition, treatment, or study design. Overall, inter-individual responsiveness reflects the combined influence of vitamin D metabolism, immune cell composition, inflammatory state, chromatin organization, inherited variation, and exposure history, with current evidence favoring continuous heterogeneity over fixed responder classes.

Recurrent immune programs across human contexts

Across human studies, vitamin D-responsive changes recur across four broad immune programs: i) antimicrobial defense, ii) selective inflammatory modulation, iii) adaptive immune remodeling, and iv) immune regulation. The evidence spans myeloid/innate, DC, lymphoid, infection/disease, and *in vivo*/longitudinal settings

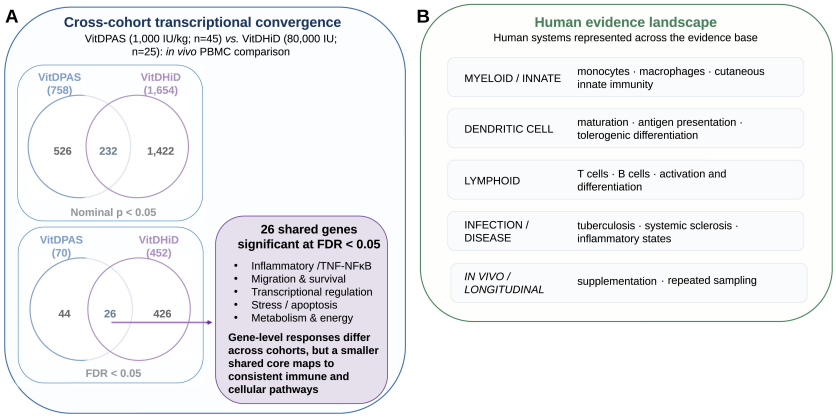


FIGURE 2
Human evidence converges on recurrent but context-dependent vitamin D immune programs. Cross-cohort comparison of vitamin D-responsive PBMC transcriptomes in the VitDPAS and VitDHiD studies **(A)**. Panel **(A)** summarizes PBMC transcriptional responses 24 hours after vitamin D_3 supplementation (37). Although overlap between significant gene lists was limited, the 26 genes shared at FDR < 0.05 mapped to recurrent immune and cellular functions. This is consistent with partial transcriptional convergence in genes associated with recurrent immune and cellular processes, although greater reproducibility at the pathway level requires direct comparison of pathway responses across cohorts. Because the comparative analysis includes a reused dataset, it is interpreted as cross-cohort convergence rather than independent replication. Evidence landscape spanning myeloid/innate, DC, lymphoid, infection/disease, and *in vivo*/temporal/longitudinal contexts **(B)**. Panel **(B)** summarizes the cellular and study contexts represented in the evidence base; it does not rank their evidential strength.

(Figure 2B) and is summarized across 25 study programs representing 37 eligible primary-human evidence units in Figure 3A. These programs can occur in different combinations, while transcriptional, chromatin, and metabolic adaptation act as cross-cutting mechanisms rather than separate directional outcomes.

Antimicrobial defense

Among the four programs, antimicrobial defense shows the clearest directional coherence. Early mechanistic work showed that $1,25(OH)_2D_3$ induces expression of human cathelicidin and

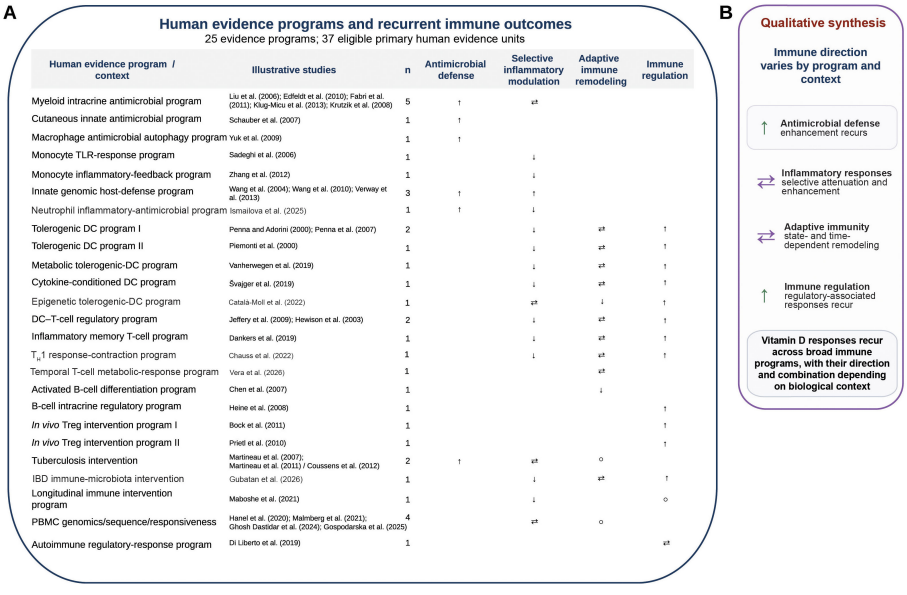


FIGURE 3
Human evidence programs and recurrent immune outcomes. Twenty-five study programs representing 37 eligible primary-human evidence units are summarized across four recurrent outcome domains: antimicrobial defense, selective inflammatory modulation, adaptive immune remodeling, and immune regulation **(A)**. Related experimental findings are grouped into study programs; links among publications, datasets and reused cohorts are documented in Supplementary Table S1. The column n denotes eligible evidence units, not publications, participants or independently recruited cohorts. Symbols summarize reported molecular, phenotypic or functional responses: ↑, predominant enhancement; ↓, predominant attenuation; ⇄, active remodeling without a single net direction; ○, the outcome was assessed but no meaningful overall direction could be assigned. Blank cells indicate that the outcome was not directly established in the mapped evidence. Regulatory-associated changes do not by themselves demonstrate suppressive function or durable tolerance. Qualitative synthesis of recurring response patterns in the included literature; the panel does not represent pooled effect estimates or a formal assessment of evidence certainty **(B)**.

DEFB4A through functional vitamin D response elements in their gene promoters (40). Across monocyte and macrophage systems, antimicrobial competence depends on pathogen sensing, local vitamin D metabolism, and cellular differentiation rather than on vitamin D exposure alone (1, 14). Different settings recruit different effectors: IFNγ can support CYP27B1-dependent antimicrobial competence, whereas IL4 promotes vitamin D catabolism (12); T cell-derived IFNγ recruits antimicrobial peptides, autophagy, and phagolysosomal maturation against *M. tuberculosis* (10); and the ligand for CD40 provides an adaptive-to-innate route that induces CYP27B1, VDR, cathelicidin, and DEFB4 and promotes restriction of intracellular mycobacteria (41).

A mechanistic link between antimicrobial peptide induction and autophagy was demonstrated in human monocytes/macrophages, where $1,25(OH)_2D_3$ induced cathelicidin-dependent expression of the autophagy-related genes *BECN1* and *ATG5* and promoted colocalization of mycobacterial phagosomes with autophagosomes (42). Both cathelicidin and autophagy were required for vitamin D-mediated antimycobacterial activity, connecting molecular responses to a directly measured host-defense function.

In wounded human skin, CYP27B1, TLR2, and cathelicidin participate in a local vitamin D-responsive defense system (23), while vitamin D-enhanced IL1β from infected macrophages can stimulate epithelial DEFB4 and contribute to antimycobacterial activity in macrophage-epithelial co-culture (24). These examples show that antimicrobial enhancement can depend on selected inflammatory signals rather than on generalized inflammatory suppression. A randomized trial in adult tuberculosis contacts provides an intervention-based functional readout: a single oral dose of vitamin D_2 improved 24 hour whole-blood restriction of growth of a luminescent Bacillus Calmette-Guérin reporter strain at 6 weeks, although the effect was not detected at 96 hours and antigen-stimulated IFNγ was unchanged (43). This does not establish prevention or treatment of clinical tuberculosis, and most mechanistic evidence remains *ex vivo* or *in vitro*.

Selective inflammatory modulation

Inflammatory responses are less uniform. Many are attenuated, but enhancement and active remodeling also occur, making selective inflammatory modulation a better description than generalized anti-inflammatory activity. In primary human monocytes, $1,25(OH)_2D_3$ can reduce TLR2/TLR4 expression and downstream nuclear factor kappa B (NFκB), p38 MAPK, extracellular signal-regulated kinase, and tumor necrosis factor (TNF) responses (20). It can also reinforce endogenous MAPK negative feedback through induction of DUSP1, attenuating LPS-driven p38 MAPK activation and IL6/TNF production (21). In dendritic and T cell systems, reduced inflammatory or T helper 1/T helper 17-associated outputs can accompany acquisition of regulatory characteristics, showing that inflammatory attenuation may form part of broader immune remodeling (17).

Regulatory responses can also involve increased activity of selected cytokine pathways. In human monocyte-derived DCs, vitamin D-induced IL6 production and JAK2-STAT3 activation accompany IL10 secretion and acquisition of $CD8^+$ T cell-suppressive activity (26). This illustrates how a cytokine pathway commonly associated with inflammation can operate within a tolerogenic program, reinforcing the need to interpret individual cytokine changes in their cellular and functional context.

Inflammatory activity can also be preserved or enhanced when it contributes to host defense. Vitamin D cooperates with nucleotide-binding oligomerization domain-containing protein 2 (NOD2) signaling to enhance antimicrobial-effector expression (44) and can increase IL1β-dependent macrophage-epithelial communication during *M. tuberculosis* infection (24). Intervention studies show a similar selectivity. In pulmonary tuberculosis, adjunctive vitamin D_3 altered the kinetics of treatment-associated inflammatory resolution without uniformly reducing all measured responses (45). In healthy adults receiving low-dose vitamin D_3 for 43 weeks, stimulated IFNγ was attenuated at selected time points while proliferation, IL10, and natural regulatory T cell (Treg) frequency were unchanged (46). Thus, inflammatory effects are better described by the specific components affected, their direction of change, and their timing than by a single inflammatory label.

Adaptive immune remodeling

Adaptive immune effects are better understood as remodeling of existing cellular states than as uniform immunosuppression. For example, $1,25(OH)_2D_3$-conditioned human monocyte-derived DCs show reduced maturation, antigen presentation, IL12 production, and T cell-stimulatory activity (2, 15). Myeloid and plasmacytoid DCs differ in their susceptibility to tolerogenic modulation (16). IFNγ can cooperate with $1,25(OH)_2D_3$ to generate DCs expressing high levels of leukocyte immunoglobulin-like receptor B4 (LILRB4) and programmed death-ligand 1 (PD-L1), with low T cell-stimulatory activity (47). These changes can shift DC function toward a more regulatory phenotype, but reduced antigen presentation or expression of regulatory markers does not by itself demonstrate durable antigen-specific tolerance. In activated $CD4^+$ T cells, $1,25(OH)_2D_3$ reduces inflammatory cytokines while increasing expression of cytotoxic T lymphocyte-associated protein 4 (CTLA4), an inhibitory receptor that restrains T cell costimulatory signaling. In the presence of IL2, vitamin D also promotes a functionally suppressive phenotype (3). Established $CCR6^+$ inflammatory memory T cells from healthy donors and early rheumatoid arthritis also reduce IL17/IFNγ and increase IL10/CTLA4 and show suppressive activity in functional assays (17).

B cells show a related dependence on activation state. $1,25(OH)_2D_3$ preferentially constrains proliferation of activated B cells and subsequent plasma cell and post-switch memory cell differentiation (19), whereas activated B cells can acquire VDR/CYP27B1 competence and increase IL10 through a VDR-dependent mechanism (18).

Host-microbiota interactions provide a further example of adaptive immune remodeling. In a prospective, uncontrolled study of 48 patients with inflammatory bowel disease (IBD) and low vitamin D status, 12 weeks of vitamin D_2 supplementation (50,000 IU/week) were associated with increased IgA-only and decreased IgG binding to gut bacteria, shifts in selected B and T cell receptor clonotypes, and enrichment of circulating $\alpha4\beta7^+$ lymphocyte populations with regulatory signatures (48). Single-cell analyses inferred increased B cell activating factor (BAFF) signaling between plasmacytoid DCs and B cells. Complementary co-cultures showed increased IgA expression and acquisition of regulatory-associated B cell phenotypes when $1,25(OH)_2D_3$ and plasmacytoid DCs were combined. These findings connect intercellular communication and host-microbiota interactions with adaptive immune remodeling, although suppressive function and durable microbiota-specific tolerance were not demonstrated.

Immune regulation

Regulatory function has direct support in some primary-cell settings. Vitamin D-conditioned human monocyte-derived DCs suppress $CD8^+$ T-cell proliferation (26), and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 (PFKFB4)-dependent glucose metabolism contributes to the capacity of human DCs to generate functionally suppressive T cells (49). These findings establish suppressive activity in the tested systems, while persistence and antigen-specific tolerance require additional evidence.

A regulatory phenotype, however, is not the same as regulatory function or durable tolerance. Supplementation studies have reported increases in circulating phenotypic Tregs (50, 51), whereas longer low-dose supplementation did not alter natural Treg frequency (46). In systemic sclerosis, $1,25(OH)_2D_3$ increased IL10 in expanded Tregs without restoring impaired suppressive function (52). Evidence based on regulatory cytokines or surface markers is not equivalent to demonstrated suppressive function or durable antigen-specific tolerance.

Cross-cutting transcriptional, chromatin, and metabolic adaptation

Transcriptional, chromatin, and metabolic changes provide the molecular basis for the immune programs described above. Genomic studies in THP-1 cells and primary human PBMCs show dynamic VDR recruitment and chromatin remodeling, substantial inter-individual variation, and recurring functional themes among partly overlapping sets of responsive genes (4, 37, 53–55). Longitudinal primary human cell profiling adds evidence that transcriptional and chromatin responses change over time (27), while exploratory data in multiple sclerosis show that the molecular response can also differ in disease context (39). Metabolic changes can contribute directly to function: in $1,25(OH)_2D_3$-conditioned human monocyte-derived DCs, PFKFB4-dependent glucose metabolism contributes to tolerogenic properties and generation of functionally suppressive T cells (49), whereas intervention metabolomics shows substantial inter-individual variation rather than a universal metabolic signature (56).

Context-dependent combinations of immune programs

Antimicrobial defense, selective inflammatory modulation, adaptive immune remodeling, and immune regulation can occur in the same biological setting. Vitamin D may enhance antimicrobial defense, attenuate selected inflammatory pathways, remodel adaptive responses, and promote regulatory features at the same time, and the direction of one component does not determine the direction of the others. Antimicrobial defense, for example, may depend on NOD2-NFκB or IL1β-dependent signaling while other inflammatory pathways are attenuated; reduced DC maturation or inflammatory T cell cytokine production can likewise coexist with increased regulatory properties. Vitamin D responses are best described by the combination of immune functions that are enhanced, reduced, or redirected, not by a single label such as immune stimulation or suppression. This program-level synthesis is shown in Figure 3B.

Why strong immune mechanisms do not guarantee clinical benefit

Strong mechanistic evidence does not necessarily translate into population-level clinical benefit. Acute respiratory infection illustrates this well. Trials have differed in baseline vitamin D status, age, population, season, dosing schedule, and outcome definition, creating both biological and methodological heterogeneity.

Vitamin D supplementation trials require consideration of background nutrient exposure. Participants in both intervention and placebo groups obtain vitamin D from diet, non-trial supplements and cutaneous synthesis; the randomized comparison therefore estimates the effect of additional supplementation rather than exposure versus complete absence of vitamin D. Trials enrolling participants who are already sufficient or near sufficient may have limited scope to detect benefits attributable specifically to correction of deficiency. Baseline vitamin D status, achieved between-group differences in 25(OH)D, adherence, background supplementation and seasonal exposure should therefore inform interpretation. These considerations are relevant to the heterogeneous trial populations included in respiratory-infection meta-analyses, but do not themselves demonstrate benefit in a particular subgroup (57–59).

Earlier meta-analyses reported small preventive effects and suggested greater benefit in profound deficiency and with daily or weekly rather than bolus dosing (57, 59). However, the 2025 update included 46 trials involving 64,086 participants, and its principal placebo-controlled comparison, comprising 40 trials and 61,589 participants, did not demonstrate a statistically significant reduction

in acute respiratory infection risk (odds ratio 0.94, 95% confidence interval 0.88-1.00; p = 0.057) (58). Separate subgroup analyses did not establish statistically significant effect modification by baseline vitamin D status, age, dosing frequency or dose size. This does not negate the antimicrobial mechanisms described above, but it shows that demonstrating a biological response and demonstrating clinical benefit are different questions. In the IBD intervention, reductions in fecal calprotectin and clinical disease activity scores accompanied the immune changes, but the absence of randomization and a placebo control prevents attribution of clinical improvement to vitamin D (48).

The 2026 Cochrane review of supplementation during pregnancy or early childhood provides a more population-specific comparison. Across 107 trials involving 31,521 participants, the outcome-specific analyses found low-certainty evidence of a small reduction in the proportion of children under five requiring respiratory-infection-related healthcare visits, while the mean number of visits per child probably changed little or not at all (60). The different populations, comparisons and endpoints limit direct comparison with the broader 2025 synthesis. These results support careful interpretation of individual clinical outcomes rather than a general claim of preventive efficacy.

The same distinction applies within the immune response itself. Changes in cytokines, regulatory cell frequency, transcriptomic signatures, or chromatin accessibility show that vitamin D has altered molecular or cellular biology, but they do not necessarily establish a change in immune function or disease outcome. In systemic sclerosis, for example, $1,25(OH)_2D_3$ increased IL10 in regulatory T cells without restoring their impaired suppressive function (52). Clinical translation depends not only on whether VDR signaling is engaged, but also on whether the resulting change affects an immune function that is relevant to the disease process and important enough to alter outcome.

Exposure and target engagement are distinct

Administered dose, achieved vitamin D status, and immune target engagement are related but distinct. The administered dose does not by itself define biological exposure: individuals can show different changes in circulating $25(OH)D_3$ after the same oral dose, and similar circulating concentrations can still be accompanied by different intracellular metabolism or VDR-dependent responses. Dosing schedule also matters, because regular daily or weekly supplementation and intermittent high-dose boluses create different temporal patterns of exposure. Earlier respiratory-infection meta-analyses suggested greater benefit with daily or weekly dosing than with bolus regimens (57, 59). However, the 2025 update did not establish significant effect modification by dosing frequency or dose size (58). Bolus regimens should therefore be distinguished in the clinical synthesis without assuming that their findings establish either universal bolus inefficacy or the superiority of regular supplementation across outcomes. Baseline deficiency remains relevant because limited substrate availability can constrain vitamin D-dependent immune responses, but it is not a sufficient predictor of molecular responsiveness or clinical benefit. The failure of baseline status subgroup effects to remain stable in the expanded respiratory infection evidence illustrates this limitation. Studies designed around an immune mechanism should distinguish the administered exposure, the achieved vitamin D status, and evidence that the relevant molecular or functional pathway was engaged. Ligand identity adds a further dimension to exposure. Vitamin D analogs can differ from $1,25(OH)_2D_3$ in their affinity for DBP and susceptibility to metabolic inactivation, thereby altering tissue exposure and the duration of VDR-dependent responses (8). Findings obtained with active vitamin D analogs therefore require interpretation in relation to their pharmacological properties and cannot automatically be extrapolated to nutritional vitamin D supplementation.

From responder classification to prospective precision immunology

Inter-individual variation creates an opportunity for more targeted vitamin D intervention, but current responder classifications are not yet robust enough for that purpose. Molecular responses vary substantially between individuals, and current responder categories depend on the biomarkers and thresholds used to define them. For a responder phenotype to become clinically useful, it would need to be defined before treatment, reproduced in independent populations, and shown prospectively to predict a relevant molecular, functional, or clinical outcome. A useful prediction model may need to combine vitamin D availability, immune cell composition and activation state, genetic or chromatin context, and early evidence of VDR target engagement. Pretreatment predictors should be distinguished from early pharmacodynamic markers used to update predictions after exposure. Clinical selection requires prospective evidence that a marker predicts differential benefit from vitamin D compared with an appropriate control. Thus, prediction of molecular responsiveness alone is insufficient.

Responder models should also distinguish between-person differences from within-person changes caused by activation state, inflammatory challenge or previous exposure. Repeated measurements in the same individuals under defined conditions are therefore needed to determine which components of responsiveness are reproducible and which change with context. The key test is whether such information improves prediction of a meaningful response beyond baseline vitamin D status alone.

Strengths and limitations of human evidence

The six contextual domains are not supported equally well by current human evidence (Figure 4). Evidence counts describe the breadth of the studies included in this narrative synthesis and should not be interpreted as formal ratings of study quality or

Contextual domain	Included datasets Distinct experimental or observational datasets	Research-group families	Primary human evidence Primary cells, tissues or participant-level data	*In vivo* / longitudinal human evidence Beyond *ex vivo* / *in vitro*	Functional evidence Experimental functional endpoints	Disease-context or external evidence Disease settings or external population/ dataset comparisons	Priority knowledge gap Research priority
Vitamin D availability & intracellular metabolism 25(OH)D levels, uptake, binding, intracellular activation	17 datasets	12 labs	n=17	n=6	n=8	n=5	*Link metabolism to immune function*
Immune-cell identity & activation state Cell-type specific responses, differentiation, activation	11 datasets	9 labs	n=11	n=1	n=10	n=1	*In vivo lineage-resolved validation*
Inflammatory & tissue context Cytokines, PRRs, immune challenges, tissue environment	23 datasets	16 labs	n=23	n=6	n=14	n=7	*Within-donor multi-stimulus studies*
Chromatin & genetic context Chromatin accessibility, epigenetic and genetic variation	8 datasets	4 labs	n=5	n=2	n=1	n=2	*Independent primary-human genomic replication*
Timing, sequence & duration of exposure Acute *vs* chronic, sequence, circadian, time-course	11 datasets	5 labs	n=7	n=3	n=2	n=1	*Direct temporal and sequence functional tests*
Interindividual & disease-related responsiveness Heterogeneity, disease states, response phenotypes	16 datasets	9 labs	n=16	n=13	n=0	n=9	*Prospective responder validation*

FIGURE 4
Coverage of included evidence and knowledge gaps across the context-dependent vitamin D framework. The image summarizes coverage of the selected evidence across the six contextual domains proposed to shape vitamin D immunoregulation. Evidence was counted at the level of distinct experimental or observational datasets; participant-cohort reuse was documented separately. Reanalyses of the same experimental measurements were linked to the original dataset and counted once within each domain and evidence dimension. Distinct experimental datasets generated from reused participants were identified separately, while shared recruitment was explicitly documented. Laboratory families were counted using the canonical assignments in Supplementary Table S1. Distinct assays in reused participants do not imply independent recruitment. Four complementary dimensions were assessed: i) primary-human evidence, requiring direct data from primary human cells, tissues or participants, including participant-level genetic, biochemical or clinical measurements, ii) *in vivo*/longitudinal human evidence, extending beyond *ex vivo* or *in vitro* experimentation, iii) functional evidence, requiring direct measurement of an immune functional endpoint, and iv) disease-context or external evidence, requiring a disease-associated population or tissue, or an explicit external population/dataset comparison relevant to the domain; a healthy-volunteer intervention alone did not qualify. External comparisons could involve previously recruited cohorts and were not necessarily independent replication. Immortalized-cell-line data did not qualify as primary-human evidence; human components of mixed-model studies were evaluated separately. Transcriptomic, epigenomic, pathway-enrichment, and molecular-marker changes were not considered functional evidence unless paired with a direct functional endpoint. Functional experiments were counted irrespective of whether the result was positive, negative, or null. Included evidence units were grouped by count: ≥10, 5-9, 2–4 and 0-1. The categories describe the number of included evidence units within each dimension. They do not constitute formal assessments of study quality, certainty, effect magnitude or direction. The key knowledge gap column identifies the evidence most needed to strengthen each contextual domain.

certainty. Vitamin D availability, inflammatory and tissue context, and inter-individual responsiveness are supported across multiple human studies, whereas chromatin and genetic mechanisms and controlled temporal designs remain less developed. One clear gap is that molecular responsiveness is often characterized in much greater detail than immune function. Transcriptomic and chromatin changes demonstrate molecular responsiveness, but attribution to VDR requires appropriate receptor-associated measurements or perturbation. Functional consequences require separate assays. The underlying evidence units, cohort reuse, and inclusion decisions are documented in Supplementary Table S1.

The evidence base has several limitations. Study selection was purposive, and no formal risk-of-bias assessment or quantitative synthesis was performed for this review. Evidence in some domains, particularly chromatin regulation and exposure timing, is concentrated in a limited number of research programs, including those of the authors, which limits the breadth of independent replication. Small donor samples, selected responder subsets, transformed cell lines, pharmacological exposures and heterogeneous endpoints limit generalization. Multiple assays or publications from the same participants add information without providing independent recruitment. Molecular changes are more extensively characterized than direct immune function, and associations between chromatin and transcription do not establish regulatory causality. The descriptive counts therefore identify coverage and gaps within the included literature; they cannot establish certainty or comparative strength across domains.

Future directions

A direct test of the framework would compare 1,25$(OH)_2D_3$ before, during and after distinct immune challenges in primary cells from multiple donors, using controls that separate treatment order from total culture time and cumulative exposure. Factorial analysis should distinguish individual stimulus effects from their interaction. Paired chromatin and transcriptional profiling should be combined with VDR and candidate AP1 transcription factor occupancy measurements, targeted perturbation and functional assays appropriate to the challenge. Washout and resting intervals would test persistence. Parallel experiments using 25$(OH)D_3$ and 1,25$(OH)_2D_3$ should test whether context dependence arises before or after ligand activation. If differences are primarily caused by CYP27B1-dependent activation, they should diminish when this step is bypassed under controlled active-ligand exposure. Differences that persist despite comparable proximal VDR target engagement would support a contribution from downstream regulatory state. Repeated donor sampling would establish whether these effects are reproducible within individuals and improve prediction beyond baseline vitamin D status. The testable predictions and corresponding study designs are summarized in Table 2.

Sterile inflammatory settings remain a question for future work. The “reverse hygiene hypothesis” highlights metabolic stress, mitochondrial damage and cellular senescence as possible sources of endogenous inflammatory signaling (22). This gives a rationale to examine how these challenges alter vitamin D responsiveness, but it does not establish that vitamin D reverses senescence-associated

TABLE 2 Knowledge gaps and research priorities for context-dependent human vitamin D immunoregulation.

Contextual domain	What is already supported	Key unresolved question	Priority study design	Prediction tested
Vitamin D availability and intracellular metabolism	Human studies show that substrate availability, transport, CYP27B1-dependent activation, CYP24A1-mediated catabolism, and cell state determine access to $1,25(OH)_2D_3$.	How strongly do differences in circulating $25(OH)D_3$, transport, and vitamin D-related genotype alter immune cell target engagement and function?	Prospective human studies that pair serum exposure with cell-specific vitamin D metabolism, VDR target engagement, and immune functional assays across baseline status and genotype.	Cellular activation of vitamin D explains variation beyond circulating substrate concentration
Immune cell identity and activation state	Lineage, differentiation, and activation alter VDR competence and the range of downstream immune responses.	Which differences are intrinsic to immune cell lineage, and which arise from activation state or tissue environment?	Matched-donor, lineage-resolved primary-human studies combining single cell or multi-omic profiling with controlled vitamin D perturbation and direct functional readouts.	Matched donor cell populations respond differently under the same exposure, with further changes following activation.
Inflammatory and tissue context	Pattern-recognition signals, cytokines, pathogens, and tissue-derived cues can shift vitamin D responses toward attenuation, enhancement, or mixed functional effects.	Can context-dependent directionality be predicted across distinct inflammatory stimuli and tissue environments?	Within-donor, head-to-head multi-stimulus experiments in primary human cells or tissues using matched vitamin D exposure and functional endpoints matched to the response being tested.	Within the same donor and cell lineage, different inflammatory challenges alter the direction, magnitude or functional consequences of the vitamin D response under matched exposure conditions.
Chromatin and genetic context	VDR binding and chromatin accessibility are dynamic, and inherited variation can modify the host background in which vitamin D signaling occurs. Primary human DC experiments connect VDR-associated DNA demethylation and JAK2-STAT3 signaling with measured suppressive function.	How generalizable are current chromatin mechanisms in primary human immune populations, and which genetic variants causally modify immune responsiveness?	Independent studies in larger groups of primary human donors using RNA-seq, ATAC-seq, and VDR-binding analyses, integrated with genotype and targeted perturbation of candidate regulatory elements.	Perturbing candidate VDR/AP1 regulatory interactions changes the co-stimulation response
Timing, sequence, and duration of exposure	Response kinetics and treatment order influence VDR-dependent transcriptional and chromatin states; evidence for circadian and seasonal modulation is more limited.	Which temporal effects are transient, which represent persistent reprogramming, and how do molecular timing effects translate to immune function?	Dense time courses with randomized treatment sequence, repeated functional measurements, and longitudinal designs that can test seasonal or circadian contributions directly.	Sequence effects remain after controlling exposure duration; a subset may persist after washout and rest
Inter-individual and disease-related responsiveness	Human studies consistently show continuous molecular heterogeneity, while current responder classifications remain biomarker- and setting-dependent.	Can pretreatment characteristics predict a reproducible molecular or functional response, and does that prediction hold across populations and disease settings?	Prospective discovery and validation cohorts that measure baseline context, early target engagement, immune function, and clinically relevant outcomes in diverse populations and disease groups.	Context-aware models predict repeated molecular or functional responses better than baseline vitamin D status alone

Research priorities were derived from the evidence framework developed in this review, considering the breadth and independence of available human evidence, mechanistic depth, temporal and regulatory resolution, functional validation, and clinical translation. Priorities identify areas in which additional evidence would most directly test or refine the proposed context-dependent model; they are not intended as rankings of biological importance. Proposed study designs address specific limitations of the existing evidence rather than providing a general catalog of desirable experimental approaches.

inflammation or persistent inflammatory memory. Pairing VDR target-engagement measurements with metabolic characterization and direct functional assays would test whether the nature and persistence of endogenous signals modify the response.

Conclusions

Biological context contributes to the mechanism of vitamin D action by determining both the signal reaching VDR and the regulatory state in which it acts. Previous exposure can modify subsequent responsiveness, while concurrent microbial signals can change the repertoire and direction of vitamin D-responsive genes. Treatment-sequence experiments in primary PBMCs (6), joint chromatin and transcriptional responses in THP-1 cells (7), and signaling-linked epigenetic remodeling with functional suppression in human DCs (26) provide complementary experimental support for this framework. The next step is to establish which regulatory interactions operate across primary human immune populations and whether they produce reproducible changes in immune function.

The same distinction is important for translation. Molecular target engagement shows that vitamin D has acted on the system and altered immune function shows that the response has biological consequences. Clinical benefit requires that the affected function is relevant to the disease process and changes it enough to influence outcome. Future studies should keep vitamin D status, molecular responsiveness, immune function, and clinical efficacy separate and connect them experimentally. The key next step is to link the VDR response observed in a defined context to a measurable immune function and, where relevant, to clinical outcome.

Author contributions

JJ: Writing – original draft, Data curation, Visualization, Conceptualization. CC: Visualization, Supervision, Conceptualization, Funding acquisition, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This publication is part of a project that has received funding from the European Union's Horizon2020 research and innovation program under grant agreement no. 952601. In addition, this work was supported by a grant from the Polish National Science Centre NCN (grant no. 2023/49/B/NZ9/00402) to CC. The funders had no role in the literature selection, interpretation of the evidence or preparation of the manuscript.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2026.2018897/full#supplementary-material

SUPPLEMENTARY TABLE S1

Evidence inventory and coding framework. The repository documents the evidence units, study-to-evidence-unit links, reuse and exclusion decisions, Figure 3 study program matrix, Figure 4 domain-specific coverage audit, and documented counting rules underlying Figure 3 and Figure 4. Evidence is counted at the level of distinct experimental or observational datasets, with participant-cohort reuse documented separately, not publications, and reused datasets are linked rather than counted as independent replication.

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Glossary

$1,25(OH)_2D_3$	1α,25-dihydroxyvitamin D_3
$25(OH)D_3$	25-hydroxyvitamin D_3
AP1	activator protein 1
ATAC-seq	assay for transposase accessible chromatin with high-throughput sequencing
ATG5	autophagy related 5
BAFF	B cell activating factor
BECN1	beclin 1
CCR6	C-C chemokine receptor type 6
CD	cluster of differentiation
CEBPD	CCAAT enhancer binding protein delta
ChIP-seq	chromatin immunoprecipitation followed by sequencing
CTLA4	cytotoxic T-lymphocyte-associated protein 4
CYP	cytochrome P450
DBP	vitamin D binding protein
DC	dendritic cell
DEFB4	defensin beta 4
DUSP1	dual-specificity phosphatase 1
FDR	false discovery rate
IBD	inflammatory bowel disease
IFNγ	interferon gamma
IL	interleukin
JAK2	Janus kinase 2
LILRB4	leukocyte immunoglobulin-like receptor B4
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
NFκB	nuclear factor kappa B
NOD2	nucleotide-binding oligomerization domain-containing protein 2
PBMC	peripheral blood mononuclear cell
PD-L1	programmed death-ligand 1
PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4
PRR	pattern-recognition receptor
PTGS2	prostaglandin-endoperoxide synthase 2
RNA-seq	RNA sequencing
RXR	retinoid X receptor
STAT3	signal transducer and activator of transcription 3
TET2	tet methylcytosine dioxygenase 2
TLR	Toll-like receptor
TNF	tumor necrosis factor
Treg	regulatory T cell
VDR	vitamin D receptor