Bimodal Influence of Vitamin D in Host Response to Systemic Candida Infection—Vitamin D Dose Matters

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Vitamin D level is linked to susceptibility to infections, but its relevance in candidemia is unknown. We aimed to investigate the in vivo sequelae of vitamin D3 supplementation in systemic Candida infection. Implicating the role of vitamin D in Candida infections, we showed that candidemic patients had significantly lower 25-OHD concentrations. Candida-infected mice treated with low-dose 1,25(OH)2D3 had reduced fungal burden and better survival relative to untreated mice. Conversely, higher 1,25(OH)2D3 doses led to poor outcomes. Mechanistically, low-dose 1,25(OH)2D3 induced proinflammatory immune responses. This was mediated through suppression of SOCS3 and induction of vitamin D receptor binding with the vitamin D–response elements in the promoter of the gene encoding interferon γ. These beneficial effects were negated with higher vitamin D3 doses. While the antiinflammatory effects of vitamin D3 are well described, we found that, conversely, lower doses conferred proinflammatory benefits in Candida infection. Our study highlights caution against extreme deviations of vitamin D levels during infections.

Keywords. 1,25(OH)2D3; cytokine; suppressor of cytokine signaling (SOCS); interferon gamma.
with downregulation of interleukin 6, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) [11]. However, the biological relevance of these effects was not known. Hence, we aim to investigate and validate the sequelae of the immunomodulatory properties of vitamin D₃ in vivo.

**METHODS**

**Stimuli and Reagents**

*Candida albicans* cells (SC5314) were grown overnight at 30°C in yeast extract–peptone–dextrose broth. Cells were washed twice in phosphate-buffered saline (PBS) and diluted to 5 × 10⁶ microorganisms/mL. Heat-killed *C. albicans* blastoconidia were used at a concentration of 1 × 10⁶ blastoconidia/mL. 1,25(OH)₂D₃ was purchased from Fluka Biochemika, Sigma-Aldrich (St. Louis, Missouri), and dissolved in absolute ethanol. The pharmaceutical preparation of 1,25(OH)₂D₃, Calcijex (1 µg/mL ampoule), was from Abbott Laboratories (Quebec, Canada).

**Mice**

Eight weeks old Balb/c mice weighing 20–24 g were infected with 2.5 × 10⁵ live *C. albicans* (SC5314) via tail vein injection. One hundred microliters of vitamin D₃ (Calcijex) was administered intraperitoneally from day 2 after infection at doses of 0.001 µg/kg, 0.01 µg/kg, 0.1 µg/kg, or 1 µg/kg for 3 days; 3-day administration of PBS served as a control. Weight and survival were charted daily, and the fungal burden in the kidneys was assessed 6 days after infection. The number of viable *Candida* organisms was determined by plating serial dilutions on Sabouraud dextrose agar plates, and the colony forming units (CFUs) were counted after incubation for 24 hours at 37°C. For the survival studies, following *Candida* infection (on day 0), the mice were treated from day 2 onward with the stipulated daily doses of Calcijex for either 3 days or continuously.

**PBMC Isolation**

Separation and stimulation of PBMCs from healthy volunteers were performed as previously described [12]. Blood specimens were collected into sodium heparin tubes (BD Vacutainer, Franklin Lakes, New Jersey) after informed consent was received. PBMCs were isolated by density centrifugation on Ficoll-Hypaque (GE Healthcare, Uppsala, Sweden). Cells were washed, counted, and adjusted to a concentration of 5 × 10⁶ cells/mL in culture medium (Roswell Park Memorial Institute 1640 medium, Dutch modification, supplemented with gentamicin, l-glutamine, and sodium pyruvate). Stimulation assays were performed in 96-well round-bottomed plates, using 100 µL of PBMCs with *C. albicans* and the respective fractions to achieve a total volume of 200 µL/well. After incubation for 24 hours or 48 hours in a humidified atmosphere (5% CO₂) at 37°C, the supernatants were collected and stored at −20°C until further assay.

**Vitamin D Level Measurement**

Vitamin D levels were measured using deidentified serum specimens from patients with *Candida* BSI in our hospital against anonymized serum specimens from hospitalized and healthy subjects archived in our hospital’s clinical laboratory. The Roche Elecsys vitamin D commercial assay was used to determine 25-hydroxyvitamin D levels in human serum, and the assay revealed that >95% of the vitamin D (25-OHD) measured in serum was vitamin D₃ [13]. This electrochemiluminescence-based immunoassay was read on a Cobas e411 analyzer.

**Recruitment and Phagocytosis Assay**

Neutrophil and macrophage recruitment were assessed following intraperitoneal injection of mice with 1 × 10⁶ inactivated *Candida* microorganisms/mL at 4 hours and 72 hours, respectively. The peritoneal cells were extracted by injecting 4 mL of ice-cold PBS-heparin into the peritoneal cavity. After washing, cytospin of the sample extract, followed by enumeration of the cell types, was performed under microscopy. To ascertain phagocytosis and killing, peritoneal phagocytes as obtained were washed and counted in a counting chamber. The processes of phagocytosis and intracellular killing were studied in an adherent monolayer of phagocytes [14].

**Histology**

The mouse kidneys were studied by light microscopy with hematoxylin and eosin and Gomori methenamine silver staining after being fixed in 4% formalin, processed and embedded in paraffin, and sectioned at thicknesses of 3 µm. Sections were assessed via light microscopy for the presence of *Candida* and the locations in the kidney where the organisms were present (in the glomeruli, cortex, medulla, and pélvicalyceal regions).

**Stimulation and Cytokine Measurement**

Spleen cells (5 × 10⁶ cells/mL) obtained from mice on day 6 of infection were stimulated with heat-killed *C. albicans* (1 × 10⁶ organisms/mL) in 96-well microtiter plates at 37°C. After 24 or 48 hours, supernatant was collected, and cytokine measurements were made using an enzyme-linked immunosorbent assay according to the instructions of the manufacturer (eBioscience, San Diego, California). Detection limits were 20 pg/mL (for interleukin 1β [IL-1β], TNF-α, and IFN-γ) and 10 pg/mL (for interleukin 17 [IL-17] and IL-10).

**RNA Isolation and Quantitative Polymerase Chain Reaction (PCR) Analysis**

Splenocytes were harvested from mice pretreated with 3 days of vitamin D₃ (or PBS as control) while human PBMCs were preincubated with vitamin D₃ (or PBS as control) for 30 minutes. The effects of both low-dose (0.01 µg/mL) and high-dose (1 µg/mL) 1,25(OH)₂D₃ were studied. The cells were then stimulated with heat-inactivated *C. albicans* for 4 hours. RNA was extracted from the splenocytes and PBMCs, using 500 µL of
TRIzol reagent (Sigma, St. Louis, Missouri). Subsequently, 200 μL of chloroform and 500 μL of 2-propanol were used to separate the RNA from DNA and proteins. After washing with 75% ethanol, the dry RNA was dissolved in 50 μL of diethylpyrocarbonate water. To obtain complementary DNA, we reverse transcribed 1 μg of DNase-treated total RNA with oligo(dT) primers (0.01 μg/mL) in a reverse transcription PCR mixture with a total volume of 20 μL. Quantitative PCR was performed using the ABI Prism 7000 Thermocycler and SYBR Green. The primers we used are listed in the Supplementary Materials. Quantification of the PCR signals for each sample was performed by comparing the cycle threshold (Ct) values, in duplicate, for the gene of interest with the Ct values for B2M as a housekeeping gene. Mean relative messenger RNA expression was calculated using the comparative Ct method. Values are expressed as a ratio of fold increase to mRNA levels of unprimed cells.

Chromatin Immunoprecipitation (CHIP)
To investigate vitamin D receptor (VDR) binding to the IFN-γ promoter, PBMCs from healthy volunteers were or were not treated with vitamin D3 for 30 minutes, followed by stimulation with C. albicans for 4 hours. Nuclear proteins were cross-linked to DNA with 1% formaldehyde and incubated at room temperature for 10 minutes on a rocking platform. Cross-linking was stopped by adding glycine to a final concentration of 0.2 M for 5 minutes. The cells were collected by centrifugation and washed twice with ice-cold PBS. The cell pellets were resuspended in 500 μL of sodium dodecyl sulfate lysis buffer, and the lysates were sonicated to yield DNA fragments of 200–500 bp. Cell debris was removed by centrifugation. Five percent of the sonicated chromatin was set aside for input control and the rest was divided equally into 2 tubes, one for anti-VDR antibody and the other for the control immunoglobulin G (IgG). The lysates were diluted 1:10 in ChIP dilution buffer. One microgram of anti-VDR antibody (catalog no. sc-1008; Santa Cruz, Dallas, Texas) was added to one of the tubes, and 1 μg of nonspecific IgG (catalog no. sc-2027; Santa Cruz) was added to the other, and the tubes were incubated overnight at 4°C on a rotating platform. The immunocomplexes were collected using Recombinant Protein A-Sepharose beads (Invitrogen, Frederick, Maryland). The beads were washed sequentially for 10 minutes at 4°C with rotation, using low-salt buffer, high-salt buffer, and LiCl wash buffer. The beads were finally washed twice with TE buffer, and the immune complexes were eluted using 300 μL of elution buffer at room temperature for 20 minutes with rotation. The immunocomplexes and the input samples were reverse cross-linked at 65°C overnight in the presence of proteinase K (Invitrogen). DNA was extracted with phenol chloroform extraction and ethanol precipitation.

Real-time PCR reactions were performed using Sybr Green qPCR master mix (Applied Biosystems, Warrington, United Kingdom) with genomic primers for the BED promoter regions [15] of the gene encoding IFN-γ (Supplementary Materials), using the ABI Prism 7000 apparatus. The results were normalized with respect to the input. Nonspecific IgG results were subtracted using the formula $2^{-\Delta C_{T}^*100 (VDR \text{ ab})} - 2^{-\Delta C_{T}^*100 (IgG)}$, where $\Delta C_{T}$ is calculated as $C_{T} (\text{ChIP DNA}) - C_{T} (\text{Input})$, and $C_{T}$ is the cycle number.

Flow Cytometry
Splenocytes were harvested and washed twice with 1 × PBS supplemented with 0.2% (w/v) bovine serum albumin, stained with CD4-APC/CD25-PE fluorochromes, and fixed and permeabilized using the FoxP3 staining kit (catalog no. 320018; Biolegend, San Diego, California). The cells were then intracellularly stained using Foxp3-Alexa Fluor 488 or isotype control and were analyzed on the BD LSRII Flow Cytometer (BD Biosciences, San Jose, California). Data analysis was performed using FlowJo software (version 7.6.5; Tree Star, Ashland, Oregon).

Statistics
Results were pooled from at least 3 sets of experiments (unless otherwise stated) and are presented as means ± standard error of the mean (SEM). Statistical analysis was performed using the Mann–Whitney U test or the paired Wilcoxon signed rank test, when relevant. $P$ values of <.05 were considered statistically significant.

Ethics Statement
Institutional review board approval (from the Domain Specific Review Boards, National Healthcare Group, Singapore) was obtained to perform the studies involving human subjects. Ethics approval for conduct of the animal study had been attained through the Biological Resource Centre Institutional Animal Care and Use Committee (Singapore).

**Figure 1.** Sera 25-hydroxyvitamin D levels in candidemic patients, hospitalized patients without candidemia, and healthy subjects. *$P < .05$ for comparisons between the indicated groups.
RESULTS

Candidemia Patients Have Lower Vitamin D Levels

To underline the role of vitamin D in candidemia in the clinical setting, we measured 25-OHD levels in sera obtained from 28 hospitalized patients with Candida BSI and compared them to levels in anonymized sera from 75 hospitalized patients in our hospital. As shown in Figure 1, patients with Candida BSI had significantly lower 25-OHD levels (mean ±SEM, 12.31 ± 1.69 ng/mL vs 17.04 ± 1.28 ng/mL; P = .045). The mean 25-OHD level (±SEM) measured in a cohort of 30 healthy volunteers was 20.88 ± 1.51 ng/mL, and this was significantly higher than in candidemic patients (P < .01) but was not significantly different from values for the anonymized hospitalized patients.

1,25(OH)2D3 Reduces Candida Fungal Burden but Not at High Dose

In the following experiments, we assessed whether vitamin D might influence susceptibility to Candida infection in vivo. Over a treatment range of 0.001–1 µg/kg, Candida-infected mice that received 0.01 or 0.1 µg/kg of Calcijex had significantly decreased kidney fungal burden, compared with untreated mice (Figure 2A). The weight loss trends mirrored that of fungal burden in the lower-dose vitamin D3–treated mice (Figure 2B). On the other hand, mice that received high dose of Calcijex (1 µg/kg) had increased Candida growth. Findings in histological sections of the mouse kidneys corresponded to the CFUs obtained through cultures. There were fewer germinating yeast in mice treated with 0.01 or 0.1 µg/kg of Calcijex (Figure 2C). In terms of overall survival (Figure 2D), Candida-infected
mice that received 3 daily doses of 1,25(OH)2D₃ had increased survival, compared with untreated mice ($P = .006$). The administration of daily doses of vitamin D3 (0.01 µg/kg of Calcijex intraperitoneally) did not confer an additional survival benefit, compared with the 3-day Calcijex regimen described above at the same dose ($P = .5$). Not unexpectedly, mice given a high dose of Calcijex (1 µg/kg) showed poor survival, consistent with the increased fungal burden observed.

**Recruitment Capability and Phagocytic Function**

To investigate whether vitamin D₃ might influence the influx kinetics of inflammatory cells and phagocyte function, cells were harvested from the peritoneal cavity 3 and 72 hours after intraperitoneal injection with *Candida* in control mice and 1,25(OH)₂D₃-treated mice. There was no significant difference in the recruitment capacity of neutrophils, monocytes, lymphocytes, and macrophages in mice treated with low-dose

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**Figure 3.** A, Recruitment of neutrophils, lymphocytes, monocytes (at 3 hours), and macrophages (at 24 hours) to the peritoneal cavity following intraperitoneal injection of *Candida albicans* in mice pretreated with 0.01 µg/kg Calcijex for 2 days and in control mice, as measured by cytopin assay. B, Phagocytosis and killing capacity of extracted neutrophils (at 3 hours) and macrophages (at 72 hours) from Calcijex-treated and control (untreated) mice. Data are pooled from 2 sets of experiments. *$P < .05$, compared with the untreated group.
Calcijex (Figure 3A). In the 1,25(OH)2D3-treated group, there was a trend toward increased phagocytosis of Candida, but overall, there was no significant enhancement of neutrophil function. A marginal but significant increase in the killing of Candida was observed in macrophages extracted from mice treated with 1,25(OH)2D3 (Figure 3B). However, the enhanced anti-Candida capabilities observed were modest and less likely to be of relevant physiological significance.

**Vitamin D3 Dose-Related Cytokine Response to Candida**

Using splenocytes from infected control mice and mice primed with 3 daily doses of 1,25(OH)2D3, we studied cytokine induction by C. albicans. The splenocytes of mice that received the lower Calcijex doses of 0.01 and 0.1 µg/kg showed a significantly elevated IFN-γ response (Figure 4B). At the higher dose of 1 µg/kg, levels of cytokines were largely suppressed. IL-17 levels showed a dose-related trend similar to that of IFN-γ and a maximal response with 0.01 µg/kg Calcijex (Figure 4C). The induction of TNF-α and IL-10 by low-dose 1,25(OH)2D3 was limited. Evaluation of whether vitamin D3 has an influence on regulatory T cells revealed no significant changes in expression of CD4+CD25+FoxP3+ cells in splenocytes of 1,25(OH)2D3-treated mice (Figure 4E).

**Modulation of STAT and SOCS mRNA Transcription by Vitamin D3**

In view of the unanticipated proinflammatory response induced by 1,25(OH)2D3 during Candida infection, we studied the
possible mechanisms through which this response may be mediated. In particular, the STAT and SOCS pathways are known to be central mediators of the host type 1 T helper cell (Th1) response. Vitamin D3 exerted a differential dose-related effect on SOCS3 transcription: only low-dose vitamin D3 inhibited SOCS3 mRNA transcription. Correspondingly, STAT-3 mRNA expression was enhanced (Figure 5A). On the other hand, there was loss of effect of vitamin D3 at higher doses. There was also no influence on the STAT1 and SOCS1 pathway (Figure 5B). These effects were seen in both the human PBMC system and in mouse splenocytes (Figure 5C and 5D).

**VDR Binding to the Promoter Region of the Gene Encoding IFN-γ**

The accentuated proinflammatory response, particularly the IFN-γ response, induced by vitamin D3 was not mediated to a significant extent by the STAT1/SOCS1 pathway. Binding and activation of the VDR could induce its interaction with specific vitamin D response elements present in the BED promoter of the gene encoding IFN-γ [15]. Using ChIP-PCR, we showed that low-dose 1,25(OH)2D3 induced increased VDR binding to the IFN-γ BED promoter during *Candida* infection in PBMCs (Figure 6A). In contrast, high-dose vitamin D3 led to reduced binding. Cells treated with low-dose 1,25(OH)2D3 had increased IFN-γ production downstream, validating the sequelae of the mechanistic modulation that we had elucidated above (Figure 6B).

**DISCUSSION**

In the present study, we showed that vitamin D3 exerts a bimodal effect on host immune response against *Candida*. At low doses, vitamin D3 conferred resistance against candidemia. On the other hand, supplementation of the host with high-dose vitamin D3 could be detrimental. The beneficial effects observed with low-dose vitamin D3 were mediated through the enhancement of the host immune response. This was through potentiation of the proinflammatory cytokine response, among which IFN-γ, TNF-α, and IL-17 are known to have important roles in antifungal host defense [16, 17]. Mechanistically, low doses of vitamin D3 suppressed SOCS3 at the level of mRNA transcription and correspondingly increased STAT3 activity. Furthermore, we showed that vitamin D3 also exerted an effect upstream through increased binding to the IFN-γ promoter BED to induce IFN-γ production. The vitamin D nuclear receptor was involved in mediating these responses. These findings are both novel and unanticipated. The reported observations to date generally describe the propensity of
vitamin D3 to skew the immune response away from a Th1 type response toward interleukin 4- and IL-10–driven Th2 responses [3, 18]. This has been attributed to the capacity of vitamin D3 to inhibit T-cell proliferation through suppression of interleukin 2 through the NFATp/AP-1 complex [19, 20]. Similarly, vitamin D3 inhibits IL-12 in dendritic cells [21]. This results in suppression of not just Th1, but also Th17 inflammatory responses. We and others have attributed this partly to vitamin D3’s capacity to downregulate transcription and expression of surface pattern-recognition receptors [11, 22]. However, many of those in vitro experiments, including our own earlier studies on the immunomodulatory effects of vitamin D3, had used higher doses of vitamin D3 that, in retrospect, might be supra-physiological in vivo [23].

On reassessment of our published data in Candida-infected human PBMCs, we observed a trend toward increased IFN-γ production with low-dose 1,25(OH)2D3 and cytokine suppression at higher doses [11]. As such, our current in vivo and ex vivo results were largely consistent with our earlier observation. Besides the bimodal cytokine trends elicited in the Candida-stimulated splenocytes, more important was our ability to demonstrate the corresponding sequelae of such immunomodulation by vitamin D3 in the host, as evidenced by the fungal burden trends (both microbiologically and histologically). While the recruitment of inflammatory cells during infection and phagocytic function did not seem significantly altered, there was a marginal increase in intracellular Candida-killing capacity. We showed, in turn, that low-dose vitamin D3 enhanced host inflammatory response most clearly through IFN-γ in the anti-Candida Th1 effector arm and likely through induction of Th17 responses. This was mediated through the suppression of SOCS3 transcription. A resultant increase in IFN-γ could be accounted for by a reported capacity of SOCS3 to inhibit Th1 differentiation [24]. The corresponding upregulation of STAT3 expression is seen with the trend toward increased production of IL-17, as seen in our study.

The VDR played a central role in mediating these responses. As a member of the nuclear receptor superfamily, the VDR can be activated by low concentrations of 1,25(OH)2D3 [25]. The VDR is a nuclear receptor that can act as a transcription factor to bind to specific vitamin D response elements within the regulatory regions of specific primary target genes [26]. To further account for the increased IFN-γ immunogenicity induced by vitamin D3, we demonstrated that low-dose vitamin D3 exerted a more profound effect on IFN-γ production upstream. We showed that 1,25(OH)2D3 induced the increased binding of VDR to the BED promoter region of the gene encoding IFN-γ and that this led to increased production of IFN-γ.

This observation of a bimodal effect suggests the differential susceptibility to modulation of the various immune targets and pathways by 1,25(OH)2D3. SOCS3 transcription, for instance, could be suppressed by low doses of 1,25(OH)2D3. However, it is only with higher doses that we saw general attenuation or even inactivation of other target genes of interest. This reasonably accounts for the differences between results of our study and those of other studies performed earlier. Most of such earlier studies on the immunomodulatory effects of 1,25(OH)2D3 had been derived from in vitro studies using higher doses of 1,25(OH)2D3 that might be supraphysiological. Thus, while the consensus remained in that the effects of high concentrations of 1,25(OH)2D3 are largely antiinflammatory [27], the intrinsic higher susceptibility of some immune targets (eg, SOCS3) to low levels of vitamin D3 and the increased 1,25(OH)2D3-induced VDR binding to the IFN-γ BED promoter warrant highlighting.

The above observations are very relevant in the clinical context. Vitamin D3 deficiency has been well described to be associated with various disease states, especially susceptibility to...
infections [28–31], as we have also demonstrated here in the context of candidemia. However, epidemiological data studying the association between vitamin D levels, supplementation, and specific infectious diseases, such as tuberculosis and viral infections, have been inconclusive [32–38]. This may be attributable to the differences in the supplemental doses used, as our findings suggest that the immunomodulatory effect of vitamin D₃ is dose dependent. Nonetheless, the bimodal effect as demonstrated in our animal studies raises the questions about what constitutes a low dose of vitamin D₃ (with its potentially beneficial effects of supplementation) and what constitutes a higher dose of vitamin D₃ (with its perhaps excessively immunosuppressive effects) in the human host. Indeed, while a higher vitamin D₃ level with its anticipated immunosuppressive influence may be a requisite against inflammatory conditions such as multiple sclerosis or inflammatory diseases, immunomodulation at such levels may not be appropriate in the context of infections. It remains to be determined as to what ought to be the optimal physiological level of vitamin D₃ in the body, although a recent recommendation had been to increase circulating 25(OH)D₃ concentration to >75 nmol/L [39]. In light of this study, however, a more prudent approach and more-specific in vivo studies need to be conducted to answer this question.

In conclusion, we have demonstrated that patients with candidemia have lower levels of vitamin D. In turn, limited supplementation of vitamin D augments host immunity and enhances resistance against C. albicans infection. On the other hand, excessive high-dose vitamin D suppresses the host immune response. On the background of the findings of this study and the continued debate over recommendations for vitamin D supplementation [40] against morbidity and mortality risk reduction, the balance between vitamin D supplementation and potential caution against hypervitaminosis D needs to be highlighted.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**

5. Daniel C, Sartory NA, Zahn N, Radeke HH, Stein JM. Immune modulatory treatment of trinitrobenzenic sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 and regulatory T cell profile. J Pharmacol Exp Ther 2008; 324:23–33.