ACS Infectious Diseases

pubs.acs.org/journal/aidcbc

Review

Vitamin D and Streptococci: The Interface of Nutrition, Host Immune Response, and Antimicrobial Activity in Response to Infection

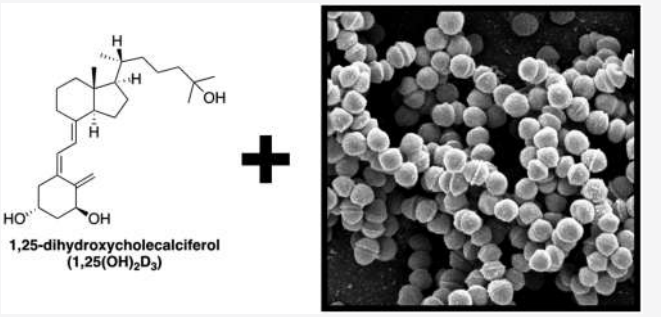
Miriam A. Guevara, Jacky Lu, Rebecca E. Moore, Schuyler A. Chambers, Alison J. Eastman, Jamisha D. Francis, Kristen N. Noble, Ryan S. Doster, Kevin G. Osteen, Steven M. Damo, Shannon D. Manning, David M. Aronoff, Natasha B. Halasa, Steven D. Townsend,* and Jennifer A. Gaddy*

Cite This: https://dx.doi.org/10.1021/acsinfecdis.0c00666

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: *Streptococcus* species are common causes of human infection. These Gram-positive, encapsulated bacterial pathogens infect diverse anatomic spaces, leading to infections including skin and soft tissue infection, endocarditis, pneumonia, meningitis, sinusitis, otitis media, chorioamnionitis, sepsis, and even death. Risk for streptococcal infection is highest in low- and middle-income countries where micronutrient deficiency is common. Epidemiological data reveal that vitamin D deficiency is associated with enhanced risk of streptococcal infection and cognate disease outcomes. Additionally, vitamin D improves antibacterial defenses by stimulating innate immune processes such as phagocytosis and enhancing production of reactive oxygen species (oxidative burst) and antimicrobial peptides (including cathelicidin and lactoferrin), which are important for efficient killing of bacteria. This review presents the most recent published work that studies interactions between the micronutrient vitamin D, the host immune system, and pathogenic streptococci as well as comparisons with other relevant infection models.



KEYWORDS: *Streptococcus, pathogenic bacteria, vitamin D, innate immunity, micronutrient deficiency, epidemiology, calciferol, 1,25(OH)2D*

Downloaded via SAN FRANCISCO STATE UNIV on November 11, 2020 at 12:51:34 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

INTRODUCTION

Vitamin D is a secosteroid, a subclass of steroids with a broken ring structure, which regulates calcium and phosphorus homeostasis in a variety of organisms and maintains the skeleton in vertebrates.[1] Extraskeletal functions of vitamin D have become apparent as studies have implicated vitamin D in cellular proliferation, differentiation, and immune system regulation.[1] Studies of vitamin D deficiency and supplementation revealed its protective role against hypertension, infections, autoimmune diseases, cardiovascular diseases, diabetes, and some cancers in addition to its antirachitic activities.[1]

The genus *Streptococcus* contains both commensal bacteria that are part of the human microbiota in nearly every part of the human body and also several important human pathogens. Individual species in this genus are capable of causing invasive infection at every stage of human life from the perinatal period to advanced age. Among different age cohorts, streptococcal species cause diverse infections from skin and soft tissue infections to meningitis and endocarditis.

Vitamin D deficiency is quite common, and prior studies have suggested an association between the frequency and severity of streptococcal infections in individuals that are deficient in vitamin D.[1,2] In this review, we will comprehensively cover vitamin D physiology with a focus on how vitamin D's immunomodulatory activity impacts the pathogenesis of streptococcal infections and how vitamin D supplementation may play an important role to improve perinatal health outcomes as a common manifestation of streptococcal infection.

STREPTOCOCCUS

The *Streptococcus* genus is a diverse group of encapsulated catalase negative, Gram-positive bacteria which exhibit a round, or cocci, chained or diplo-arranged morphology. The genus is further divided into 49 species, many of which are

Received: September 22, 2020

© XXXX American Chemical Society

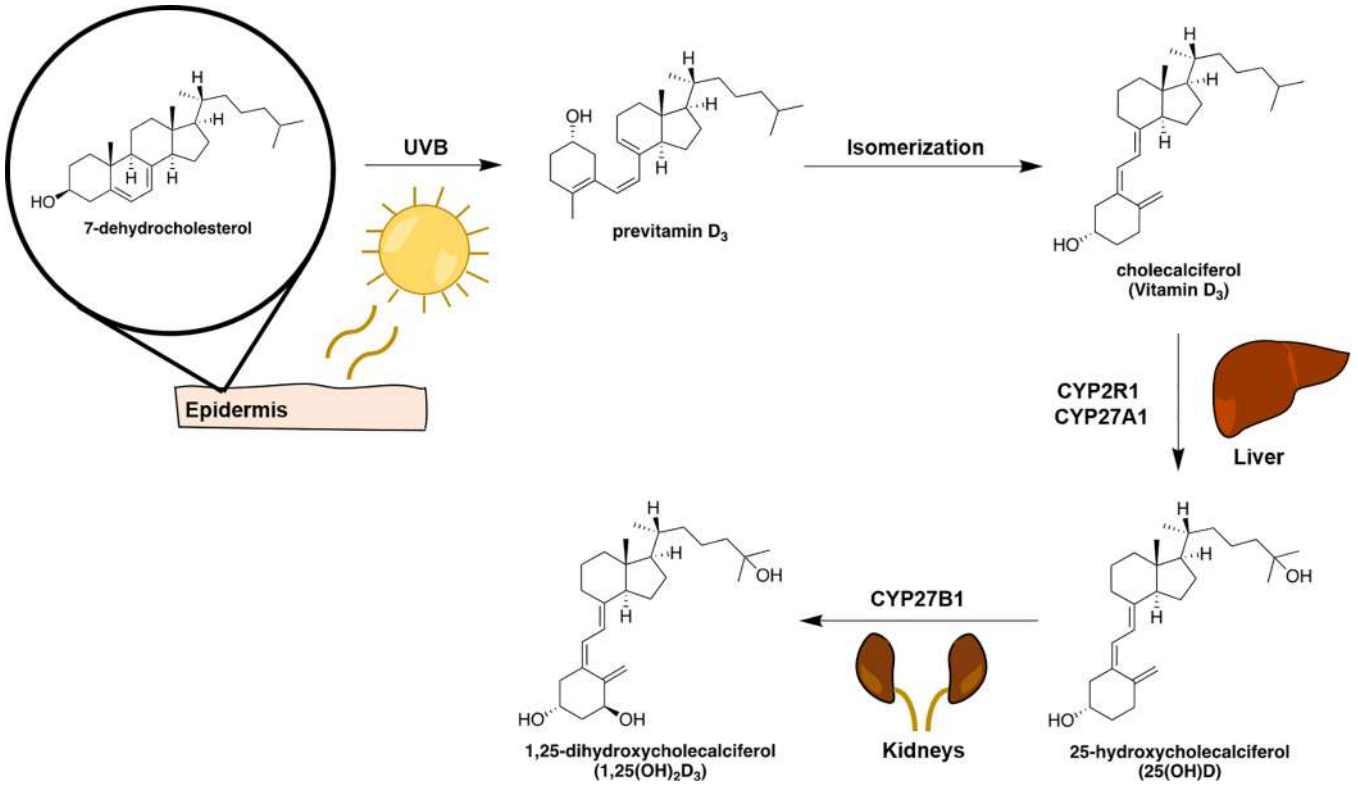


Figure 1. Diagram of vitamin D biosynthesis. Vitamin D biosynthesis begins with the cholesterol precursor 7-dehydrocholesterol, which can be found in the epidermal layer of the skin. When the epidermis is exposed to ultraviolet light B (UVB) irradiation, 7-dehydrocholesterol structurally undergoes a photochemical ring opening to yield the previtamin D_3 intermediate. In the lower layers of the skin, previtamin D_3 then undergoes thermal isomerization to generate vitamin D_3 (cholecalciferol). Vitamin D_3 is then transported throughout the body via binding to vitamin D binding proteins (DBP). Subsequently, 25-hydroxylase enzymes in the liver, specifically CYP2R1 and CYP27A1, catalyze the oxidation of vitamin D_3 to 25-hydroxycholecalciferol (25(OH)D). This 25(OH)D isoform is the primary storage form of vitamin D and is oxidized by CYP27B1 to yield the active hormone form of vitamin D (1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$)) in the kidneys.

found as part of the normal human microbiota colonizing the respiratory, gastrointestinal, and genitourinary tracts. Thirty-five of these species have been associated with diverse infections in humans ranging from limited skin/soft tissue infections to invasive infections such as endocarditis and meningitis.[3,4] Four of these species cause the majority of human infections: Group A *Streptococcus* (including *Streptococcus pyogenes*), Group B *Streptococcus* (including *Streptococcus agalactiae*), oral streptococci (including *Streptococcus mutans*), and *Streptococcus pneumoniae* (commonly called pneumococcus).

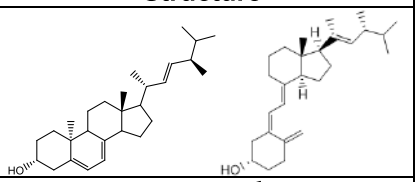
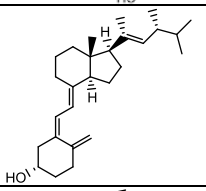
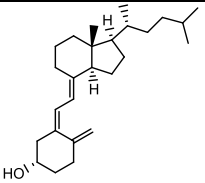
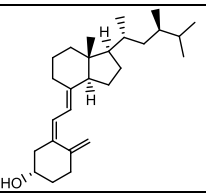
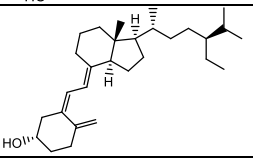
Group A Streptococcus. Group A *Streptococcus* (GAS) causes human disease ranging from mild infection such as pharyngitis and impetigo to life threatening infections including necrotizing fasciitis and streptococcal toxic shock syndrome, which causes widespread systemic vasodilation and/or hypoperfusion of vital organs. Globally, GAS causes disease in up to 7 million people annually, with most cases (6 million) presenting as pharyngitis, commonly referred to as "strep throat."[5,6] Serious immune sequelae may be triggered after repeated GAS infections, including acute glomerulonephritis and rheumatic heart disease, leading to a significant number of deaths attributed to GAS.[5] A report by the World Health Organization estimated that GAS is the ninth leading infectious cause of human mortality.[6]

Group B Streptococcus. *Streptococcus agalactiae* or Group B *Streptococcus* (GBS) is a common cause of perinatal infections but also infects elderly patients or those with underlying conditions such as diabetes.[7−9] GBS is an especially large health burden to mothers and neonates in developing countries, with an estimated 21.7 million pregnant mothers colonized with GBS in 2015;[10] GBS vaginal colonization increases the incidence of perinatal infections including chorioamnionitis, funisitis, neonatal sepsis, mastitis, and increases risk for infection-related preterm birth. GBS infection predominately manifests as soft tissue infections, although other complications may arise. An epidemiological study in the USA from 2008 to 2016 found increasing incidence of invasive GBS infection in nonpregnant patients, and most patients had at least one underlying condition with obesity, diabetes, and older age being common.[11]

Oral Streptococci (Including *Streptococcus mutans*). Streptococci are the dominant species in the oral cavity and upper respiratory tract, and many of the oral streptococcal species have been historically classified as "viridans streptococci" because many display only partial hemolysis when cultured on blood agar.[12] This group was further separated based on biochemical properties and now phylogenetic approaches. Despite being part of the normal commensal microbiota, oral streptococci can cause human disease ranging from dental caries to invasive diseases such as bacteremia or endocarditis.[12] *S. mutans*, a common oral *Streptococcus* species, is best known for causing human dental caries.[13] *S. mutans* adapted to be a commensal member of the oral microbiota, where this organism is a component of oral biofilms present on teeth and is the prime initiator of plaque and a potent producer

https://dx.doi.org/10.1021/acsinfecdis.0c00666
ACS Infect. Dis. XXXX, XXX, XXX−XXX

Table 1. Five Major Forms of Vitamin D and Their Uses

Name	Notes	Structure
Vitamin D_1 (1:1 mixture of lumisterol and ergocalciferol)	• Precursor of vitamin D_2 • No longer used as a vitamin D supplement	
Vitamin D_2 (ergocalciferol)	• Plant-derived or synthetically made • Less effective at raising serum levels compared to vitamin D_3	
Vitamin D_3 (cholecalciferol)	• Produced in sun-exposed skin or found in animal sources such as egg yolk, oily fish, and liver • Most effective form of vitamin D	
Vitamin D_4 (22-dihydroergocalciferol)	• Produced in mushrooms upon UV irradiation • Used to raise serum calcium levels	
Vitamin D_5 (sitocalciferol)	• Studied for its effectiveness as an antitumor agent in prostate cancer	

of acid, leading to exacerbation of tooth decay and oral disease.[14] *S. mutans* is able to colonize injured heart valves and cause endocarditis because it readily moves into the bloodstream during dental operations.[15] Dental caries are the most common and costly oral disease worldwide, with *S. mutans* as the etiological agent underlying a large proportion of this burden.[16]

Streptococcus pneumoniae. *Streptococcus pneumonia*, also referred to as pneumococcus, causes up to 4 million cases of illness within the United States and 450 000 hospitalizations per year.[17] These infections carry a high mortality rate as studies indicate that 10% of patients with invasive pneumococcal diseases succumb to their infection.[18] While *S. pneumoniae* commensally colonizes the pharynx and upper respiratory tract of healthy people, invasive pneumococcal infection may establish at a variety of sites, including the lung parenchyma and meninges.[19] Depending on the location, *S. pneumoniae* can be isolated from approximately 5−90% of the healthy human population.[20] *S. pneumoniae* has been widely linked to respiratory infections in immunocompromised and low- and middle-income populations and is the major cause of community acquired pneumonia, an important cause of bacteremia in infants and older adults.[4]

BIOSYNTHESIS OF VITAMIN D

Vitamin D is an essential nutrient that can either be biosynthesized or absorbed through dietary ingestion. Vitamin D biosynthesis begins with the cholesterol precursor 7-dehydrocholesterol, which can be found in the epidermal layer of the skin (Figure 1). When the epidermis is exposed to ultraviolet light B (UVB) irradiation, 7-dehydrocholesterol structurally undergoes a photochemical ring opening to yield the previtamin D_3 intermediate.[21] In the lower layers of the skin, previtamin D_3 undergoes thermal isomerization to generate vitamin D_3 (cholecalciferol). Vitamin D_3 traffics throughout the body via vitamin D binding proteins (DBP).[22] Subsequently, 25-hydroxylase enzymes in the liver, specifically CYP2R1 and CYP27A1, catalyze oxidation of vitamin D_3 to 25-hydroxycholecalciferol (25(OH)D). 25(OH)D is the primary storage form of vitamin D within the body.[22] In the kidneys, 25(OH)D is oxidized by CYP27B1 to yield 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$), the active form of the vitamin D hormone.[22] Catalysis to $1,25(OH)_2D_3$ is tightly controlled by calcium, phosphate, and fibroblast growth factor 23 (FGF23) in a negative-feedback control mechanism to regulate calcium homeostasis. As such, the active form of vitamin D ($1,25(OH)_2D_3$) aids in maintenance of serum calcium levels in conjunction with parathyroid hormone.[21] Additionally, $1,25(OH)_2D_3$ increases intestinal calcium absorption and promotes mineralization of the skeleton.[21]

Aside from biosynthesis, vitamin D_3 is absorbed in the gastrointestinal tract after ingestion of foods such as fatty fish (salmon, sardines, and mackerel) or egg yolks.[23] Comparatively, vitamin D_2 (ergocalciferol) is a plant-derived source of vitamin D, which is often supplemented into nutritional products and marketed as metabolically analogous to vitamin

https://dx.doi.org/10.1021/acsinfecdis.0c00666
ACS Infect. Dis. XXXX, XXX, XXX−XXX

D_3. However, research suggests that vitamin D_2 supplementation is not as effective at increasing 25(OH)D concentrations in serum, and supplementation with vitamin D_3 remains the superior dietary vitamin D source.[24]

THE FIVE MAJOR FORMS OF VITAMIN D

While vitamins D_2 and D_3 are the predominant forms of vitamin D, chemically, the molecule exists in five different forms, vitamin D_1 through D_5 (Table 1). First discovered by Windaus and Lisert in 1931, vitamin D_1 was later found to be a 1:1 mixture of two compounds, lumisterol and ergocalciferol (vitamin D_2).[25] Due to its lack of purity, vitamin D_1 is no longer recognized as a source of vitamin D supplementation. Vitamin D_2(ergocalciferol) is largely found in fungi but is also commercially fortified in foods such as cereal, bread, and milk products. Structurally, the only difference between vitamin D_2 and D_3 (cholecalciferol) is a double bond between carbons 22 and 23 and the methyl group on carbon 24 of the vitamin D_2 side chain. In contrast to vitamin D_2, which is produced in plants and fungi exposed to sunlight, vitamin D_3 is produced in the skin of humans when exposed to sunlight. A derivative of vitamin D_3, vitamin D_4 (22-dihydroergocalciferol), is found in select mushrooms after the precursor 22,23-dihydroergosterol is exposed to UV irradiation. This form of vitamin D is effective in raising serum calcium levels and known to treat hypocalcemia. Derived from 7-dehydrositosterol, vitamin D_5 (sitocalciferol) has been studied for its effect on prostate cancer.[26] In previous studies, vitamin D_3 has been successful as an antitumor agent in prostate cancer, but it also has the ability to cause hypercalcemia in patients.[26] A vitamin D_5 analogue, 1α-hydroxyvitamin D_5, has shown to have similar potency to vitamin D_3 without added side effects.[26]

VITAMIN D HAS IMMUNOMODULATORY ACTIVITY

Innate Immunity and Vitamin D. In recent years, research has shown that the active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), is not only critical for calcium and bone homeostasis, but also modulates innate immunity.[27] $1,25(OH)_2D$ represses pro-inflammatory cytokines and chemokines *in vitro* in response to inflammatory and/or infectious insults.[28−31] In addition to its anti-inflammatory actions, $1,25(OH)_2D$ stimulates phagocytosis[32−34] and enhances production of reactive oxygen species (oxidative burst)[35] and release of antimicrobial peptides (including cathelicidin and lactoferrin),[36,37] thereby aiding innate immune cells in efficient killing of invading bacteria. Interestingly, vitamin D can be used to differentiate monocyte-like THP-1 cells into macrophages.[38,39] $1,25(OH)_2D$ may play an important role in limiting bacterial infection pathology and tissue damage (such as in tuberculosis) by downregulating levels of matrix metalloproteinases (MMPs) and upregulating levels of tissue inhibitor of MMP-1 (TIMP-1).[40] As such, the ability of $1,25(OH)_2D$ to suppress MMPs and induce TIMPs may play a crucial role in GBS induction of MMPs and TIMPs in the fetal membranes, leading to weakening of the membranes.[41]

Vitamin D has a number of immunomodulatory properties against *Streptococcus* species, in both innate and adaptive immunity. In primary human neutrophils, vitamin D upregulates expression of pattern recognition receptors (PRRs) in response to *S. pneumoniae* stimulation, particularly TLR2 and NOD2.[42] In addition, vitamin D enhances neutrophil killing of *S. pneumoniae* and secretion of antimicrobial peptides HNP1−3 and LL-37 while also inducing an anti-inflammatory program whereby NFκB signaling is suppressed by inducing suppressor of cytokine signaling (SOCS) proteins.[42] The vitamin D-induced secretion of LL-37 could increase capsule release by *S. pneumoniae* and thereby enhance neutrophil-mediated killing.[43] SOC proteins are induced in an IL-4-dependent manner, thus leading to the downregulation of NF-κB and TRAF6 and to the regulation of excessive production of inflammatory cytokines.[42] This gives rise to the possibility that vitamin D can improve killing of pneumococci via neutrophils while seemingly limiting unwarranted inflammation. However, in a different study, macrophages treated with vitamin D and GAS induced antimicrobial peptide LL-37 production but exhibited a decrease in the ability of macrophages to kill bacteria.[44] This disparity may be due to upregulation of GAS virulence factors in response to LL-37 or could be due to differences in macrophage versus neutrophil biology. In stimulation of peripheral blood mononuclear cells (PBMCs) with pneumococcal antigen, Anderson et al. found that vitamin D reduced levels of the proinflammatory interferon (IFN)-γ, interleukin (IL)-17, and IL-22 while increasing IL-10 and, interestingly, IL-1β.[45] In a similar study, Hoe et al. found that vitamin D reduced proinflammatory cytokine release in response to stimulation with bacterial products including tumor necrosis factor-α, IFNγ, and IL-8 but showed that IL-1β was reduced, not induced, as was found in Anderson et al.[45,46] This discrepancy may be due to the difference in stimulation: Hoe et al. used lipopolysaccharide instead of pneumococcal whole cell antigen, as was used in Anderson et al., which may differentially activate the inflammasome necessary for IL-1β production.[45,46] Vitamin D also affects dendritic cell (DC) activation, the bridge to adaptive immunity, in response to pneumococcal antigens.[45] Similar to neutrophils,[42] vitamin D induced greater expression of TLR2 and NOD2 in DCs treated with pneumococcal peptidoglycan and antimicrobial peptides (human beta defensin-3) and upregulated IL-1β, as found in Anderson et al.[46,47]

Vitamin D ($1,25(OH)_2D_3$) decreases inflammation in a mouse model of multiple sclerosis, whereby symptoms of experimental autoimmune encephalomyelitis are significantly resolved. Li et al. show that using vitamin D_3 as a preventative therapy or post-disease treatment suppresses spinal cord expression of an endosomal toll-like receptor, TLR8, which is known to be upstream of inflammatory cytokine expression.[48] This group also found that spinal cord expression of TLR3, 4, and 7 is suppressed in the post-disease treatment group. Interestingly, expression of a common downstream TLR mediator, MyD88, is also lower in response to vitamin D_3. Therefore, vitamin D_3 treatment leads to decreased spinal cord expression of cognate TLR8-mediated inflammatory responses in monocytes such as TNF-α, IFN-γ, and IL-17 induction. The proposed mechanism for vitamin D_3 suppression is through direct effect on innate immune cells including monocytes. Accordingly, vitamin D_3 treatment of THP-1 monocyte-like cells not only significantly reduced TLR8 expression but also expression or activity of MyD88, IRF-4, IRF-7, and NF-κB in cells challenged with TLR8 ligands.[48] Importantly, these results suggest the TLR8 pathway is a target of $1,25(OH)_2D_3$ and that TLR8 may play a role in the anti-inflammatory activity of vitamin D. This is likely

https://dx.doi.org/10.1021/acsinfecdis.0c00666
ACS Infect. Dis. XXXX, XXX, XXX−XXX

important in the context of streptococcal infection because TLR8 has been implicated as a critical sensor of bacterial RNA in human monocyte cells and has been shown to facilitate host recognition of *S. pyogenes*, *S. pneumoniae*, and *S. agalactiae*.[49−51]

Adaptive Immunity and Vitamin D. While an IL-17-dominated CD4 T helper cell program (Th17) adaptive response is associated with streptococcal infection,[52] DC stimulation with vitamin D suppresses Th17 programming (also seen in Anderson et al.) in favor of inducing a T regulatory phenotype. Concerning the humoral response, individuals with asthma had serum levels of 25-hydroxyvitamin D (25[OH]D) that correlated positively with antipneumococcal antibodies, but this correlation was far weaker in nonasthmatic study participants. In some non-*Streptococcus* bacterial infections (i.e., *Mycobacterium tuberculosis*), vitamin D stimulates IFNγ expression from T cells to promote autophagy, phagosomal maturation, and antimicrobial peptide production in macrophages.[53] However, other studies also using *M. tuberculosis* are more consistent with the findings in streptococcal infections and have shown that vitamin D downregulates IFNγ from CD4 T cells along with perforin, granulysin, and granzyme-B from natural killer cells while increasing IL-10 production.[54] Vitamin D has also been shown to alter the proliferation of lymphocytes stimulated with streptococci derivatives.[55,56] Additionally, vitamin D has been shown to improve immune responses to vaccines (such as those utilized against influenza) in patients that are vitamin D-insufficient at baseline.[57] However, vitamin D status did not differ between immunoglobulin responders and hypo-responders in patients infected with *Streptococcus pneumoniae* as well as patients vaccinated against *S. pneumoniae*, *Neisseria meningitidis* type C (MenC), and/or *Haemophilus influenzae* type b (Hib).[58]

VITAMIN D HAS ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY

Vitamin D has been recognized as a potential chemotherapeutic treatment for bacterial infections for decades.[49] Within the host, vitamin D has been shown to have immunomodulatory effects that likely prevent bacterial growth and biofilm formation. However, vitamin D has also been shown to have direct antimicrobial and antibiofilm activity against several pathogens, including streptococci. *In vitro* studies have shown that vitamin D_3 exhibits strong growth inhibition of *S. pyogenes*, *Klebsiella pneumoniae*, and *Escherichia coli*.[59] Recent reports have demonstrated that vitamin D analogues induce lysis of planktonic cultures of *Streptococcus mutans* and inhibit biofilm formation, a critical process for full virulence. Saputo et al. reported that the vitamin D analogues, alfacalcidol, calcitriol, and doxercalciferol, induced lysis of *S. mutans* planktonic cells at least 2-fold greater than DMSO. Interestingly, only calcitriol and doxercalciferol inhibited biofilm formation with calcitriol preventing formation by 40-fold greater than DMSO. The same report also demonstrated that doxercalciferol sensitized bacitracin-resistant *S. mutans* to bacitracin, reducing the minimum inhibitory concentration (MIC) from 128 to 4 μg/mL.[60]

The antimicrobial activity of vitamin D is not confined to *Streptococcus* species. Early reports demonstrated that vitamin D supplementation had potent antibacterial activity against *Mycobacterium tuberculosis* infections *in vivo*.[61] Expression of vitamin D receptor and the vitamin D-1 hydroxylase genes by TLR activation leads to the induction of the antimicrobial peptide cathelicidin, which promotes intracellular *M. tuberculosis* death in human macrophages and monocytes.[36] Tubercle bacilli were shown to be reduced over days to weeks when vitamin D in both oil and propylene glycol were added topically to *M. tuberculosis* cultures. In addition, *M. tuberculosis* was unable to grow on media supplemented with vitamin D. Interestingly, cod fish oil (a prominent source of vitamin D) was used in Europe in the 1700s to treat tuberculosis, long before its bactericidal activity was known.[62]

Vitamin D has been shown to inhibit the growth and expression of virulence factors of the dental pathogen *Porphyrmonas gingivalis*.[63] The expression of genes *fimA*, *hagA*, and *hagB*, which are virulence factors involved in colonization, were reduced by 43, 48, and 21%, respectively, when 1 μg/mL vitamin D ($1,25(OH)_2D_3$) was added. In addition, the same report described an additive antimicrobial effect against *P. gingivalis* when vitamin D was used in concert with antibiotics such as tetracycline and metronidazole.

Vitamin D degradation products have been shown to exert strong bactericidal activity against the gastric pathogen *Helicobacter pylori*.[64] While vitamin D (cholecalciferol) can be metabolized by CYP2R1, CYP27A1, and CYP27B1 to produce 25(OH)D and $1,25(OH)_2D_3$, natural (nonbiologic) degradation can occur via high humidity and high temperature. 25(OH)D and $1,25(OH)_2D_3$ produced by nonbiologic degradation were shown to reduce *H. pylori* growth by 10^4- and 10^6-fold, respectively, compared to undegraded cholecalciferol treated cultures. These vitamin D degradation products were shown to act by destabilizing the membrane of *H. pylori* and ultimately inducing the loss of structural integrity, causing cell lysis. Taken together, these results indicate that vitamin D can have antibacterial activity against a wide range of bacterial pathogens, including *Streptococcus spp*.

VITAMIN D DEFICIENCY AND SUSCEPTIBILITY TO STREPTOCOCCAL INFECTION

Vitamin D deficiency is associated with increased risk of infection and supplementation alters host−pathogen interactions. Micronutrient deficiencies, such as vitamin D inadequacy, has been associated with increased risk of infection, including those caused by *Streptococcus*.[65,66] Specifically, vitamin D deficiency has been correlated with increased risk of community acquired streptococcal pneumonia and tonsillopharyngitis.[1,65] Neutrophils upregulate CYP27B1 in the presence of IFN-y thus resulting in the upregulation of alpha-defensins (HNP1−3) that mediates the killing of pneumococci.[42] A retrospective study of 54 patients with recurrent tonsillopharyngitis caused by GAS demonstrated that serum levels of vitamin D (25(OH)D) less than 20 ng/mL were associated with the disease.[67] These results further support an earlier study in which men with 25(OH)D serum levels less than 40 nmol/L were found to have more severe respiratory tract infections.[67,68] In addition, a cross-sectional study of 300 patients found that serum concentration of $1,25(OH)_2D_3$, the active form of vitamin D, was inversely correlated with severity of community associated pneumonia caused by various pathogens, including pneumococcus. However, no correlation was found between serum concentration of 25(OH)D, the storage form of vitamin D, and severity of community associated pneumonia. The results from this study suggest that the inability to convert the storage form of vitamin D to the active form, leading to low serum levels of $1,25(OH)_2D_3$, is a risk factor for streptococcal pneumonia.[65]

https://dx.doi.org/10.1021/acsinfecdis.0c00666
ACS Infect. Dis. XXXX, XXX, XXX−XXX

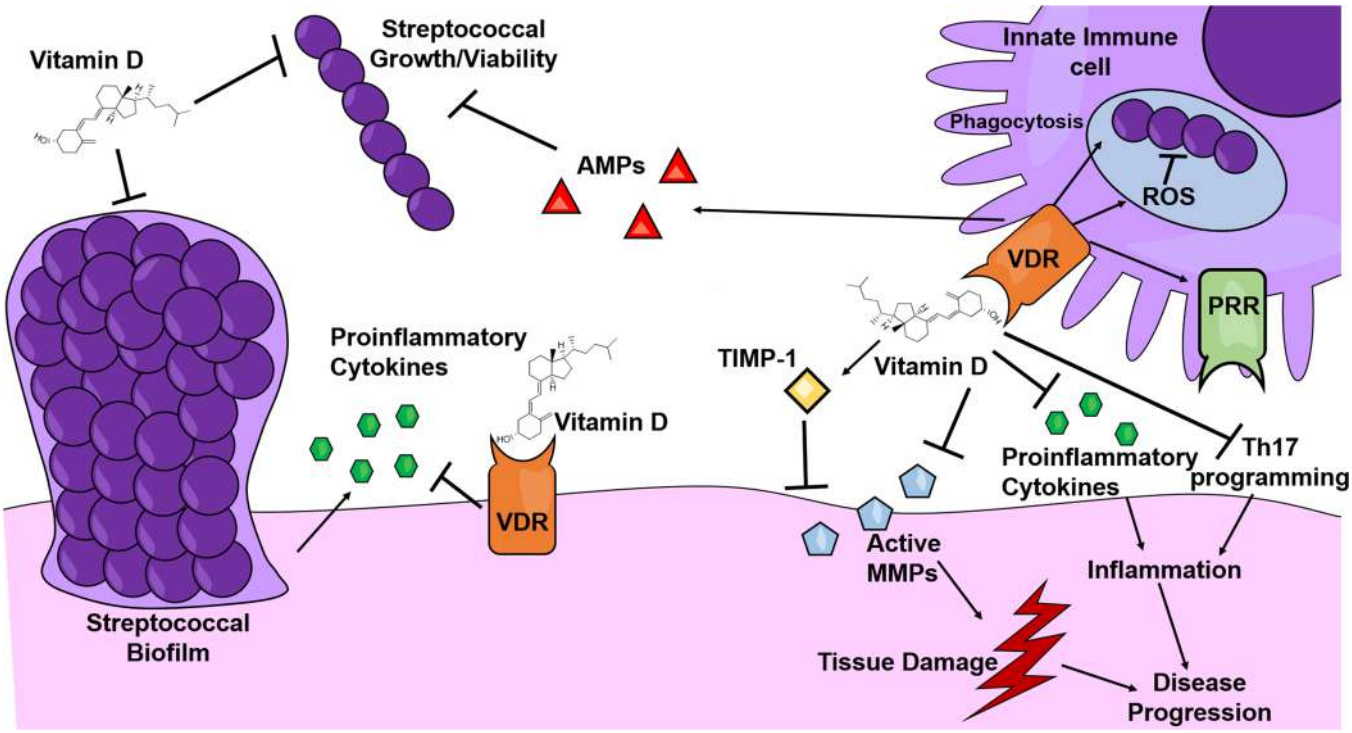


Figure 2. Conceptual model of the role of vitamin D in *Streptococcus*-host interactions. Vitamin D interacts with both host and pathogen cells. Vitamin D inhibits streptococcal growth, viability, and biofilm formation, which are important for colonization and persistence in the host niche. Vitamin D signals through the VDR and promotes innate immune cell functions including phagocytosis, production of AMPs such as lactoferrin, LL-37, and cathelicidin, as well as ROS, upregulation of PRR, and production of TIMP-1, which negatively regulates MMPs that contribute to tissue damage. Vitamin D also represses production of MMPs and proinflammatory cytokines and leads to repression of Th17 programming favoring a regulatory T cell phenotype instead. Thus, vitamin D can decrease inflammation and disease progression.

Lower vitamin D levels have been associated with decreased expression of antimicrobial peptides in the placenta and increased incidence of GBS carriage in the recto-vaginal niche.[66,69] A study of pregnant adolescents revealed that maternal recto-vaginal GBS colonization was associated with significantly lower mRNA levels of vitamin D regulatory proteins (cubulin, megalin, CYP27B1, and CYP24A1) as well as lower levels of vitamin D receptor protein abundance and placental cathelicidin mRNA compared to non-GBS colonized pregnant adolescents. It was shown that this effect of GBS colonization on cathelicidin mRNA levels was largely mediated by cubulin and CYP27B1 mRNA expression, further supporting the idea that vitamin D and its metabolites support antimicrobial peptide production.[66] In another study, vitamin D levels in cord blood at birth were used to investigate the link between vaginal carriage of GBS and maternal vitamin D levels. GBS vaginal carriage was significantly greater in women whose newborns showed deficient (<10 mg/mL) or insufficient (10−30 mg/mL) vitamin D levels at birth compared to women whose newborns showed sufficient (>30 mg/mL) vitamin D levels at birth.[70] The results from these studies demonstrate the association between vitamin D deficiency and susceptibility to infection by *Streptococcus* species.

VITAMIN D DEFICIENCY IS ASSOCIATED WITH PRETERM BIRTH

Invasive Group B *Streptococcus* infections during pregnancy are one of the major causes of adverse pregnancy outcomes such as preterm birth.[7,9,10] A variety of factors can influence vaginal colonization and invasive infection, which can lead to preterm birth. For example, epidemiological studies have frequently linked micronutrient deficiency with preterm birth. A recent meta-analysis compiling data from over 10 000 pregnant patients shows that maternal vitamin D deficiency (maternal serum 25(OH)D < 20 ng/mL) is associated with a significant increase in preterm birth with an odds ratio of 1.29.[71] Another meta-analysis reveals that vitamin D supplementation may reduce a subset of preterm deliveries.[72] Interestingly, some mothers who delivered preterm had particular vitamin D receptor (VDR) gene variants which are associated with lower serum vitamin D concentrations. Specifically, women with *Taq*I/AG, *Apa*I/AA, and *Fok*I/AG VDR genotypes had lower serum vitamin D levels and higher rates of preterm delivery. Genotypes *Bsm*I/TT and *Apa*I/AA were associated with true vitamin D deficiency, and those mothers were respectively 2.36 and 7.99 times more likely to deliver preterm.[73] Two other single nucleotide polymorphisms (*Fok*I, *Cdx2*) in the VDR have been associated with spontaneous preterm birth.[74] Of note, mothers with both of these SNPs also had higher odds ratios of chorioamnionitis and infection during pregnancy, suggesting an intersection of nutritional predisposition for infection and preterm birth. Work in animal models further delineates an association of dietary vitamin D with gestational age at delivery. Mice maintained on a reduced vitamin D diet had a 47% increase in preterm birth compared to controls that consumed regular diets with vitamin D.[75] Further studies to elucidate the mechanism(s) of vitamin D in preterm birth are warranted.

https://dx.doi.org/10.1021/acsinfecdis.0c00666
ACS Infect. Dis. XXXX, XXX, XXX−XXX

DOES VITAMIN D SUPPLEMENTATION MITIGATE THE RISK OF *STREPTOCOCCUS*-ASSOCIATED DISEASES?

Vitamin D supplementation has been shown to enhance neutrophil killing of pathogenic streptococcal bacteria while concomitantly dampening excessive inflammatory responses and apoptosis, indicating vitamin D has chemotherapeutic potential against streptococcal infections. However, most research thus far on streptococcal and vitamin D interactions focuses on *in vitro* cell work and very little *in vivo* work exists. Most studies with vitamin D supplementation focus on human diseases outside of infectious diseases, but there are few exceptions. For instance, in one study with *Candida* infection in mice, low levels of vitamin D decreased fungal burden and improved survival outcomes.[76]

In an *ex vivo* study with bladder tissue from postmenopausal women, a group found that vitamin D enhanced production of cathelicidin, an antimicrobial peptide, when the tissues were infected with uropathogenic *E. coli*.[77] Using colocalizing experiments, Hertting et al. demonstrated that the bladder cells treated with vitamin D had increased expression of cathelicidin. This observation that vitamin D leads to increased production of cathelicidin and other antimicrobial peptides is encouraging to connect vitamin D with streptococcal infections.[36] In fact, cathelicidins have been linked to protection against Group A *Streptococcus* infection of the soft skin.[78] *S. pyogenes* has evolved two-component systems and virulence factors to overcome immune stress by cathelicidins.[79,80]

Evidence linking vitamin D levels and infectious diseases takes form in one human study of inflammatory bowel disease patients. The research group observed an inverse correlation between serum vitamin D levels and instances of *Clostridium difficile* infections, suggesting that vitamin D availability is linked with risk of infection.[81] With potential links between vitamin D and streptococcal infections, more research investigating the potential of using vitamin D supplementation to prevent or combat streptococcal infections is warranted.

Vitamin D supplementation is an inexpensive intervention shown to decrease adverse outcomes in a wide range of infectious diseases. Studies have demonstrated that vitamin D supplementation of mothers and infants can reduce the risk of sepsis in neonates.[82] A double-blind individually randomized placebo-controlled trial involving young children showed that risk of a repeat episode of pneumonia within 90 days of supplementation of oral vitamin D_3 was lower in the intervention than in the placebo group.[83] Taken together, the available data regarding vitamin D supplementation suggest a potential cost-effective chemotherapeutic intervention to mitigate risk associated with infection-associated illnesses such as those caused by *Streptococcus* species.

CONCLUSIONS

Vitamin D exerts a wide range of effects on both host and pathogen cells (Figure 2). Vitamin D inhibits streptococcal growth, viability, and biofilm formation. Vitamin D likely signals through the vitamin D receptor and promotes innate immune cell functions, including phagocytosis, production of antimicrobial peptides (AMPs) and reactive oxygen species (ROS), upregulation of pattern recognition receptors (PRR), and production of TIMP-1, which negatively regulates matrix metalloproteinases (MMPs) that contribute to tissue damage. Vitamin D also represses production of MMPs and proinflammatory cytokines and leads to repression of Th17 programming, favoring a regulatory T cell phenotype instead. Together, these data indicate that vitamin D promotes innate immune clearance of streptococcal cells and immunoregulation of the inflammatory response to ameliorate disease outcome. Thus, a close review of existing literature suggests that vitamin D homeostasis influences the outcome of streptococcal infections.

AUTHOR INFORMATION

Corresponding Authors

Jennifer A. Gaddy − *Department of Pathology, Microbiology and Immunology and Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee 37232, United States; Department of Veterans Affairs, Tennessee Valley Healthcare Systems, Nashville, Tennessee 37212, United States;* orcid.org/0000-0002-2192-4224; Phone: (615) 873-7884; Email: jennifer.a.gaddy@vumc.org; Fax: (615) 343-6160

Steven D. Townsend − *Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37232, United States;* orcid.org/0000-0001-5362-7235; Phone: (615) 322-8171; Email: Steven.d.townsend@vanderbilt.edu

Authors

Miriam A. Guevara − *Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee 37232, United States*

Jacky Lu − *Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee 37232, United States*

Rebecca E. Moore − *Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37232, United States*

Schuyler A. Chambers − *Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37232, United States;* orcid.org/0000-0002-3557-830X

Alison J. Eastman − *Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee 37232, United States*

Jamisha D. Francis − *Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee 37232, United States*

Kristen N. Noble − *Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee 37232, United States*

Ryan S. Doster − *Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee 37232, United States*

Kevin G. Osteen − *Department of Pathology, Microbiology and Immunology and Department of Obstetrics and Gynecology, Vanderbilt University Medical Center, Nashville, Tennessee 37232, United States; Department of Veterans Affairs, Tennessee Valley Healthcare Systems, Nashville, Tennessee 37212, United States*

Steven M. Damo − *Department of Chemistry, Fisk University, Nashville, Tennessee 37208, United States; Department of Biochemistry, Vanderbilt University, Nashville, Tennessee 37232, United States*

Shannon D. Manning − *Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan 48824, United States*

https://dx.doi.org/10.1021/acsinfecdis.0c00666
ACS Infect. Dis. XXXX, XXX, XXX−XXX

David M. Aronoff − *Department of Pathology, Microbiology and Immunology, Department of Medicine, and Department of Obstetrics and Gynecology, Vanderbilt University Medical Center, Nashville, Tennessee 37232, United States;* orcid.org/0000-0003-4587-6121

Natasha B. Halasa − *Department of Pathology, Microbiology and Immunology, Department of Medicine, and Department of Pediatrics, Vanderbilt University Medical Center, Nashville, Tennessee 37232, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsinfecdis.0c00666

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was funded by the National Institutes of Health grant R01 HD090061 (to J.A.G.) and by NIH T32 HL007411-36S1 (supporting J.L.), 2T32AI112541-06 (supporting J.F.), K08AI151100 (supporting R.S.D.), R35GM133602 (to S.D.T.), and F32HD100087 (to A.J.E.). Additional funding from the National Science Foundation Awards 1547757 and 1400969 and NIH grant GM05551 (to S.M.D.) supported this work. Additional support was provided by NIH U01TR002398, NIH R01AI134036, and the March of Dimes (to D.M.A.) from the Emerging Infections Program's cooperative agreement (1U50CK000491), from the Centers for Disease Control and Prevention (to N.B.H.), and from Vanderbilt Institute for Clinical and Translational Research program supported by the National Center for Research Resources, Grant UL1 RR024975-01, and the National Center for Advancing Translational Sciences, Grant 2 UL1 TR000445-06.

■ REFERENCES

(1) Parva, N. R., Tadepalli, S., Singh, P., Qian, A., Joshi, R., Kandala, H., Nookala, V. K., and Cheriyath, P. (2018) Prevalence of Vitamin D Deficiency and Associated Risk Factors in the US Population (2011−2012). *Cureus 10* (6), No. e2741.

(2) Nseir, W., Taha, M., Nemarny, H., and Mograbi, J. (2013) The Association between Serum Levels of Vitamin D and Recurrent Urinary Tract Infections in Premenopausal Women. *Int. J. Infect. Dis. 17* (12), No. e1121.

(3) Krzyaściak, W., Pluskwa, K. K., Jurczak, A., and Koaścielniak, D. (2013) The Pathogenicity of the *Streptococcus* Genus. *Eur. J. Clin. Microbiol. Infect. Dis. 32*, 1361−1376.

(4) Parks, T., Barrett, L., and Jones, N. (2015) Invasive Streptococcal Disease: A Review for Clinicians. *Br. Med. Bull. 115* (1), 77−89.

(5) Walker, M. J., Barnett, T. C., McArthur, J. D., Cole, J. N., Gillen, C. M., Henningham, A., Sriprakash, K. S., Sanderson-Smith, M. L., and Nizet, V. (2014) Disease Manifestations and Pathogenic Mechanisms of Group A *Streptococcus*. *Clin. Microbiol. Rev. 27* (2), 264−301.

(6) Carapetis, J. R. (2004) The Current Evidence for the Burden of Group A Streptococcal Diseases. *World Heal. Organ. 5* (11), 685−694.

(7) Patras, K. A., and Nizet, V. (2018) Group B Streptococcal Maternal Colonization and Neonatal Disease: Molecular Mechanisms and Preventative Approaches. *Front. Pediatr.. 6*(27). DOI: 10.3389/fped.2018.00027.

(8) Kwon, K. T., and Armstrong, D. G. (2018) Microbiology and Antimicrobial Therapy for Diabetic Foot Infections. *Infect. Chemother. 50* (1), 11−20.

(9) Koumans, E. H. A., Rosen, J., Van Dyke, M. K., Zell, E., Phares, C. R., Taylor, A., Loft, J., and Schrag, S. (2012) Prevention of Mother-to-Child Transmission of Infections during Pregnancy: Implementation of Recommended Interventions, United States, 2003−2004. *Am. J. Obstet. Gynecol. 206* (2), 158.E1−158.E11.

(10) Russell, N. J., Seale, A. C., O'Driscoll, M., O'Sullivan, C., Bianchi-Jassir, F., Gonzalez-Guarin, J., Lawn, J. E., Baker, C. J., Bartlett, L., Cutland, C., Gravett, M. G., Heath, P. T., Le Doare, K., Madhi, S. A., Rubens, C. E., Schrag, S., Sobanjo-Ter Meulen, A., Vekemans, J., Saha, S. K., and Ip, M. (2017) Maternal Colonization with Group B *Streptococcus* and Serotype Distribution Worldwide: Systematic Review and Meta-Analyses. *Clin. Infect. Dis. 65* (Suppl 2), S100−S111.

(11) Francois Watkins, L. K., McGee, L., Schrag, S. J., Beall, B., Jain, J. H., Pondo, T., Farley, M. M., Harrison, L. H., Zansky, S. M., Baumbach, J., Lynfield, R., Snippes Vagnone, P., Miller, L. A., Schaffner, W., Thomas, A. R., Watt, J. P., Petit, S., and Langley, G. E. (2019) Epidemiology of Invasive Group B Streptococcal Infections among Nonpregnant Adults in the United States, 2008−2016. *JAMA Int. Med. 179* (4), 478−488.

(12) Abranches, J., Zeng, L., Kajfasz, J. K., Palmer, S. R., Chakraborty, B., Wen, Z. T., Richards, V. P., Brady, L. J., and Lemos, J. A. (2018) Biology of Oral Streptococci. *Microbiol. Spectrum. 6* (5) DOI: 10.1128/microbiolspec.GPP3-0042-2018.

(13) Ahn, S. J., Wen, Z. T., and Burne, R. A. (2006) Multilevel Control of Competence Development and Stress Tolerance in *Streptococcus mutans* UA159. *Infect. Immun. 74* (3), 1631−1642.

(14) Smith, E. G., and Spatafora, G. A. (2012) Gene Regulation in S. Mutans: Complex Control in a Complex Environment. *J. Dent. Res. 91* (2), 133−41.

(15) Moreillon, P., Que, Y. A., and Bayer, A. S. (2002) Pathogenesis of Streptococcal and Staphylococcal Endocarditis. *Infectious Disease Clinics of North America. 16* (2), 297−318.

(16) Marsh, P. D. (2003) Are Dental Diseases Examples of Ecological Catastrophes? *Microbiology 149* (2), 279−294.

(17) Murray, J., Agócs, M., Serhan, F., Singh, S., Deloria-Knoll, M., O'Brien, K., Mwenda, J. M., Mihigo, R., Oliveira, L., Teleb, N., Ahmed, H., Wasley, A., Videbaek, D., Wijesinghe, P., Bhadra Thapa, A., Fox, K., Paladin, F. J., Hajjeh, R., Schwartz, S., Van Beneden, C., Hyde, T., Broome, C., and Cherian, C. (2014) Global Invasive Bacterial Vaccine-Preventable Diseases Surveillance–2008−2014. *MMWR. Morb. Mortal. Wkly. Rep. 63* (49), 1159−1162.

(18) CDC. (2010) Streptococcus Pneumoniae 2009. *Act. Bact. Core Surveill. Rep. Emerg. Infect. Progr. Network, Centers Dis. Control Prev..*

(19) Bogaert, D., De Groot, R., and Hermans, P. W. M. (2004) Streptococcus Pneumoniae Colonisation: The Key to Pneumococcal Disease. *Lancet Infect. Dis. 4* (3), 144−154.

(20) CDC. (2017) Streptococcus Pneumoniae. *Epidemiol. Prev. Vaccine-Preventable Dis..*

(21) Holick, M. F., Maclaughlin, J. A., Clark, M. B., Holick, S. A., Potts, J. T., Anderson, R. R., Blank, I. H., Parrish, J. A., and Elias, P. (1980) Photosynthesis of Previtamin D3 in Human Skin and the Physiologic Consequences. *Science 210* (4446), 203−205.

(22) Holick, M. F. (2009) Vitamin D Status: Measurement, Interpretation, and Clinical Application. *Annals of Epidemiology. 19* (2), 73−78.

(23) Holick, M. F. (2004) Sunlight and Vitamin D for Bone Health and Prevention of Autoimmune Diseases, Cancers, and Cardiovascular Disease. *Am. J. Clin. Nutr. 80* (6), 1678S−1688S.

(24) Tripkovic, L., Lambert, H., Hart, K., Smith, C. P., Bucca, G., Penson, S., Chope, G., Hypponen, E., Berry, J., Vieth, R., and Lanham-New, S. (2012) Comparison of Vitamin D2and Vitamin D3 Supplementation in Raising Serum 25-Hydroxyvitamin D Status: A Systematic Review and Meta-Analysis. *Am. J. Clin. Nutr. 95* (6), 1357−1364.

(25) Windaus, A., Lüttringhaus, A., and Deppe, M. (1931) Über Das Krystallisierte Vitamin D1. *Justus Liebigs Ann. Chem. 489* (1), 252−269.

(26) Mooso, B., Madhav, A., Johnson, S., Roy, M., Moore, M. E., Moy, C., Loredo, G. A., Mehta, R. G., Vaughan, A. T. M., and Ghosh, P. M. (2010) Androgen Receptor Regulation of Vitamin D Receptor in Response of Castration-Resistant Prostate Cancer Cells to 1α-Hydroxyvitamin D5: A Calcitriol Analog. *Genes Cancer 1* (9), 927−940.
(27) Baeke, F., Takiishi, T., Korf, H., Gysemans, C., and Mathieu, C. (2010) Vitamin D: Modulator of the Immune System. *Curr. Opin. Pharmacol. 10* (4), 482−496.
(28) Cohen-Lahav, M., Shany, S., Tobvin, D., Chaimovitz, C., and Douvdevani, A. (2006) Vitamin D Decreases NFκB Activity by Increasing IκBα Levels. *Nephrol., Dial., Transplant. 21* (4), 889−897.
(29) Golden, G. A., Wyatt, T. A., Romberger, D. J., Reiff, D., McCaskill, M., Bauer, C., Gleason, A. M., and Poole, J. A. (2013) Vitamin D Treatment Modulates Organic Dust-Induced Cellular and Airway Inflammatory Consequences. *J. Biochem. Mol. Toxicol. 27* (1), 77−86.
(30) Hansdottir, S., Monick, M. M., Lovan, N., Powers, L., Gerke, A., and Hunninghake, G. W. (2010) Vitamin D Decreases Respiratory Syncytial Virus Induction of NF-KB-Linked Chemokines and Cytokines in Airway Epithelium While Maintaining the Antiviral State. *J. Immunol. 184* (2), 965−974.
(31) Zhang, Y., Leung, D. Y. M., Richers, B. N., Liu, Y., Remigio, L. K., Riches, D. W., and Goleva, E. (2012) Vitamin D Inhibits Monocyte/Macrophage Proinflammatory Cytokine Production by Targeting MAPK Phosphatase-1. *J. Immunol. 188* (5), 2127−2135.
(32) Goldman, R. (1984) Induction of a High Phagocytic Capability in P388D1, a Macrophage-like Tumor Cell Line, by 1α,25-Dihydroxyvitamin D3. *Cancer Res. 44* (1), 11−19.
(33) Tokuda, N., and Levy, R. B. (1996) 1,25-Dihydroxyvitamin D3 Stimulates Phagocytosis but Suppresses HLA-DR and CD13 Antigen Expression in Human Mononuclear Phagocytes. *Exp. Biol. Med. 211* (3), 244−250.
(34) Xu, H., Soruri, A., Gieseler, R. K. H., and Peters, J. H. (1993) 1,25-Dihydroxyvitamin D3 Exerts Opposing Effects to IL-4 on MHC Class-II Antigen Expression, Accessory Activity, and Phagocytosis of Human Monocytes. *Scand. J. Immunol. 38* (6), 535−540.
(35) Sly, L. M., Lopez, M., Nauseef, W. M., and Reiner, N. E. (2001) 1α,25-Dihydroxyvitamin D3-Induced Monocyte Antimycobacterial Activity Is Regulated by Phosphatidylinositol 3-Kinase and Mediated by the NADPH-Dependent Phagocyte Oxidase. *J. Biol. Chem. 276* (38), 35482−35493.
(36) Liu, P. T., Stenger, S., Li, H., Wenzel, L., Tan, B. H., Krutzik, S. R., Ochoa, M. T., Schauber, J., Wu, K., Meinken, C., Kamen, D. L., Wagner, M., Bals, R., Steinmeyer, A., Zügel, U., Gallo, R. L., Eisenberg, D., Hewison, M., Hollis, B. W., Adams, J. S., Bloom, B. R., and Modlin, R. L. (2006) Toll-like Receptor Triggering of a Vitamin D-Mediated Human Antimicrobial Response. *Science (Washington, DC, U. S.) 311* (5768), 1770−1773.
(37) Wang, T.-T., Nestel, F. P., Bourdeau, V., Nagai, Y., Wang, Q., Liao, J., Tavera-Mendoza, L., Lin, R., Hanrahan, J. W., Mader, S., and White, J. H. (2004) Cutting Edge: 1,25-Dihydroxyvitamin D 3 Is a Direct Inducer of Antimicrobial Peptide Gene Expression. *J. Immunol. 173* (5), 2909−2912.
(38) Hmama, Z., Nandan, D., Sly, L., Knutson, K. L., Herrera-Velit, P., and Reiner, N. E. (1999) 1α,25-Dihydroxyvitamin D3-Induced Myeloid Cell Differentiation Is Regulated by a Vitamin D Receptor-Phosphatidylinositol 3-Kinase Signaling Complex. *J. Exp. Med. 190* (11), 1583−1594.
(39) Schwende, H., Fitzke, E., Ambs, P., and Dieter, P. (1996) Differences in the State of Differentiation of THP-1 Cells Induced by Phorbol Ester and 1,25-Dihydroxyvitamin D3. *J. Leukocyte Biol. 59* (4), 555−561.
(40) Anand, S. P., and Selvaraj, P. (2009) Effect of 1, 25 Dihydroxyvitamin D3 on Matrix Metalloproteinases MMP-7, MMP-9 and the Inhibitor TIMP-1 in Pulmonary Tuberculosis. *Clin. Immunol. 133* (1), 126−131.
(41) Zaga-Clavellina, V., Merchant-Larios, H., Garcia-Lopez, G., Maida-Claros, R., and Vadillo-Oretega, F. (2006) Differential secretion of matrix metalloproteinase-2 and −9 after selective infection with group B streptococci in human fetal membranes. *J. Soc. Gynecol. Invest. 13* (4), 271−279.
(42) Subramanian, K., Bergman, P., and Henriques-Normark, B. (2017) Vitamin D Promotes Pneumococcal Killing and Modulates Inflammatory Responses in Primary Human Neutrophils. *J. Innate Immun. 9*, 375−386.
(43) Kietzman, C. C., Gao, G., Mann, B., Myers, L., and Tuomanen, E. I. (2016) Dynamic capsule restructuring by the main pneumococcal autolysin LytA in response to the epithelium. *Nat. Commun. 7*, 10859.
(44) Love, J. F., Tran-Winkler, H. J., and Wessels, M. R. (2012) Vitamin D and the Human Antimicrobial Peptide LL-37 Enhance Group A *Streptococcus* Resistance to Killing by Human Cells. *mBio 3* (5), No. e00394.
(45) Hoe, E., Nathanielsz, J., Toh, Z., Spry, L., Marimla, R., Balloch, A., Mulholland, K., and Licciardi, P. (2016) Anti-Inflammatory Effects of Vitamin D on Human Immune Cells in the Context of Bacterial Infection. *Nutrients 8* (12), 806.
(46) Anderson, J., Do, L. A. H., Toh, Z. Q., Hoe, E., Reitsma, A., Mulholland, K., and Licciardi, P. V. (2020) Vitamin D Induces Differential Effects on Inflammatory Responses During Bacterial and/or Viral Stimulation of Human Peripheral Blood Mononuclear Cells. *Front. Immunol. 11*, 602.
(47) Olliver, M., Spelmink, L., Hiew, J., Meyer-Hoffert, U., Henriques-Normark, B., and Bergman, P. (2013) Immunomodulatory Effects of Vitamin D on Innate and Adaptive Immune Responses to *Streptococcus pneumoniae*. *J. Infect. Dis. 208* (9), 1474−1481.
(48) Li, B., Baylink, D. J., Deb, C., Zannetti, C., Rajaallah, F., Xing, W., Walter, M. H., Lau, K. H. W., and Qin, X. (2013) 1,25-Dihydroxyvitamin D3 Suppresses TLR8 Expression and TLR8-Mediated Inflammatory Responses in Monocytes *In Vitro* and Experimental Autoimmune Encephalomyelitis In Vivo. *PLoS One 8* (3), No. e58808.
(49) Eigenbrod, T., Pelka, K., Latz, E., Kreikemeyer, B., and Dalpke, A. H. (2015) TLR8 Senses Bacterial RNA in Human Monocytes and Plays a Nonredundant Role for Recognition of *Streptococcus pyogenes*. *J. Immunol. 195* (3), 1092−9.
(50) Dowling, D. J., van Haren, S. D., Scheid, A., Bergelson, I., Kim, D., Mancuso, C. J., Foppen, W., Ozonoff, A., Fresh, L., Theriot, T. B., Lackner, A. A., Fichorova, R. N., Smirnov, D., Vasilakos, J. P., Beaurline, J. M., Tomai, M. A., Midkiff, C. C., Alvarez, X., Blanchard, J. L., Gilbert, M. H., Aye, P. P., and Levy, O. (2017) TLR7/8 adjuvant overcomes newborn hyporesponsiveness to pneumococcal conjugate vaccine at birth. *JCI Insight. 2* (6), No. e91020.
(51) Ehrnström, B., Beckwith, K. S., Yurchenko, M., Moen, S. H., Kojen, J. F., Lentini, G., Teti, G., Damås, J. K., Espevik, T., and Stenvik, J. (2017) Toll-Like Receptor 8 Is a Major Sensor of Group B *Streptococcus* But Not *Escherichia coli* in Human Primary Monocytes and Macrophages. *Front. Immunol. 8*, 1243.
(52) Lundgren, A., Bhuiyan, T. R., Novak, D., Kaim, J., Reske, A., Lu, Y. J., Qadri, F., and Malley, R. (2012) Characterization of Th17 Responses to *Streptococcus pneumoniae* in Humans: Comparisons between Adults and Children in a Developed and a Developing Country. *Vaccine 30* (26), 3897−3907.
(53) Fabri, M., Stenger, S., Shin, D. M., Yuk, J. M., Liu, P. T., Realegeno, S., Lee, H. M., Krutzik, S. R., Schenk, M., Sieling, P. A., Teles, R., Montoya, D., Iyer, S. S., Bruns, H., Lewinsohn, D. M., Hollis, B. W., Hewison, M., Adams, J. S., Steinmeyer, A., Zügel, U., Cheng, G., Jo, E. K., Bloom, B. R., and Modlin, R. L. (2011) Vitamin D Is Required for IFN-γ-Mediated Antimicrobial Activity of Human Macrophages. *Sci. Transl. Med. 3* (104), 102.
(54) Afsal, K., Selvaraj, P., and Harishankar, M. (2018) 1, 25-Dihydroxyvitamin D3 Downregulates Cytotoxic Effector Response in Pulmonary Tuberculosis. *Int. Immunopharmacol. 62*, 251−260.
(55) Usui, K., Okubo, Y., Hirano, T., and Tsuboi, R. (2017) Vitamin D3 derivatives, alone or in combination with glucocorticoids, suppress streptococcal pyrogenic enterotoxin A-stimulated proliferation of

https://dx.doi.org/10.1021/acsinfecdis.0c00666
ACS Infect. Dis. XXXX, XXX, XXX−XXX

peripheral blood mononuclear cells in patients with psoriasis. *J. Dermatol. 44* (5), 567−572.
(56) Arai, K., Uchiyama, T., Okubo, Y., Tsuboi, R., Oka, K., and Hirano, T. (2007) Comparative study of the effects of betamethasone butyrate propionate, vitamin D3 derivatives, and cyclosporine on human lymphocyte-proliferation stimulated with a hemolytic streptococci-derived superantigen. *Eur. J. Pharmacol. 571* (2−3), 222−230.
(57) Patel, N., Penkert, R. R., Jones, B. G., Sealy, R. E., Surman, S. L., Sun, Y., Tang, L., DeBeauchamp, J., Webb, A., Richardson, J., Heine, R., Dallas, R. H., Ross, A. C., Webby, R., and Hurwitz, J. L. (2019) Baseline Serum Vitamin A and D Levels Determine Benefit of Oral Vitamin A&D Supplements to Humoral Immune Responses Following Pediatric Influenza Vaccination. *Viruses 11* (10), 907.
(58) Peelen, E., Rijkers, G., Meerveld-Eggink, A., Meijvis, S., Vogt, M., Cohen Tervaert, J. W., Hupperts, R., and Damoiseaux, J. (2013) Relatively high serum vitamin D levels do not impair the antibody response to encapsulated bacteria. *Eur. J. Clin. Microbiol. Infect. Dis. 32* (1), 61−69.
(59) Feindt, E., and Ströder, J. (1977) Studies on the Antimicrobial Effect of Vitamin D3. *Klin. Wochenschr. 55*, 507−508.
(60) Saputo, S., Faustoferri, R. C., and Quivey, R. G. (2018) Vitamin D Compounds Are Bactericidal against Streptococcus Mutans and Target the Bacitracin-Associated Efflux System. *Antimicrob. Agents Chemother. 62*, No. e01675.
(61) Raab, W. (1946) Tuberculous Empyema Treated with Vitamin A-D Concentrate; a Preliminary Report. *Dis. Chest 12* (1), 68−71.
(62) Guy, R. A. (1923) The History of Cod Liver Oil as a Remedy. *Arch. Pediatr. Adolesc. Med. 26* (2), 112−116.
(63) Grenier, D., Morin, M. P., Fournier-Larente, J., and Chen, H. (2016) Vitamin D Inhibits the Growth of and Virulence Factor Gene Expression by Porphyromonas Gingivalis and Blocks Activation of the Nuclear Factor Kappa B Transcription Factor in Monocytes. *J. Periodontal Res. 51* (3), 359−365.
(64) Hosoda, K., Shimomura, H., Wanibuchi, K., Masui, H., Amgalanbaatar, A., Hayashi, S., Takahashi, T., and Hirai, Y. (2015) Identification and Characterization of a Vitamin D3 Decomposition Product Bactericidal against *Helicobacter pylori. Sci. Rep. 5*, 8860.
(65) Pletz, M. W., Terkamp, C., Schumacher, U., Rohde, G., Schütte, H., Welte, T., and Bals, R. (2014) Vitamin D Deficiency in Community-Acquired Pneumonia: Low Levels of 1,25(OH)2 D Are Associated with Disease Severity. *Respir. Res. 15*, 53.
(66) Van Winden, K. R., Bearden, A., Kono, N., Frederick, T., Operskalski, E., Stek, A., Pandian, R., Barton, L., and Kovacs, A. (2019) Low Bioactive Vitamin D Is Associated with Pregnancy-Induced Hypertension in a Cohort of Pregnant HIV-Infected Women Sampled Over a 23-Year Period. *Am. J. Perinatol.* DOI: 10.1055/s-0039-1694007.
(67) Nseir, W., Mograbi, J., Abu-Rahmeh, Z., Mahamid, M., Abu-Elheja, O., and Shalata, A. (2012) The Association between Vitamin D Levels and Recurrent Group A Streptococcal Tonsillopharyngitis in Adults. *Int. J. Infect. Dis. 16* (10), No. e735.
(68) Laaksi, I., Ruohola, J. P., Tuohimaa, P., Auvinen, A., Haataja, R., Pihlajamäki, H., and Ylikomi, T. (2007) An Association of Serum Vitamin D Concentrations < 40 Nmol/L with Acute Respiratory Tract Infection in Young Finnish Men. *Am. J. Clin. Nutr. 86* (3), 714−717.
(69) Akoh, C. C., Pressman, E. K., Whisner, C. M., Thomas, C., Cao, C., Kent, T., Cooper, E., and O'Brien, K. O. (2017) Vitamin D Mediates the Relationship between Placental Cathelicidin and Group B *Streptococcus* Colonization during Pregnancy. *J. Reprod. Immunol. 121*, 42−48.
(70) Chhin, D., Pozzetto, B., Teyssier, G., Mteirek, M., Diehl, R., Trombert-Paviot, B., Varlet, M. N., Goffaux, P., and Patural, H. (2013) Relationship between Cord Blood Vitamin D Level and Group B *Streptococcus* Vaginal Carriage Rate in Pregnant Women. *ESPEN. J. 8* (4), No. e150.
(71) Qin, L. L., Lu, F. G., Yang, S. H., Xu, H. L., and Luo, B. A. (2016) Does Maternal Vitamin D Deficiency Increase the Risk of Preterm Birth: A Meta-Analysis of Observational Studies. *Nutrients 8* (5), 301.
(72) Zhou, S. S., Tao, Y. H., Huang, K., Zhu, B. B., and Tao, F. B. (2017) Vitamin D and Risk of Preterm Birth: Up-to-Date Meta-Analysis of Randomized Controlled Trials and Observational Studies. *Journal of Obstetrics and Gynaecology Research. 43* (2), 247−256.
(73) Dutra, L. V., Affonso-Kaufman, F. A., Cafeo, F. R., Kassai, M. S., Barbosa, C. P., Santos Figueiredo, F. W., Suano-Souza, F. I., and Bianco, B. (2020) Association between Vitamin D Plasma Concentrations and VDR Gene Variants and the Risk of Premature Birth. *BMC Pregnancy Childbirth 20*, 3.
(74) Javorski, N., Lima, C. A. D., Silva, L. V. C., Crovella, S., and de Azêvedo Silva, J. (2018) Vitamin D Receptor (VDR) Polymorphisms Are Associated to Spontaneous Preterm Birth and Maternal Aspects. *Gene 642* (5), 58−63.
(75) Wilson, R. L., Phillips, J. A., Bianco-Miotto, T., McAninch, D., Goh, Z., Anderson, P. H., and Roberts, C. T. (2020) Reduced Dietary Calcium and Vitamin D Results in Preterm Birth and Altered Placental Morphogenesis in Mice During Pregnancy. *Reprod. Sci. 27*, 1330−1339.
(76) Lim, J. H. J., Ravikumar, S., Wang, Y.-M., Thamboo, T. P., Ong, L., Chen, J., Goh, J. G., Tay, S. H., Chengchen, L., Win, M. S., Leong, W., Lau, T., Foo, R., Mirza, H., Tan, K. S. W., Sethi, S., Khoo, A. L., Chng, W. J., Osato, M., Netea, M. G., Wang, Y., and Chai, L. Y. A. (2015) Bimodal Influence of Vitamin D in Host Response to Systemic *Candida* Infection-Vitamin D Dose Matters. *J. Infect. Dis. 212* (4), 635−644.
(77) Hertting, O., Holm, Å., Lüthje, P., Brauner, H., Dyrdak, R., Jonasson, A. F., Wiklund, P., Chromek, M., and Brauner, A. (2010) Vitamin D Induction of the Human Antimicrobial Peptide Cathelicidin in the Urinary Bladder. *PLoS One 5* (12), No. e15580.
(78) Di Nardo, A., Yamasaki, K., Dorschner, R. A., Lai, Y., and Gallo, R. L. (2008) Mast Cell Cathelicidin Antimicrobial Peptide Prevents Invasive Group A *Streptococcus* Infection of the Skin. *J. Immunol. 180* (11), 7565−7573.
(79) Lauth, X., Von Köckritz-Blickwede, M., McNamara, C. W., Myskowski, S., Zinkernagel, A. S., Beall, B., Ghosh, P., Gallo, R. L., and Nizet, V. (2009) M1 Protein Allows Group a Streptococcal Survival in Phagocyte Extracellular Traps through Cathelicidin Inhibition. *J. Innate Immun. 1*, 202−214.
(80) LaRock, C. N., Döhrmann, S., Todd, J., Corriden, R., Olson, J., Johannssen, T., Lepenies, B., Gallo, R. L., Ghosh, P., and Nizet, V. (2015) Group A Streptococcal M1 Protein Sequesters Cathelicidin to Evade Innate Immune Killing. *Cell Host Microbe 18* (4), 471−477.
(81) Ananthakrishnan, A. N., Cagan, A., Gainer, V. S., Cheng, S. C., Cai, T., Szolovits, P., Shaw, S. Y., Churchill, S., Karlson, E. W., Murphy, S. N., Kohane, I., and Liao, K. P. (2014) Higher Plasma Vitamin D Is Associated with Reduced Risk of *Clostridium difficile* Infection in Patients with Inflammatory Bowel Diseases. *Aliment. Pharmacol. Ther. 39* (10), 1136−1142.
(82) Grant, W. B. (2010) Vitamin D Supplementation Could Reduce Risk of Sepsis in Infants. *World J. Pediatr. 6*, 185.
(83) Manaseki-Holland, S., Qader, G., Isaq Masher, M., Bruce, J., Zulf Mughal, M., Chandramohan, D., and Walraven, G. (2010) Effects of Vitamin D Supplementation to Children Diagnosed with Pneumonia in Kabul: A Randomised Controlled Trial. *Trop. Med. Int. Health 15* (10), 1148−1155.

https://dx.doi.org/10.1021/acsinfecdis.0c00666
ACS Infect. Dis. XXXX, XXX, XXX−XXX